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MiniReview

Ambient Air Pollution and Adverse Birth Outcomes: Methodologic Issues in an Emerging Field

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Abstract: Since the mid-1990s, the number of studies linking air pollutants to low birthweight, small for gestational age, preterm birth and cardiac birth defects has grown steadily. This *MiniReview* (in conjunction with the May 2007 International Conference on Foetal Programming and Development Toxicity) highlights key methodological issues surrounding this research area, based on our experiences in Southern California. All 'criteria' air pollutants have been linked to birth outcomes. Our own studies found most consistent associations for carbon monoxide and particles. Traffic exhaust toxins are possible causative agents, but air monitoring data relied on by almost all existing studies inadequately capture their intracommunity variability in concentrations. Exposure assessment might be improved by biomarkers and land use-based regression modelling or information on time-activity patterns. Foetal development provides unique opportunities to study exposures acting during narrow susceptibility windows. A time-series approach by design controls for confounders that do not vary temporally but can only address short-term acute effects. Studies employing spatial or medium-term (trimester-specific) temporal contrasts may be more susceptible to residual confounding, and studies adjusting only for risk factors recorded on birth certificates have been criticized. Findings from our recent study in Southern California indicate that air pollution effect estimates are not markedly influenced by risk factors not provided on birth certificates. Yet, studies collecting detailed risk factor information in other geographic regions may be needed to further evaluate the extent of residual confounding in record-based analyses. Investigating biological mechanisms (e.g. using ultrasound measurements and biomarkers for hypothesized pathways) is an important remaining issue.

Even though evidence linking ambient air pollution with mortality and respiratory morbidity in human beings has accumulated over the past 30 years [1], researchers have only recently begun to explore its influence on less traditional end-points and organs such as the cardiovascular system and heart [2] and adverse pregnancy outcomes [3–6]. Pregnancy may constitute a period of human development particularly susceptible to toxins contained in air pollution because of high cell proliferation, organ development and the changing capabilities of foetal metabolism [7]. Importantly, the foetal origins hypothesis suggests that growth and developmental delays *in utero* may influence not only childhood mortality and morbidity but also the risk for diseases of the heart and metabolism, including diabetes in adulthood [8]. For example, in support of the Barker hypothesis [9], a recent study reported that adults who were born moderately preterm (32–36 gestational weeks) experienced increased blood pressure and insulin resistance at 30 years of age [10].

The goal of this *MiniReview* (published in conjunction with the International Conference on Foetal Programming and Development Toxicity in May 2007) is to highlight some key methodological issues surrounding this area of research. We do not intend for this to be a formal literature review or meta-analysis, but rather a discussion of issues we feel are particularly important based on experiences from our studies in Southern California.

The well-accepted link between maternal smoking and adverse birth outcomes lends support for a role of ambient (outdoor) air pollution impacts on pregnancy. In the past decade, linkage of registry data such as birth certificates, with exposure measures based on routine monitoring data has resulted in a fast-growing body of evidence of air pollution's harmful impact on foetal development. These studies have linked a number of air pollutants to outcomes, including low birthweight (LBW) and small for gestational age (SGA, often referred to as intrauterine growth retardation, IUGR), prematurity, and cardiac birth defects. More recently,

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researchers also started to investigate pre-eclampsia [11] and spontaneous abortion, the latter possibly mediated through DNA fragmentation in sperm [12].

