Meeting report: atmospheric pollution and human reproduction.

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Running title:

Atmospheric Pollution and Human Reproduction

Abbreviations:

CO: Carbon monoxide

GIS: Geographic Information System

IUGR: Intra-Uterine Growth Restriction

LBW: Low Birth Weight (birthweight below 2,500 g)

NO_2: Nitrogen dioxide

PAH: Polycyclic Aromatic Hydrocarbons

PM_{10}: Particulate Matter with an aerodynamic diameter below 10 μm

$PM_{2.5}$: Particulate Matter with an aerodynamic diameter below 2.5 μm (fine PM)

ROS: Reactive Oxygen Species

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ABSTRACT

Background: There is a growing body of epidemiologic literature reporting associations between atmospheric pollutants and reproductive outcomes, in particular birthweight and gestational duration. Objectives: The objectives of our international workshop were to discuss the current evidence, to identify the strengths and weaknesses of published epidemiologic studies and to suggest future directions for research. Discussions: Participants identified promising exposure assessment tools, including exposure models with fine spatial and temporal resolution that take into account time-activity patterns. More knowledge on factors correlated with exposure to air pollution, such as other environmental pollutants with similar temporal variations, and assessment of nutritional factors possibly influencing birth outcomes would help evaluate importance of residual confounding. Participants proposed a list of points to report in future publications on this topic to facilitate research syntheses. Nested case-control studies analyzed using two-phase statistical techniques and development of cohorts with extensive information on pregnancy behaviors and biological samples are promising study designs. Issues related to the identification of critical exposure windows and potential biological mechanisms through which air pollutants may lead to intrauterine growth restriction and premature birth were reviewed. Conclusions: To make progress, this research field needs input from toxicology, exposure assessment and clinical research, especially to aid in the identification and exposure assessment of feto-toxic agents in ambient air, in the development of early markers of adverse reproductive outcomes, and of relevant biological pathways. In particular, additional research using animal models would help better delineate the biological mechanisms underpinning the associations reported in human studies.

INTRODUCTION

After a seminal publication in 1977 (Williams et al. 1977), few studies addressing the possible effects of air pollutants on human reproduction were published prior to the late 1990s, a time when the number of publications sharply increased. A brief summary of the main findings is given in Table 1. Several reviews exist (Glinianaia et al. 2004a, b; Lacasana et al. 2005; Maisonet et al. 2004; Sram et al. 2005). While it is still too early to draw firm conclusions, these data suggest adverse associations between air pollution, specifically carbon monoxide (CO), nitrogen dioxide (NO_2), sulfur dioxide (SO_2) and particulate matter (PM, in particular fine particulate matter, $PM_{2.5}$), and measures of fetal growth (assessed at birth) and gestational duration. For other pollutants (e.g. ozone) and outcomes (e.g. semen quality or birth defects) the evidence to date is either weaker or little data exist.

OBJECTIVES

An international workshop was convened in May 2007 to discuss the current body of evidence for effects of atmospheric pollution on human reproduction, to identify the strengths and weaknesses of published epidemiologic studies, to suggest future directions for research, to foster collaboration and to promote dialogue between epidemiologists, toxicologists, clinicians and biostatisticians. Several outcomes related to human reproduction were the focus of the discussions, including pregnancy outcomes (intra-uterine growth restriction, IUGR, gestational age) and male reproductive health (semen quality). We report here on the issues discussed by

the speakers, workshop participants and working groups; many of these issues and ideas were raised and discussed without any formal process of consensus building and should therefore not be seen as being endorsed by all workshop participants.

RESULTS

Study design related issues

Study Designs- An approach commonly employed in epidemiologic studies of air pollution and birth outcomes is linkage of outcome and covariate data from birth certificate records with ambient air quality monitoring data. Its main advantage is that it allows conducting large size studies at a very low cost as it relies on routinely collected data. Its limitations are exposure misclassification and possibly confounding (see below). For these reasons, prospective cohort studies with recruitment of women before delivery (e.g. Choi et al. 2006) hold promise; they allow use of biomarkers of exposure or outcome and conduct personal monitoring and collection of detailed information on behaviors related to exposure and on confounders, at a much higher cost. These two designs can be coupled by conducting case-control studies with collection of additional information at the individual level for a sample nested within a cohort constituted from birth records (Ritz et al. 2007); nested studies combine the strength of the larger sample size with more detailed information for a subset of pregnancies.