All of the routinely measured 'criteria' air pollutants [i.e. carbon monoxide (CO), PM_{10}, $PM_{2.5}$, nitrogen dioxide (NO_2), O_3, sulfur dioxide (SO_2)] and, in addition, polycyclic aromatic hydrocarbons (PAHs), have been linked to various measures of IUGR mostly in urban areas throughout the world [3–6]. In our studies conducted in the South Coast Air Basin of California, we found positive associations between last trimester exposures to CO and particulate matter less than 10 μm in aerodynamic diameter (PM_{10}) and term LBW [13,14]. Two additional California studies reported associations for $PM_{2.5}$ but not CO when examining births throughout the entire state [15], and for O_3 and CO when examining births during 1975–1987 in several Southern California cities located from Santa Barbara to San Diego County [16]. Associations have also been reported – by us and others – between early and late pregnancy exposures to CO, particles [total suspended particulate matter (TSP), PM_{10}, $PM_{2.5}$], SO_2, and NO_2 and preterm birth [3,6,14,17–19]. It should be noted that associations between air pollution and preterm and low weight birth have been documented not only in more polluted regions like the Los Angeles basin (e.g. mean entire pregnancy averages of 34 μg/m^3 and 20 μg/m^3 for PM_{10} and $PM_{2.5}$, respectively, during 2002–2003 [19]), but also in other urban areas with lower air pollution levels (e.g. mean entire pregnancy averages of 22 μg/m^3 and 12 μg/m^3 for PM_{10} and $PM_{2.5}$, respectively, in Connecticut and Massachusetts during 1999–2002 [20]). A limited number of studies have reported associations between air pollution exposure and cardiac birth defects, specifically between CO and isolated ventricular septum defects [21], CO and tetralogy of Fallot, PM_{10} and isolated atrial septal defects, and SO_2 and isolated ventricular septal defects [22]. Furthermore, certain subpopulations of women and foetuses may be especially susceptible to air pollutants, such as individuals with a compromised general health status or those with social disadvantages that translate into increased exposure to toxins environmentally or occupationally, adverse behaviours (poorer diet, alcohol use and smoking) and lack of adequate access to health care and preventative health measures. In our study in Los Angeles, we observed that traffic-related air pollution exposure disproportionately affected low-income and disadvantaged neighbourhoods in the winter, when pollutant levels tend to peak due to meteorological conditions. These associations resulted in the highest odds of preterm birth for African Americans, Hispanics, and mothers <20 years and 35 years or older [23].

Although several review articles summarizing the literature on air pollution and birth outcomes have been published [3–5], only one study attempted a formal meta-analysis of the existing data for LBW [6]. Further work in this regard has been hampered by difficulties in rectifying differences between studies in definition of outcomes, number and type of pollutants and covariates considered, pregnancy exposure periods evaluated, and due to inconsistencies in reporting (e.g. scaling of pollutants or use of quartiles to define exposure categories).

Outcome definitions

Low birthweight (<2500 g at birth) is the outcome studied most in relation to air pollution and includes preterm as well as full-term infants, with some studies further distinguishing infants with very low birthweight (<1500 g). Term LBW is sometimes considered a marker for IUGR under the assumption that babies born at or after 37 weeks of gestation and weighing less than 2500 g were growth-restricted and not small just because they were born early. Some studies instead tried to differentiate growth-retarded from premature infants by using SGA as the outcome of interest, defined as a weight below the 10th percentile of infants born at a given gestational age (and with the same sex and race/ethnicity). The SGA measure intends to identify infants with inadequate intrauterine growth yet this is based solely on the weight distribution of all infants born at a specific gestational age rather than all foetuses of the gestational age. Therefore, using SGA might confuse the issue of premature births of adequate gestational size/weight with growth restriction (i.e. abnormal intrauterine growth and development). Others have preferred to examine preterm birth (<37 weeks completed gestation) as its own end-point, regardless of birthweight.

Some have argued against the use of a <2500 g birthweight cut-off, because analyses have shown that the strongest predictor of perinatal mortality in a population is not the proportion of newborns with a LBW, but the proportion of newborns whose birthweight falls outside the population specific Gaussian birthweight distribution (the residual) that is mostly comprised of preterm births [24,25]. Olsen and Basso [25] also argue that SGA is misleading, because it is a purely descriptive population concept: the infant being among the smallest in this particular population. Thus, an infant that has achieved its full genetic growth potential could be considered SGA just because of its genetically determined small size. They argue further that our interest in birthweight from a health perspective should focus on a deviation from the genetic growth potential rather than the absolute birthweight. For example, based on a Norwegian study [26], a birthweight lower than the expected based on siblings' and mother's birthweight, was the most important risk factor for perinatal mortality over the entire birthweight distribution. However, implementation of such alternate outcome definitions to help identify truly growth-restricted and high-risk infants may be impossible to establish in studies that rely on birth certificates or other public records that document only birthweight and gestational age of the index infant. Prospective cohort studies may provide opportunities to examine alternate birth outcome definitions. For example, in a recent cohort study, Slama et al. [27] employed ultrasound measures of head circumference twice during pregnancy and showed that growth restrictions before the end of the second trimester

were related to levels of outdoor NO_2 (a marker of traffic exhaust pollutants) during the first trimester.