The time-series approach has proven useful to study the acute cardio respiratory effects of air pollution and has been adapted to studies of preterm birth and fetal death (Pereira et al. 1998; Sagiv et al. 2005). However, unlike the traditional time-

series analysis in which the population at risk (e.g. of cardiac death) remains relatively stable across time, the population at risk of adverse birth outcomes is constantly changing throughout the year. Given that the seasonality of birth has been reported to differ by factors related to socioeconomic status (Bobak and Gjonca 2001), composition of the population at risk may differ across seasons. Thus, application of time-series or case-crossover designs to reproductive outcomes may require additional considerations. Generally, these approaches relying only on temporal variations in exposure appear complementary to the above-mentioned designs relying on cohorts or birth records, which usually take advantage of both spatial and temporal exposure contrasts.

Confounding- Since air pollution levels vary in time and space, any factor influencing reproduction and varying with time or space in a way similar to air pollutants is a potential confounder (Figure 1). However, some common pregnancy complications (e.g. pre-eclampsia) associated with adverse birth outcomes might be caused by air pollutants and should therefore probably not be treated as confounders. The workshop discussions focused on socioeconomic status, season and nutrition.

Socioeconomic status and related factors are associated with the occurrence of adverse reproductive outcomes (Parker et al. 1994). Part of this association may be explained by variables that we can control for, such as active or passive smoking, parity, body mass index, occupational and residential exposures to other pollutants. However, some residual influence of socioeconomic status on reproductive outcomes may remain after controlling for these factors. Since socioeconomic status may also be associated with air pollution levels in neighborhoods (Woodruff et al. 2003), it is a potential confounder. Higher levels of primary traffic-related air pollutants are often

observed in the city center than in the suburbs; in many U.S. cities, people from poorer socioeconomic classes more often live in the city center than in the suburbs and thus are exposed to higher levels of these pollutants. An opposite pattern may exist in some European cities, where city centers are more often inhabited by residents with higher socioeconomic status. Thus, as exemplified for typical U.S. and European cities, the direction of the implied confounding bias in studies without efficient adjustment for socioeconomic status might depend on the study area. An issue remains about how to measure socioeconomic status in order to control for it in studies of air pollution and reproduction, i.e. about the best way to combine characteristics such as income, educational level or occupation of either partner, ethnicity, type of health insurance, etc.; different measures of socioeconomic status probably need to be constructed in each country.

Season is associated with air pollution levels. Moreover, some data suggest that premature births are associated with season (Lee et al. 2006), although part of this association might in fact be due to seasonality in air pollutants levels. The underlying cause for an association between premature birth and season might also be exposure to other environmental factors that also vary with season, e.g. drinking water pollutants or infectious diseases; in this case, season should be seen as a potential confounder in studies of air pollution and premature birth. Since season of birth is influenced by the duration of pregnancy, which in turn may be shortened by exposure to air pollutants and since confounders should, by definition, not be affected by exposure (Rothman and Greenland 1998), the more appropriate adjustment might be for season of conception rather than season of birth. To minimize residual confounding, it may also be necessary to explore smoothing approaches such as spline regression (Salam et al. 2005) rather than employing a

simple qualitative approach for coding season. In some settings, the association of season with air pollution might be very strong (in particular with trimester-specific air pollution levels); in this case, controlling for season might produce over-adjustment or make the estimates associated with air pollution unstable. Ideally, it would be more appropriate to adjust for the seasonally-varying factors underlying any association between season and birth outcome. For similar reasons, season is also a potential confounder in studies of semen quality.

Maternal nutrition before and during pregnancy may vary strongly by geographic area, ethnicity, socioeconomic status, and possibly season and hence with air pollution levels. Animal experiments suggest an influence of maternal nutrition on measures of IUGR (Kind et al. 2006). There are currently very few epidemiologic studies supporting an effect of variations in maternal diet as currently encountered in industrialized countries on IUGR (Stein et al. 2004), however in general it would be biologically plausible. There is also recent work showing possible effect measure modification between nutrition and air pollutants (see below). Contrary to season or socioeconomic factors, any confounding by nutritional factors might be difficult to quantify and remove because of measurement errors in the assessment of nutritional factors.

Studying separately the apparent effects of the temporal and spatial components of exposure might also constitute an option for examining potential residual confounding (Janes et al. 2007). The use of a control exposure window after pregnancy (see below) might be another way to examine potential for residual confounding by factors spatially correlated with exposure.

Effect measure modification (Figure 1)- In theory, all potential confounders are candidates for effect measure modification (VanderWeele and Robins 2007). So far, one study estimated stronger effects of air pollution in neighborhoods with low socioeconomic status in winter (Ponce et al. 2005), suggesting an increased vulnerability in these populations. A stronger effect of air pollution on birth weight was also reported for parous than for nulliparous women in a study in which exposure was estimated for the home address; the authors interpreted this heterogeneity in effects to suggest that the home address-based exposure estimate was more accurate for parous pregnant women since they are more likely to stay at home to take care of their other children than nulliparous women (Ritz and Yu 1999). A study using biomarkers of exposure to air pollutants and passive smoking reported a stronger association between air pollutants and IUGR among women exposed to passive smoking (Perera et al. 2005) than among women not exposed to passive smoking. It has also been suggested that the sizes of air pollutant effect measure differ for male and female offspring (Ghosh et al. 2007). Concerning nutrition, a review (Kannan et al. 2006) and a recent study (Jedrychowski et al. 2007) hypothesized that maternal pre-pregnancy and gestational nutrition may modulate the harmful effects of prenatal exposures to $PM_{2.5}$ on birth outcomes. Experiments based on transcriptome analysis indicate that several groups of genes involved in immunity and metabolism of xenobiotics are repressed in the placentas of rats with diet-induced IUGR (Buffat et al. 2007). This suggests that the mechanisms of resistance to xenobiotics such as air pollutants may be altered in the case of IUGR induced by a poor diet, and gives some support to a stronger sensitivity to air pollutants for fetuses exposed to other environmental stressors.

Gene-environment interactions with functional genetic polymorphisms implied in the possible biological pathways of action of air pollutants are also worth considering; Wang et al. (2000) highlighted different size effects for maternal occupational exposure to benzene on gestational duration depending on polymorphisms in genes coding for enzymes involved in phase I and phase II metabolism of xenobiotics (*CYP1A1* and *GSTT1*).

Exposure assessment

Pollutants considered- Most studies have focused on routinely measured "criteria" pollutants for which data are more easily available (i.e. CO, NO_2, O_3, $PM_{2.5}$, and PM_{10}). Future studies may want to address specific pollutant sources such as road traffic (distinguishing truck and diesel traffic from the other types of vehicles) or pollutants with specific hypotheses regarding biological mechanisms such as ultrafine particulate matter (<0.1 μm in aerodynamic diameter, either mass or particle number concentration) or polycyclic aromatic hydrocarbons (PAHs). They may also consider expanding their scope to include the evaluation of mixtures of pollutants and possibly determine the composition of PM since the composition, source and toxicity of equal-size PM can vary according to time and location (Hopke et al. 2006). This may help explain similarities or differences in results for the same criteria pollutant type reported for different regions.

Finally, while most studies have focused on average exposures, considering the effect of peaks in exposure might provide additional insights.

Traditional approaches- Air pollution measurements from existing networks of ambient monitoring stations are often used to assess exposure to air pollution within a given distance from a station (typically, studies have used limits from less than 1.7 km up to 8 km) or within a given administrative unit (e.g. county). Such approaches allow including large numbers of births. However, they are hindered by exposure misclassification due to unmeasured time-activity patterns, time spent indoors and local heterogeneity for certain pollutants. Furthermore, a fairly large proportion of women (20-30%) may move during pregnancy (Canfield et al. 2006) making exposure assessment based only on delivery residence problematic.

In principle, simulation studies could be conducted to estimate the extent of exposure variability and contribution of various sources to the total exposure to optimize the exposure assessment (see e.g. Whitaker et al. 2003 for an example from another field). Since one cannot *a priori* predict the effect of exposure measurement error (Jurek et al. 2005), sensitivity analyses (Lash and Fink 2003; Zeger et al. 2000) with detailed information concerning the direction and degree of exposure misclassification (e.g. from studies in which several approaches are simultaneously used to assess exposure) would allow quantifying the bias induced by the different sources of measurement error in each study.

GIS (Geographic Information Systems)-based approaches- Several approaches allow taking into account small area variations in pollution (for example, presence of a road). Indices such as distance from the closest road or distance weighted traffic density (Wilhelm and Ritz 2003), constitute a simple source model potentially available in many locales. Exposure estimates can also be derived with land-use regression (LUR) methods, air dispersion models (Brauer et al. 2003;

Nieuwenhuijsen et al. 2006) or two-stage geostatistical approaches incorporating monitoring station data and information on temporally or spatially varying covariates (Fanshawe et al. 2007). The resulting increase in spatial resolution of exposure models should not be achieved at the cost of a poorer temporal resolution. Indeed, the critical exposure window for many reproductive outcomes may be short (days, months or trimesters) and LUR models typically yield yearly exposure estimates. One option is to incorporate temporal variability into LUR models based on measures from background monitoring stations (Brauer et al. In press; Slama et al. 2007). However, further studies may be needed to determine how well background stations reflect temporal variability at traffic locations.