For population-based research, birth defects generally need to be actively ascertained in a registry for all live born infants and foetal deaths (typically diagnosed after 20 weeks of gestation) and live born infants and, importantly, after birth in order to capture anomalies not apparent at delivery. Furthermore, the records need to allow distinguishing between isolated, multiple, syndromic or chromosomal defects and also by anatomical sub-categories. For cardiac defects, the diagnoses should preferably be confirmed by autopsy or surgical reports, catheterization or echocardiogram. Therefore, studying the influence of air pollutants on birth defects in a valid manner will not be possible unless such a system is already in place for a given geographic area and can be linked to extensive air monitoring data for a large enough population with enough births at risk for these rare outcomes.

Susceptible windows

The relatively short 9-month period during which the foetus develops *in utero* provides unique opportunities to study exposures acting during narrow susceptibility windows. Networks of continuous monitoring stations in urban areas allow for exposure assessment on a fine time scale often exploited in time-series studies. However, this approach assumes that exposure effects are acute and necessarily short-term (i.e. acting within days or a few weeks at most). For many birth outcomes, a more appropriate scale may be months or trimesters. The importance of short-term exposures versus more long-term, cumulative doses may vary by the end-point of interest [7]; currently, there is a lack of toxicological information to help guide selection of relevant exposure periods for most foetal growth end-points (except perhaps for specific birth defects). For LBW and preterm birth, first and third trimester air pollution exposures have been implicated as having the most relevance, while for birth defects, the development time of the specific organ has to be considered. In fact, one might also need to consider high exposures that can lead to competing outcomes such as spontaneous abortions or still births of damaged foetuses that do not survive. This contributes to selective survival that removes foetuses at risk for an adverse birth outcome such as LBW or preterm birth from the live births, translating to non-linearity in dose effects [28].

Clinical studies have shown associations between first trimester ultrasound and biochemical parameters (circulating maternal concentrations of pregnancy-associated plasma protein A, a trophoblast-derived regulator of the insulin-like growth factor system) and the risk of later adverse perinatal outcomes (including still birth, growth restriction, preterm birth and pre-eclampsia), possibly reflecting a defect in early pregnancy placentation [29]. A recent study of assisted technology pregnancies [30] with known dates of conception also suggested that duration of pregnancy and complications of late pregnancy may be the ultimate consequence of conditions in the earliest stages of placental and foetal development underlining the importance of the periconceptional period and first trimester.

Air pollution exposure assessment

Early studies conducted in China [31,32] and the Czech Republic [33,34] mostly identified particles (measured as TSP) and SO_2 as being associated with adverse birth outcomes, with the major pollutant sources being high-sulfur content coal used in homes for heating and cooking, and in power plants and other industry. This implicated high levels of air pollution from these combustion sources but did not allow determining which component was most important. Only PAHs were specifically identified [35] and, when measured, showed effects independent of PM_{10} and $PM_{2.5}$ levels in the Teplice and Prachatice areas of the Czech Republic [36]. This observation for outdoor air pollution may also be further supported by recent reports of observed reductions in birthweight from indoor burning of biomass for cooking (another major source of PAHs) in rural Guatemala [37] and Zimbabwe [38].