Considering each micro-environment- Since women may spend a considerable amount of their time outside their residence, exposure estimates need to be derived for other locations, such as at work and in transport to create an integrated personal exposure estimate. The transport environment may make a significant contribution to total exposure, even when the time spent in this environment is short (Kaur et al. 2007; Zhu et al. 2007). Time micro-environment activity diaries have been used to capture people's movement; global positioning systems (GPS) also offer possibilities (Nethery et al. 2007).

Personal dosimetry- When a sufficient number of measurements are taken (e.g. during the course of pregnancy), personal monitoring (e.g. Choi et al. 2006; Jedrychowski et al. 2007) may provide an estimate of exposure less prone to misclassification than ecological or semi-individual approaches; implementation costs for the latter are however an order of magnitude smaller per individual. Simulation

studies that address power (Armstrong 1987) and bias considerations might help determine if the financial resources in a given study are best invested into increasing sample size or improving accuracy of exposure assessment.

Biomarkers of exposure- The use of biomarkers of exposure for outdoor air pollutants is currently limited. Some applications include measurement of adducts between polycyclic aromatic hydrocarbons (PAH) and DNA in maternal or cord blood (Perera et al. 2005), urinary metabolites of benzene, pulmonary markers of combustion of fossil fuels (Kulkarni et al. 2006) and assessment of cotinine, a metabolite of nicotine, in blood or urine. Compared to studies of respiratory morbidity, studies of human reproduction involve special considerations because of physiological filters (lung epithelium, placental barrier…) between the environment and the target organs (e.g., the placenta, gonads, hypothalamo-pituitary axis). Environmental levels may poorly approximate the dose absorbed by these target organs; for example, correlations of 0.5 to 0.7 between personal exposure to PAH present in $PM_{2.5}$ and PAH-DNA adducts in white blood cells have been reported among women (Binkova et al. 1996); more moderate correlations (in the 0.2-0.3 range) have been reported in white blood cells PAH-DNA adducts between maternal blood collected within one day postpartum and umbilical cord blood collected at delivery (e.g. Perera et al. 2004). Consequently, correlations between atmospheric PAH levels and PAH-DNA levels in cord blood might be weak. Further work is probably warranted to identify and validate biomarkers specific of traffic-related air pollutants.

A limitation is that metabolites of pollutants usually have short half-lives in the body. Thus, researchers employing such biomarkers need to target the relevant exposure window, or perform repeated measurements, unless validation studies show little

intra-individual variations in the concentration of the biomarkers considered. In this regard, the assay of adducts between pollutant metabolites and either DNA or proteins (Castano-Vinyals et al. 2004) constitutes an interesting option, as the half-life of these DNA or protein adducts may be longer than that of unbound metabolites.

Critical exposure windows

Due to typically strong seasonal variations in air pollution levels, there are opportunities to study whether specific periods of pregnancy and of spermatogenesis are more sensitive to air pollutants than others. However, teasing out the critical windows of exposure is challenging because: 1) different pollutants may act during different periods of pregnancy, 2) routinely measured (and thus evaluated) pollutants may only be proxy markers of the pollutant(s) affecting health and 3) pollutant mixtures differ across locations and time. Windows of highest sensitivity reported in studies on air pollution and IUGR that assessed all trimesters or months of pregnancy are presented in Supplemental Material, Figure 1: for each pollutant, there have been very few studies with similar methodologies (e.g. as far as mutual adjustment for other time windows is concerned), which limits between-studies comparisons. Most studies on IUGR used trimester-specific exposure windows. Yet, when there are no strong *a priori* biologic hypotheses, investigating finer time scales (e.g. months) might be a more informative and appropriate approach.

Identification of critical exposure windows from biological knowledge- Animal experiments (Rocha E Silva et al. 2008) and biological knowledge should guide the

definition of exposure windows. In the case of cardiac malformations for example, one may focus specifically on exposure that occurred no later than in the second month of pregnancy, which corresponds to a period of rapid fetal heart formation. Because current biological knowledge is more limited for other reproductive outcomes, epidemiologic studies sometimes also use a data-driven approach (relying on models summarized in Supplemental Material, Table 1) by reporting the effect estimates associated with different exposure windows. We will now focus on methodological issues raised by this approach.

Methodological issues- In studies of preterm delivery relying on binomial regression, a methodological issue in exposure assessment was pointed out by Clarice Weinberg at the workshop: the time window is sometimes defined with respect to the date of birth (e.g. a six-week period before birth). In the case of a birth at 34 gestational weeks, this will correspond to the period from 29 to 34 gestational weeks, whereas for a birth at 41 gestational weeks, this corresponds to the period from weeks 36 to 41, which includes the period from 37 to 41 weeks, when a premature birth cannot occur anymore by definition. Alternatively one could employ a matched case-control design in which exposures are averaged over the same gestational period (e.g. from 29 to 34 gestational weeks) for the case and the matched controls (Huynh et al. 2006). A survival model is another recommended analytical approach, possibly incorporating time-dependent variables (O' Neill et al. 2003). Lastly, one could simply truncate exposure at the gestational cut-off for premature births. Such approaches are also recommended when studying spontaneous abortion or stillbirth.

Other methodological issues were mentioned. Exposures earlier in pregnancy may be more prone to measurement error than those later in pregnancy, both because

maternal residence - often used to assign exposure - is usually known only at birth and because women may spend more time at home later compared to earlier in pregnancy (Nethery 2007).

Another issue is that correcting gestational age using first trimester ultrasound measurements may lead to underestimating effects of environmental pollutants on birth outcomes, if these effects already manifest early in pregnancy and influence fetal growth at the time of the first ultrasound measurement (Slama et al. In press).

Pre- and post-event exposures- Studies could examine pre- and post-pregnancy windows of exposure. Pre-pregnancy exposure to air pollutants might entail genetic or epigenetic effects on the male or female gametes (Somers et al. 2004), which might in turn influence pregnancy outcomes.

It has been suggested (Slama et al. 2007) that comparing the estimated effect of pregnancy exposure with that of postnatal exposure (e.g. the 9 months following birth if one assumes that the relevant exposure window corresponds to the whole pregnancy) may help discarding specific biases as the explanation of the association between air pollution and reproductive outcomes. Depending on the correlations between postnatal and pregnancy exposures, associations of postnatal exposure with pregnancy outcome would be expected to be weaker than that of pregnancy exposure, if pregnancy exposure has a causal effect. While there was no consensus among participants on this issue, the idea might be further explored by simulations.

Biological mechanisms

Alteration of maternal-placental exchanges- Alterations of utero-placental and umbilical blood flow, and transplacental glucose and oxygen transport influence fetal growth (Pardi et al. 2002). PM levels have been associated with plasma viscosity and endothelial function in non-pregnant adults (Pope and Dockery 2006). Further investigation is necessary to document whether these effects also exist among pregnant women - who differ from other adults in terms of heart rate, plasma viscosity and insulin resistance (Kaaja and Greer 2005). If so, air pollution-induced changes in plasma viscosity and artery vasoconstriction may in turn influence maternal-placental exchanges and hence fetal growth (Figure 2). This hypothesis could be tested in studies with Doppler measurements of umbilical artery blood flow, which have already been used in studies on maternal exposure to cigarette smoke (Kalinka et al. 2005). Also, some of the studies linking short-term changes in air pollutants to endothelial function or inflammatory response (reviewed e.g. in Pope and Dockery 2006) could be repeated among pregnant women.

Endocrine disruption- Air pollutants such as heavy metals (cadmium) or diesel exhaust as a whole may interfere with steroidogenesis, affect progesterone production (Takeda et al. 2004; Tomei et al. 2007) and may thus act as endocrine disrupters. Among pregnant women, endocrine disruption might be involved in causing IUGR (Kanaka-Gantenbein et al. 2003). Endocrine disruption is also a potentially relevant mechanism for effects on male fecundity; male exposures in adulthood, but also during fetal life should be considered (Sharpe and Irvine 2004).

Oxidative pathways and alteration of maternal host-defense mechanisms- PM can induce a broad polyclonal expression of cytokines and chemokines in respiratory

epithelium (Sioutas et al. 2005), but also maybe at extrapulmonary sites. Engel et al. (2005) reported that common genetic variants in proinflammatory cytokine genes were associated with spontaneous preterm birth. Future work could study if the effect of PM on preterm birth is modified by polymorphisms in pro-inflammatory cytokine genes. Oxidative stress pathways are also possibly relevant for male reproductive outcomes, as reactive oxygen species (ROS) levels have been found to be negatively correlated with sperm motility and concentration (Agarwal et al. 2006). Finally, PM-induced inflammatory processes may modulate host defenses and alter maternal immunity, thus leading to increased susceptibility to infections. These infections may in turn induce pre-term labor or IUGR (Figure 2).