In more recent studies, all of the routinely measured 'criteria' air pollutants have been linked to adverse birth outcomes; however, the most frequent positive reports have been for particles (TSP, PM_{10}, $PM_{2.5}$), CO and SO_2. One difficulty in isolating a specific pollutant of concern for foetal development based on this body of evidence stems from inconsistencies between studies. Specifically, studies varied in the pollutants that were considered (based on pollutant data availability and *a priori* hypotheses) and also did not consistently use multipollutant models to try to disentangle effects. The latter may not be possible in many locations where levels of certain pollutants originating from the same source (e.g. CO and NO_x from motor vehicles, or particles and SO_2 from industry) are correlated in space and time. An additional problem when evaluating multiple pollutants is that typically not every pollutant is measured at every station within a given region. Multipollutant modelling restricting to subjects linked to stations measuring all pollutants alters the study population and the impact on results may not be straightforward due to selection issues. Extrapolations over large distances may not be adequate either and complex modelling approaches would need to be validated appropriately. Pollutants of importance may vary by reproductive end-point and/or geographic area and synergistic effects from pollutant mixtures may also be important.

Based on our studies in the South Coast Air Basin, we observed associations for pregnancy outcomes most consistently for CO and particles (PM_{10} and $PM_{2.5}$) [13,14,17,19]. A major source of pollutant emissions in this region, especially for CO, NO_x and PAHs, is motor vehicles (SO_2 levels in this area are generally low). These findings, along with previously reported associations between pregnancy exposures to PAHs and alterations in foetal growth based on a pregnancy cohort in New York City [39] point to toxins in motor vehicle exhaust as possible causative agents. In the

Los Angeles basin, concentrations of ultrafine particles (<0.1 μm), CO and NO_x are correlated spatially and decrease exponentially with distance from freeways, with 60–80% decay within approximately 150 m (500 feet) [40]. Similarly, measurement data for PM_{10} and $PM_{2.5}$ absorbance, black smoke, particle-bound PAHs and elemental carbon (EC) – all markers of exhaust particle emissions – indicate strong spatial gradients in concentrations with peaks near roadway sources [41–43]. Our research linking residential traffic density (a surrogate measure of exposure to motor vehicle exhaust that tries to account for these spatial gradients) to LBW and preterm birth suggests traffic pollutants warrant further study [44]. While air monitors placed in urban areas allow estimation of community-wide average exposures, they are less apt to provide quality data for spatially heterogeneous air pollutants such as CO and PAHs sorbed to ultrafine particles where proximity to sources (within a few hundred metres of a heavy traffic roadway) may result in very high exposures. Thus, additional information is needed to resolve this spatial heterogeneity and reduce exposure misclassification for such pollutants. Two alternative exposure assessment methods are biomarkers and geographic information system (GIS) techniques such as land use-based regression (LUR).

Biomarkers

Short-term personal monitoring and biomarkers promise in-depth exposure information at an individual level, and even at a physiologic and target organ level. Yet, these approaches are not only extremely costly and labour intensive and, therefore, feasible only in small samples, but one also needs to clearly define the toxin of interest and determine whether personal or biologic monitoring would be possible over time periods of relevance for pregnancy. Because air pollution, like tobacco smoke, is a mixture of many potential toxins, a decision must be made about which toxin to choose for monitoring. For example, one needs to decide whether it would be better to use a good indicator for a mixture from a specific source of interest (such as traffic exhaust) or an indicator of a suspected foetal toxicant. To date, there is little consensus on appropriate biomarkers for ambient air pollutants except possibly for some PAHs, mostly through measurement of DNA adducts, although measurement of hydroxylated PAH metabolites in urine is another option [45]. Other issues that need to be considered carefully regarding use of biomarkers are the ability to discern source contributions (e.g. dietary versus inhalation sources of PAHs) and appropriate timing of measurements. For example, urinary PAH metabolites measured at a single point in time during pregnancy will reflect exposures that occurred in the previous few days unless exposure is continuous [46] while DNA adducts will reflect a longer-time period of approximately 1 month. Multiple measurements during pregnancy would likely be needed to determine appropriateness of spot measurements to reflect longer-term (pregnancy month or trimester) exposures in subsequent studies. This is also the case for personal monitoring of airborne exposures. Multiple 48-hr measures (assuming measurement periods longer than this are not feasible for pregnancy studies) would probably be needed, at least in a validation study, to determine the ability of one-time short-term measures to reflect exposures throughout pregnancy.