Paternally-mediated effects on birth outcomes- Paternal influences should be considered because of the possible influence of air pollution on semen quality and on heritable mutation rates of male origin (Somers et al. 2004). These male effects might in turn influence reproductive outcomes, although the evidence is currently limited. Attempts to examine in human the influence of air pollution on heritable mutation rates such as done by Somers et al. (2004) in mice are worth considering.

Animal models- Animal experiments, as well as studies of pregnant women with collection of biological samples may help examine the relevance of these mechanisms. Experimental studies reported alterations of reproductive function in rodents in relation to air pollution (Archibong et al. 2002; Mohallem et al. 2005; Rocha E Silva et al. 2008). The relevance of such results for human reproduction is difficult to discern due to great differences in rodent and human placentation and fetal development (Carter 2007). The guinea pig is a good model for studying placental

transfer and fetal growth restriction and the sheep is a well established model for fetal physiology but of limited value for placental research (Carter 2007). The best animal models are non-human primates even though their placentation is somewhat different due to their paucity of interstitial trophoblast cells.

Public health implications

Pregnant women often want to know what they can do to increase the likelihood of the delivery of a healthy child (see University of British Columbia 2007 for examples of recommendations given to pregnant women). Air pollution is also a societal concern. Exposure to ambient air pollution is ubiquitous, and even if increased risks of adverse reproductive outcomes due to such exposures are relatively small, they can have a relatively big impact measured in terms of attributable cases at the population level. One cost-benefits analysis estimated that 200 cases of post-neonatal mortality and 10,000 low birthweight deliveries would be prevented in the U.S. between 1990 and 2010 due solely to the reduction in air pollutant concentrations expected to occur because of the U.S. Clean Air Act (Wong et al. 2004).

Possible effects of air pollutants to consider- Adverse reproductive outcomes might have long-term consequences. IUGR and prematurity have both been linked to increased risk of neonatal mortality, to childhood diseases and to adult diseases such as heart diseases and diabetes (Barker 2004). The associations between IUGR and

neonatal mortality (Wilcox 2001) and between IUGR and health in adulthood (Sinclair et al. 2007) may not be due to a causal effect of IUGR *per se* but rather to some of the determinants of IUGR. Therefore, the long term health consequences of air pollution-mediated adverse birth outcomes can probably not be well predicted from the known associations between birth weight and health in adulthood and need to be directly assessed. The situation might differ for the long-term consequences of air pollution-mediated premature births.

CONCLUSION

Research exploring the effects of air pollution on human reproduction is a young field. Many of the current methodological issues are shared with other research areas focused on health effects of air pollutants. We will indicate here some of its specificities.

Both air pollution levels and probably fetal sensitivity to environmental pollutants vary sharply over time, so that exposure models should aim towards a fine temporal resolution (this also applies to other reproductive outcomes such as menstrual cycle function). Pregnancy is a period of life with specific time-activity, work and residential mobility patterns, which needs to be taken into account. Not only maternal but also paternal exposures are possibly important. In addition to *spatial* confounding (i.e. by factors spatially correlated with exposure), which may also exist in other environmental studies, reproductive studies can be affected by *temporal* confounding due to risk factors that vary seasonally with exposure. In terms of identifying biological mechanisms, close collaboration between epidemiology and other basic science disciplines is still missing. The identification of a plausible set of biological mechanisms by biologists, toxicologists and epidemiologists may give more weight to the associations reported in human observational studies. Given the heterogeneous chemical and physical nature of pollutants such as PM, there is no reason to believe in the existence of a unique biological mechanism likely to explain PM effects on complex events such as fetal growth and premature birth.

RECOMMENDATIONS

1) In addition to the already broadly targeted reproductive outcomes discussed above, there are other perinatal endpoints which may be sensitive to air pollutant exposures and could be considered in future studies to broaden the case for reproductive outcomes (see Table 2).

2) We suggested points to report (possibly in online supplements of journals) in the interest of facilitating comparisons across studies in future epidemiologic studies on air pollution and human reproduction (Table 3).

3) The spatial resolution of exposure models is often inadequate and is in need of improvement, e.g. by using dispersion and LUR models; these models should also include a temporal component. Time-activity patterns of subjects should be taken into account.

4) The development of biomarkers of exposure to traffic-related air pollutants should be encouraged, specifically biomarkers reflecting the dose absorbed by relevant target organs such as the feto-placental unit. This would allow quantification of how the feto-placental dose relates to maternal dose, to environmental levels of pollutants and to the occurrence of adverse reproductive outcomes.

5) Investigating the short-term effects of air pollution on endothelial function, inflammatory response and blood pressure of pregnant women could help

understanding if these are possible pathways for air pollutants effects on reproductive outcomes.

6) Animal experiments are needed to help identify relevant biological mechanisms.

7) The research field has developed through studies on a large number of births making use of existing air quality monitoring and electronic birth certificate data; the utility of this design has been recognized, but it should not be considered the only option. Studies that collect detailed exposure and covariate information and biological samples, possibly in nested subgroups of larger populations, should be further encouraged.

8) Study designs which have proven useful in assessing air pollution impacts on other health outcomes (e.g. time series, case-crossover designs) could be further explored in the context of reproductive outcomes.

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Table 1: Overview of current evidence concerning the possible effects of air pollutants on human reproduction.

Reproductive health outcome (strength of evidence [a])		Exposure assessment [b]	Study designs	Illustrative references
Male reproductive health				
Semen quality	(-/+)	AQMS, biomarkers	Longitudinal or cross-sectional	(Rosa et al. 2003; Rubes et al. 2005)
Female reproductive health				
Hormonal function	(LD)		Experimental (rats)	(Archibong et al. 2002)
Couples' fecundity	(LD)	AQMS	Pregnancy-based retrospective study; experimental (mice)	(Dejmek et al. 2000; Mohallem et al. 2005)
Pregnancy and fetal health				
Stillbirth	(LD)	AQMS	Time-series	(Pereira et al. 1998)
Prematurity	(-/+)	AQMS	Birth register-based study; time series	(Huynh et al. 2006; Sagiv et al. 2005; Wilhelm and Ritz 2003)
Congenital malformations	(-/+)	AQMS	Birth defect register-based study	(Ritz et al. 2002)
Intra-uterine growth, birthweight	(+)	AQMS, biomarkers, LUR, personal monitoring	Birth register-based study; cohorts of pregnant women; experimental	(Choi et al. 2006; Ritz and Yu 1999; Rocha E Silva et al. 2008; Slama et al. 2007)
Secondary sex-ratio	(LD)	AQMS	Birth register-based study and experiment (mice)	(Lichtenfels et al. 2007)
Postnatal health				
Infant death	(+)	AQMS	Case-control study relying on birth/death certificates.	(Ritz et al. 2006; Woodruff et al. 2006)
Transgenerational effects				
Heritable mutation rate	(LD)	Personal monitoring	Experimental (mice)	(Somers et al. 2004)

[a] LD (limited data) indicates outcomes little or not studied; -/+ indicates mixed or yet inconclusive results.

[b] AQMS: Air quality monitoring stations; LUR: Land-use regression models.

Table 2: Suggested reproductive outcomes to study in relation to atmospheric pollutants.

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Pre-pregnancy events	Pregnancy events	Post-pregnancy events
Time to pregnancy [a] Semen quality [a] Menstrual cycle [a] Proteomic markers of sperm function [b]	Spontaneous abortions, stillbirths Maternal hypertension, pulse pressure Pre-eclampsia Fetal ultrasound measurements Fetal growth velocity Birthweight (z-score) Transcriptomic analysis [c] Symmetric *vs.* asymmetric growth restriction Doppler umbilical artery velocimetry Birth defects Sex-ratio	Placental size, weight Testicle, penis sizes Ano-genital distance (males) Kidney size (boys and girls) Dubowitz or Ballard scores

[a] Both exposure in adulthood and during intra-uterine life are worth considering (e.g. Jensen et al. 2004).

[b] (Lefievre et al. 2007)

[c] See e.g. (Buffat et al. 2007)

Table 3: Recommended points to report in epidemiological studies of the effects of air

pollutants on human reproduction.

Topic	Points to report [a]
Population	Characteristics of excluded subjects (see e.g. table 1 in Parker et al. 2005)
Health outcome	Indicate all health outcomes examined Birthweight for gestation standards used for SGA classification Methods for determining gestational age
Exposure	Rationale behind monitoring station buffer area size, if applicable Type of monitoring stations used (background, source oriented sites, etc.) Distribution of exposure during the considered time-windows Correlation between (window-specific) exposure variables (see e.g. table 5 in Parker et al. 2005) Information used to geocode addresses (e.g. ZIP code only *versus* street address)
Other covariates	Which socioeconomic factors (or their proxies) were tested, and how do they relate to the exposure and outcome?
Statistical analysis	Check for non linear relations between exposure and outcome Indicate which adjustment factors had the greatest influence on the estimated effect of exposure

[a] For more general recommendations on points to report see e.g. von Elm et al. (2007).