There have been two studies to date that conducted personal monitoring and biomarker measures of particles ($PM_{2.5}$) and PAHs during pregnancy and examined associations with foetal growth end-points [39,47]. High prenatal exposures to eight carcinogenic PAHs (48-hr average during the third trimester measured by personal air sampling) were related to reductions in birthweight among African Americans in New York City, but not Dominicans and there was no association between benzo(α)pyrene (B(α)P)-DNA adducts measured in cord blood at delivery and size at birth in either group [48]. This study illustrates the complexities of interpreting results when multiple exposure measures collected at different pregnancy time-periods are utilized. The results may indicate that the toxin of highest relevance for birth outcomes is not B(α)P individually but either a combination of multiple PAHs (because eight compounds were assessed by personal air monitoring) or another specific PAH in the airborne mixture that was not well correlated with B(α)P. It may also indicate that an inhalation source exposure (e.g. PAHs from traffic) is more important than a dietary exposure source (e.g. PAHs from diet) that may have significantly contributed to the B(α)P cord blood biomarker measures. For women in Kraków, Poland, higher personal PAH levels (48-hr average) during the second trimester were associated with reductions in birthweight, length and head circumference (exposures were 10-fold higher in Poland than in New York City) [47]. Due to the extensive personal measures taken, these studies were relatively small in size. Moreover, the correlations of these personal PAH levels with more broadly available vehicle exhaust exposure measures could not be explored beyond reports of time spent outdoors and means of transportation used by women in the cohort. Thus, unfortunately, these studies did not allow the investigators to extrapolate the detailed personal measurement data to larger populations in the regions studied through modelling approaches. Such combined approaches applied to highly exposed urban areas may be necessary to determine the importance of specific motor vehicle exhaust toxins such as PAHs or volatile organic compounds for adverse birth outcomes.

Land use-based regression modelling

Geographic information system techniques such as LUR models may provide an important complement to personal and biomonitoring for assessing exposure to pollutants with spatially heterogeneous concentrations (e.g. toxins in traffic exhaust) in large study populations. In the LUR modelling approach, concentrations of vehicle exhaust markers, such as NO_x, are measured simultaneously at many locations throughout an urban area using relatively inexpensive passive monitors (e.g. Ogawa monitors). Various GIS parameters (such as traffic and roadway density, population density and

land use) are used to predict the measured concentrations [49]. The model can then be used to estimate concentrations at home and work locations based on the GIS parameter values at these locations. The LUR approach so far has mainly been utilized to study chronic diseases such as asthma where longer-term exposures (e.g. over years or a life-time) are of interest. Typically, multiple 2-week measurements are made through out a year in two to four seasons and then averaged. Thus, these models are meant to characterize spatial rather than temporal variability in air pollution levels. This specification may not work for pregnancy, where shorter-term exposures are of interest. These models might have to be 'seasonalized' for pregnancy end-points to capture temporal as well as spatial variability in pollutant levels, although this may vary by location. Solutions may include measuring multiple 2-week periods during the year to build season- or month-specific LURs. Alternatively, temporal patterns observed at ambient monitoring stations could be used to adjust LUR model estimates as done by Slama et al. [27]. However, this assumes the spatial surface (variability) is stable over time. Additional monitoring may be needed to verify this assumption.

Typically, NO_x has been used to build LUR models due to its relative ease of measurement. Measuring personal and residential (i.e. indoors and outdoors) levels of ultrafine particle and PAH in conjunction with NO_x would help verify whether it is a good marker for specific exhaust toxins of concern for pregnancy. Furthermore, one may want to measure additional vehicle exhaust markers (such as elemental and organic carbon, hopanes and steranes) in conjunction with PAHs to obtain speciation information to help discern vehicular sources of particles and PAHs from other outdoor sources, such as wood burning and industrial combustion, and indoor sources such as tobacco smoke and cooking [50].

Time-activity patterns

Improving exposure assessment and reducing exposure misclassification also requires consideration of women's time-activity patterns during pregnancy. Because most studies of pregnancy outcomes have used birth certificates to identify and characterize subjects, residential location at delivery was generally the only information available to assign exposures. To examine how the importance of time-activity patterns may affect exposure misclassification for pregnant women, we selected a case–control sample nested within the year 2003 birth cohort in Los Angeles County and collected detailed risk factor and residential, commuting and occupational information via a survey (the University of California Los Angeles Environment and Pregnancy Outcome Survey – EPOS). We found that accounting for time-activity patterns of pregnant women in an effort to reduce exposure misclassification tended to strengthen effect estimates [19]. For example, when we restricted our analyses to women who had not moved during pregnancy (approximately 80% of our group) our results slightly strengthened, as one would expect, assuming that non-movers suffered less from exposure misclassification and that misclassification was non-differential. Associations between CO averaged over the last 6 weeks prior to birth and prematurity were stronger for women who did not work outside their homes during pregnancy than for those who did, suggesting that shorter-term averages are sensitive to exposure misclassification (likely non-differential) introduced by a woman's actual location. This is particularly important for primary exhaust pollutants, such as CO, whose concentrations vary substantially over short distances. Formal sensitivity analyses help to verify these assumptions and the impact of exposure misclassification on effect estimates [51].

Confounding due to seasonal or social and behavioural risk factors

Similar to mortality studies of air pollution, as mentioned above, most previous pregnancy outcome studies have relied on public records, the birth certificate, as their primary or sole source of data. These studies have the advantage of using large numbers of birth records, which reduces the uncertainty due to random error common to smaller studies that collect in-depth covariate information from mothers. However, this increased precision may come at the expense of increased confounding bias, which is not reflected in the standard uncertainty estimates. Birth record studies are typically limited to routinely recorded information and may thus be limited in their ability to control for confounding by maternal or foetal risk factors. Studies employing spatial or medium-term temporal contrasts lose the advantage of the time-series approach, (i.e. that they do not have to consider as potential confounders any risk factors that do not vary on the relevant time scale). These studies must consider the possibility of seasonal and/or community specific residual confounding inherent in their approach when relying solely on a set of standard covariates provided on routine birth records. For example, records may be less than comprehensive for maternal behaviours that might adversely affect the foetus and are more common (e.g. low socioeconomic status communities that also tend to have higher air pollution levels). However, birth certificates do provide individual-level information for a number of important pregnancy risk factors (e.g. age, education, race/ethnicity, parity, prenatal care) that are proxies for behavioural risk factors. Controlling for such variables in statistical analyses may be sufficient to address such confounding.

Based on our EPOS data, we found that trimester-specific effect estimates for air pollution did not appear to be confounded by covariates not routinely collected on birth certificates [19]. Specifically, for our case–control sample nested within the year 2003 Los Angeles County birth cohort, we collected detailed information on risk factors not reported on birth certificates and used this information to estimate covariate-adjusted associations for preterm birth and gestational age-specific air pollution exposures. Adjustment for covariates reported on birth certificates had the strongest influence on the pollutant effect estimates, while additional

adjustment for a large number of EPOS survey covariates (occupation, income, maternal smoking and ETS, alcohol drinking, etc.) changed the odds ratio estimates by less than 5%. This confirmed our previous suppositions that (i) estimates of effect for pollutants that change with season and are averaged over short time intervals, such as months or pregnancy trimesters, are likely not confounded by behavioural factors that do not change seasonally, and (ii) birth certificate covariates are sufficient to remove most confounding for short- to moderate-term exposure averages. This was further supported by our results for entire pregnancy averages that rely more on spatial rather than temporal contrasts. The largest changes in estimates were observed for the entire pregnancy CO averages after adjustment for birth certificate covariates and, again, additional adjustment for covariates collected via the survey did not change estimates appreciably. These results seem to support the notion that longer-term averages are more sensitive to confounding bias due to population differences in other risk factors but also indicated that adjustment for most behavioural risk factors above and beyond the birth certificate variables (maternal age, education, race/ethnicity, parity, season, etc) is not necessary. Similar nested case–control studies in other urban areas may help to determine whether these findings apply to populations in other locations as well.

Biologic mechanisms

Low birthweight, SGA and preterm birth can be caused by maternal, foetal or placental factors or a combination; for example, air pollution may affect maternal respiratory or general health, and, in turn, impair uteroplacental and umbilical blood flow, transplacental glucose and oxygen transport, and total insulin and its trophic effects on the foetus, all known as major determinants of foetal growth [52]. A recent review article summarized potential biologic pathways that, in some animal or cell culture models or in human beings, have been shown to be affected by particulate air pollutants, including systemic oxidative stress and inflammation, changes in blood coagulation, endothelial function and haemodynamic responses. The authors hypothesized that these might be potential mechanisms also impacting the placenta and foetus in turn [53]. These pathways may or may not act independently; for example, an increase in maternal blood pressure and an impaired trophoblast invasion of the spiral arteries may induce uteroplacental hypoperfusion and a state of relative hypoxia surrounding the trophoblast, such that the resulting oxidative stress might compromise nutrient delivery to the foetus and impair foetal growth throughout pregnancy.

It is generally well accepted that active tobacco smoke exposure during pregnancy leads to increased risks of delivering LBW and preterm infants [54]. One of the major constituents of tobacco smoke, CO, crosses the placenta rapidly and is detectable in the foetal circulation, equilibrating at levels that are 15% higher than maternal levels [55]. Carboxyhaemoglobin (COHb) is formed by the binding of CO and haemoglobin, shifting the oxygen dissociation curve to the left, resulting in a decrease in the availability of oxygen to foetal tissues. Animal data indicate maternal CO exposures of 150–200 p.p.m., leading to approximately 15–25% COHb, can produce reductions in birthweight, cardiomegaly, delays in behavioural development and disruption of cognitive function in laboratory animals of several species [56]. Existing data also suggest some of these effects may be present at exposures as low as 60–65 p.p.m. (approximately 6–11% COHb) maintained throughout gestation [56]. However, these experimental CO levels and exposures received from active and passive smoking are one to two orders of magnitude higher than CO concentrations in ambient air. For example, in our earliest study, the maximum third trimester average CO concentration for women living in Southern California was approximately 5 p.p.m. [13]. Thus, it is unclear whether ambient CO concentrations are high enough to induce hypoxia and influence foetal development. Rather, CO may be reflective of the action of other toxins in motor vehicle exhaust that co-occur with this routinely monitored pollutants, such as PAHs, metals or volatile organic compounds.

Experimental and human data indicate PAHs can cross the placenta and reach foetal organs [35,57]. There are some animal data that support a role for PAH exposure in adverse reproductive outcomes, including stillbirths, reabsorptions, congenital abnormalities and decreases in foetal weight [58]. Interestingly, one study reported mouse pup weight was significantly depressed at day 20 and day 42 after birth after oral administration of B(α)P during pregnancy, suggesting the effects of transplacental PAH exposure on foetal weight may persist into the postnatal period [59]. The specific mechanisms by which PAHs may cause adverse birth outcomes in human beings are unknown. Current hypotheses include anti-oestrogenic effects, binding of constituents to the human aryl hydrocarbon receptor to induce P450 enzymes, and DNA damage resulting in activation of apoptotic pathways [3,48]. Dejmek et al. [36] proposed that PAHs may directly affect early trophoblast proliferation due to their reaction with growth factor receptors, causing sub-optimal placentation, reduction in exchange of oxygen and nutrients with the foetus, and impairment of foetal growth. PAHs may also act through oxidative stress pathways.

The majority of fine and ultrafine ($<PM_{0.1}$) particles found in the urban atmosphere derive from engine combustion [40,60]. Ultrafines have very low mass but magnitudes higher particle numbers and therefore a high surface area relative to fine and coarse particles for adsorption of toxic species [60]. Measurement studies undertaken by investigators at urban sites in Los Angeles indicate a large portion of ultrafines are made up of organic carbon, followed by elemental carbon as primary products from vehicle emissions and that ultrafines contain the largest fraction of PAHs by mass [60]. Ultrafine particles have a high respiratory deposition, can escape phagocytosis by alveolar macrophages and translocate to extrapulmonary organs [61]. Organic components of particles, which comprise a large proportion of freshly emitted exhaust and secondary

aerosols, can induce a broad polyclonal expression of cytokines and chemokines in respiratory epithelium and this effect may be due to the action of PAHs, metals and related compounds that lead to the production of cytotoxic reactive oxygen species (ROS); these inflammatory and oxidant stress responses are expected to occur at extrapulmonary sites as well [60]. Ultrafines in Los Angeles ambient air were found to be most potent towards inducing cellular haem oxygenase-1 expression and depleting intracellular glutathione, both important in oxidant stress responses and were also shown to localize in mitochondria where they induce major structural damage and may also further contribute to oxidative stress. Cho et al. [62] using a dithiothreitol assay as a quantitative measure of *in vitro* ROS formation found the highest activity for the ultrafine mode in Los Angeles basin particles and a relatively high correlation of redox activity with elemental carbon ($r^2 = 0.79$), organic carbon ($r^2 = 0.53$) and the PAH, benzo(g,h,i)perylene ($r^2 = 0.82$). Pan et al. [63] reported that diesel exhaust particle themselves were capable of catalysing the consumption of oxygen by ascorbate and thiols leading to the generation of ROS. Thus, both the particle cores as well as adsorbed species appear important to ROS formation. It has been hypothesized that preterm birth can be triggered by abnormal cytokine production favouring inflammation; however, increased concentrations of inflammatory cytokines are also linked to term deliveries, and may be a normal component of the body's preparation for parturition [64]. Susceptibility to preterm delivery may be increased by an early activation of components normally associated with delivery. Mulherin Engel et al. [64] recently reported that common genetic variants in pro-inflammatory cytokine genes (selected TNF/LTA haplotypes) were associated with spontaneous preterm birth in both African American and white women. Thus, this oxidative stress and inflammation pathway may be of interest for future studies.

Conclusions

Since the mid-1990s, the number of studies linking various ambient air pollutants to adverse birth outcomes including LBW, SGA, preterm birth and cardiac birth defects has grown steadily. Although several review articles have been published [3–5], only one study attempted a formal meta-analysis of the existing data for one end-point (LBW) [6] mainly due to difficulties in rectifying differences between studies. One of these difficulties is isolating a specific pollutant of concern for foetal development based on this body of evidence. Air pollution is a mixture of substances whose composition depends on the sources that contribute to it and, thus, pollutants of importance may vary by geographic area; synergistic effects from pollutant mixtures may also be important. The pollutants of most importance may also depend on the reproductive end-point of interest. There is currently a dearth of knowledge of biological mechanisms in the area of air pollution and birth outcomes due in part to the lack of well-established and feasible animal models and the inability to pinpoint one specific toxin of importance that should be investigated. A pragmatic approach may be to first identify the source(s) of concern (e.g. motor vehicle exhaust) and then postulate and test toxicity of specific air pollutants emitted by that source as best as possible using animals and cell systems. So far, the most work along these lines has been done for PAHs, but other air toxics such as metals and volatile organic compounds may also be important to investigate. However, if a specific source can be clearly implicated, we may not need to determine the exact biologic mechanisms of effect before acting to regulate that source more stringently.

There is also the lingering issue of possible residual confounding in studies that rely on spatial comparisons and birth records only; additional data collection nested within cohorts established from a source that comprehensively covers a large population such as birth records and/or prospective pregnancy cohort studies may help to address some of these questions. Findings from a case-control study nested within a birth cohort that we conducted in Southern California showed that air pollution effect estimates did not change markedly when controlling for additional risk factors not reported on birth certificates. Similar studies in other urban areas may help to determine whether these findings apply to other populations as well.

Few of these problems are unique to perinatal epidemiology in the context of environmental exposures, and most plague all of air pollution epidemiology. Thus, eventually a decision will need to be made as to when we have accumulated enough evidence, despite the limitations of any one study, and use this information to inform standard setting and policy-making, as has been the case with other end-points like mortality and respiratory diseases. Because foetuses are extremely susceptible, the disruption of foetal development may have an impact on child and adult health. Since millions of women throughout the world are exposed to air pollution levels similar to or greater than the levels in the studies published to date, adopting a more pre-cautionary approach would be prudent.

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