FIGURES

Figure 1: Hypothesized relations between air pollution, intra-uterine growth restriction

(IUGR) and extraneous factors possibly acting as confounders in an epidemiologic study

of air pollution effects on IUGR.

Arrows indicate plausible effects of a factor over another not mediated by another factor
present in the diagram. A dotted arrow indicates a plausible although not established
relation. An arrow from a factor A intersecting an arrow from B to C is meant to indicate
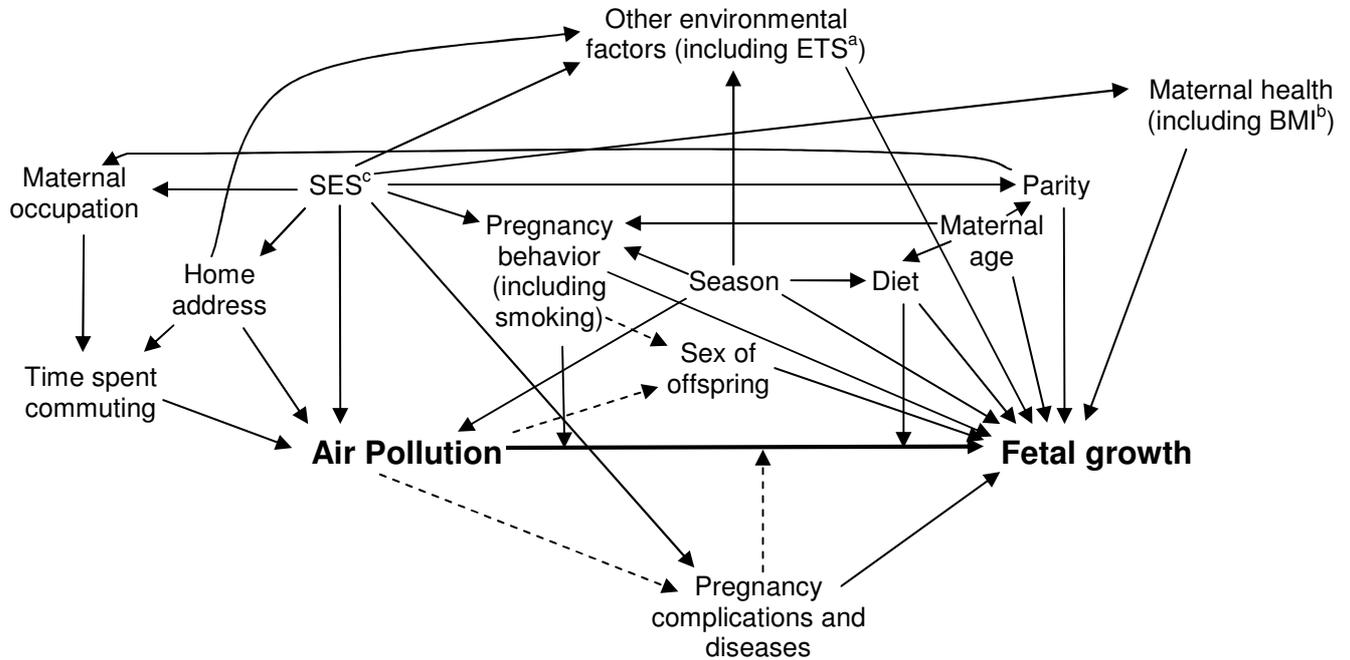that A may modify the effect of B on C (Weinberg 2007).
[a] ETS: Environmental Tobacco Smoke.
[b] BMI: Body Mass Index.
[c] SES: Marker of socioeconomic status (e.g. maternal education).

Figure 2: Possible biological mechanisms by which air pollutants could influence intra-

uterine growth restriction (IUGR) or prematurity.

Figure 1:

Arrows indicate plausible effects of a factor over another not mediated by another factor present in the diagram. A dotted arrow indicates a plausible although not established relation. An arrow from a factor A intersecting an arrow from B to C is meant to indicate that A may modify the effect of B on C (Weinberg 2007).

[a] ETS: Environmental Tobacco Smoke.

[b] BMI: Body Mass Index.

[c] SES: Marker of socioeconomic status (e.g. maternal education).

Figure 2: