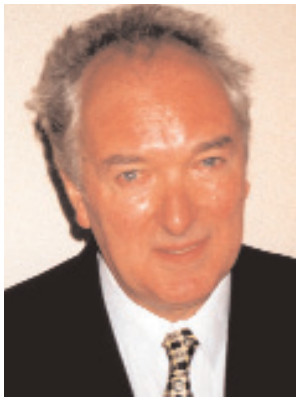
Air pollution may have an impact on adverse reproductive outcomes in both females and males.



Impact of Air Pollution on Reproductive Health

It has long been known that air pollution can affect human health. Dockery et al. (*1*) and Schwartz (*2*) reported that increasing concentrations of pollutants are responsible for the significant increase of daily mortality. Respirable particulate matter is considered to be responsible for this increase. This association has been repeatedly observed in the United States (*1,2*), Europe (*3*), Brazil (*4*), and China (*5*). The results sparked discussions on new standards for levels of particulate matter ≤ 10 µm (PM_{10}), as well as proposals to measure, separately, respirable particles < 2.5 µm ($PM_{2.5}$).

Similar data were observed on the effect of air pollution on neonatal and postneonatal mortality (*6,7*). These data raise questions about whether pollution influences birth weight or other pregnancy outcomes. The list of end points in reproductive toxicology has expanded in recent years to include many functional birth defects.

In 1987, Generoso et al. (*8*) postulated that the mutagen ethylene oxide increased the incidence of developmental abnormalities and death of mouse fetuses when early zygotic stages were exposed to the mutagen. Later, Rutledge (*9*) was able to induce fetal abnormalities by other mutagens. These results contradict the perception of experimental teratology that most induced embryonic damage resulting in adverse developmental consequences originates from treatment during organogenesis and that earlier exposure of the conceptus produces death rather than persistent developmental consequences.

If the hypothesis is correct that air pollutants can similarly affect fetal development in humans, inducing not only infant mortality and birth defects but also developmental abnormalities such as functional defects, an appropriate test area would be a highly polluted environment such as the Black Triangle, which includes the Czech Republic, the former East Germany, and Poland. This region is one of the largest sources of pollution in Europe, resulting from the combustion of brown coal in power plants and heavy industrialization.

There have only been a few reports on the potential association of pregnancy outcome with air pollution. Bobak and Leon (*6*) reported an association of total suspended particulate (TSP) and SO_2 with neonatal and postneonatal mortality in the Czech Republic. Woodruff et al. (*7*) found that early postneonatal mortality was associated with the PM_{10} level for selected causes of death in the United States. Wang et al. (*10*) reported an increasing exposure–response relationship between SO_2 and TSP levels and low birth weight (LBW; < 2,500 g). Bobak and Leon (*11*) observed an association of TSP and SO_2 with LBW in an ecologic study in the Czech Republic in 1986–1988.

Perera et al. (*12*) observed the impact of air pollution on the biomarkers used by molecular epidemiologists in polluted regions of Poland. The new potential of molecular epidemiology became apparent during the analysis of the effects of prenatal exposure to polycylic aromatic hydrocarbons (PAHs) from ambient pollution on fetal development (*13*). The increase of PAH–DNA adducts in leukocytes from umbilical cord blood was inversely correlated to the decrease in birth weight and head circumference. Air pollution also increased DNA adducts in the placenta (*14*). The bulky DNA adducts were affected by the concentration of carcinogenic PAHs in the last month of pregnancy and glutathione *S*-transferase M1 (GSTM1) and *N*-acetyltransferase 2 (NAT2) polymorphisms. Newborns with intrauterine growth retardation (IUGR) also had higher levels of DNA adducts (*15*).

The mother's lifestyle is also an important factor. For example, DNA adducts in the placenta were higher in women who smoked cigarettes or were exposed to environmental tobacco smoke, and were inversely correlated with vitamin C plasma levels (*15*). Similarly, causes of IUGR and LBW were higher in smoking mothers than in nonsmoking mothers (*13,16*).

Recently, Dejmek et al. (*16*) examined a group of mothers for the possible impact of PM_{10} and $PM_{2.5}$ on IUGR. They observed an increase in IUGR if the concentration of PM_{10} during the first month of pregnancy increased above 40 mg/m^3. Such average monthly concentrations of PM_{10} are not an exception in industrial regions and large cities during the winter. This observation corresponds to earlier experiments on mice (*8,9*) in which the period shortly after conception was most susceptible to induced developmental changes by air pollutants.

IUGR is one of the most common consequences of mutagenic exposure around the time of implantation. Reduced fetal growth is an important predictor of neonatal morbidity and mortality. In a recent study, Barker (*17*) showed a relationship between some serious adult risks (namely, noninsulin-dependent diabetes, hypertension, and coronary heart disease) and impaired growth in the prenatal and early postnatal period. In this study, Barker (*17*) implied that higher exposure to pollutants during the early stages of intrauterine life may be responsible for diseases in middle age.

These data suggest that exposure to particulate matter (or associated air pollutants) early in pregnancy may adversely affect fetal growth. Regardless of which toxicant associated with particulate matter could affect fetal growth, the biologic mechanisms remain to be explained. The active components of these complex mixtures must be inhaled and absorbed into the maternal blood stream. Highly biologically active compounds (e.g., PAHs) might interfere with some processes in the development or nourishment of the fetus. Analyzing the same mothers from an earlier study (*16*), Dejmek et al. (*18*) observed an increased risk of IUGR after exposure to carcinogenic PAHs > 15 ng/m^3 in the first gestational month. Therefore, we hypothesize that exposure to carcinogenic PAHs in early gestation may influence fetal growth. The association between PM_{10} and IUGR observed earlier may be explained, in part, by PAHs adsorbed to air particles.

Binkova et al. (*19*) analyzed the genotoxicity and embryotoxicity of urban air particulate matter from the same region where the mothers studied by Dejmek et al. (*16*) lived. The analysis (*19*) used an *in vitro* acellular assay coupled with ^{32}P-postlabeling of DNA adducts and a chick embryotoxicity screening test. In both assays, the highest activity was found for fractions containing mainly PAHs. These results are in agreement with the other studies (*20,21*) which show that PAHs account for most of the mutagenic activity from the neutral fraction of urban air. This is the first report in which the biologic activities of complex

mixtures in short-term assays with remarkably different end points, such as DNA formation and embryotoxicity, have been compared. These results indicate that PAHs are a major source of genotoxic and embryotoxic activities of organic mixtures associated with urban air particles.

These studies (*10–19*) show that air pollution acts on fetal development. Induced changes may be seen not only as morphologic birth defects but also as subtle functional changes, affecting their carriers throughout their lives. Children are very sensitive from conception. Their development is complex, influenced by various environmental factors as well as by the mother's deleterious lifestyle, such as cigarette smoking or poor diet. Susceptibility of the population to such factors is determined by genetic polymorphisms as well. We are still far from understanding the interactions of all genes responsible for the final effect. Our knowledge about metabolic polymorphisms (GSTM1, NAT2, CYP1A1) seems to be only the tip of the iceberg.

There are few data that show the effect of air pollution on human sperm. Many individual chemicals may influence the number, morphology, and motility of sperm (*22*). The decline in male reproductive health has been associated with exposure to estrogenic or other hormonally active environmental chemicals during fetal and childhood development (*23*).

Two reports have analyzed whether exposures to high levels of air pollution during the entire process of spermatogenesis are associated with abnormal semen parameters (*15,24*). This study was supported by experimental data in which PAHs altered male reproductive functions in mice. Similar effects were observed in men. A comparison of semen from young men from exposed and control districts in the Czech Republic in the late winter (after the period of high pollution) showed a significant relationship between air pollution and semen quality. Exposures to air pollution were associated with significant decreases in the percentage of motile sperm, the percentage of morphologically normal sperm, the percentage of morphologically normal sperm heads, and with abnormal sperm chromatin structure.

The main effects associated with air pollution appear to be postmeotic effects on sperm motility and morphology. Severe alterations in motility and morphology can be associated with infertility. In general, these data suggest that exposure to air pollution for one spermatogenic cycle may increase the risk of altered semen quality. However, this appears to be reversible because the young men evaluated 6 months after high pollution episodes had improved semen quality.

A new fluorescence *in situ* hybridization (FISH) technique allowed direct cytogenetic analysis of human sperm, determining changes in the number of chromosomes in gametes (aneuploidy). Aneuploidy, one of the most important chromosomal changes in men, is related to infertility, spontaneous abortions, perinatal mortality, and mental retardation. In young men from a polluted district, Y disomy was five times higher in samples collected in late winter than in samples collected in late summer, which corresponds with changes of air pollutant concentrations in the course of a year (*25*). The frequency of sperm with Y disomy was significantly elevated among smokers as compared to nonsmokers (*26*). When aneuploidy was examined in the Czech and Californian nonsmokers, the Czech nonsmokers had higher levels of X, X-Y, and eight disomies (*15*). These differences may also be related to differences in air pollutant composition or in the level of air pollution. The increased frequency of Y disomy in sperm may be related to the risk of having an aneuploid child.

These data indicate that air pollution may have an impact on adverse reproductive outcomes in both females and males. The most prominent environmental pollutants are organic compounds, especially PAHs. Pregnant women, newborn children, and young men seem to the the most susceptible to air pollution.

The evaluation of the impact of air pollution on reproductive health is only beginning. More than a decade ago, preliminary experimental data indicated that some pollutants may affect reproduction, inducing subtle functional changes. Studies showing the impact of air pollution on pregnancy outcome and the quality of sperm have only recently been published, and more studies should follow. We should try to understand the factors responsible for these observed developmental and reproductive effects. These efforts should provide valid data for risk assessment, which should aid in promoting reproductive health and healthier children.

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References and Notes

1. Dockery DW, Pope CA III, Xu X, Spengler JD, Ware JH, Fay ME, Ferris BG Jr, Speizer FE. An association between air pollution and mortality in six US cities. New Engl J Med 329:1753–1759 (1993).
2. Schwartz J. Air pollution and daily mortality: a review and meta analysis. Environ Res 64:36–52 (1994).
3. Katsouyanni K, Touloumi G, Spix C, Schwartz J, Balducci F, Medina S, Rossi G, Wojtyniak B, Summer J, Bacharova L, et al. Short-term effects of ambient sulphur dioxide and particulate matter on mortality in 12 European cities: results from time series data from the APHEA project. Br Med J 314:1658–1663 (1997).
4. Saldiva PHN, Pope CA III, Schwartz J, Dockery DW, Lichtenfels AJ, Salge JM, Barone J, Bohm GM. Air pollution and mortality in elderly people: a time-series analysis in Sao Paolo, Brazil. Arch Environ Health 50:159–163 (1995).
5. Xu X, Gao J, Dockery DW. Air pollution and daily mortality in residential areas of Beijing, China. Arch Environ Health 49:216–222 (1994).
6. Bobak M, Leon DA. Air pollution and infant mortality in the Czech Republic, 1986–1988. Lancet 340:1010–1014 (1992).
7. Woodruff TJ, Grillo J, Schoendorf KC. The relationship between selected causes of postneonatal infant mortality and particulate air pollution in the United States. Environ Health Perspect 105:608–612 (1997).
8. Generoso WM, Rutledge JC, Cain KT, Hughes LA, Braden PW. Exposure of female mice to ethylene oxide within hours after mating leads to fetal malformation and death. Mutat Res 176:269–274 (1987).
9. Rutledge JC. Developmental toxicity induced during early stages of mammalian embryogenesis. Mutat Res 396:113–127 (1997).
10. Wang X, Ding H, Ryan L, Xu X. Association between air pollution and low birth weight: a community-based study. Environ Health Perspect 105:514–520 (1997).
11. Bobak M, Leon DA. Pregnancy outcomes and outdoor air pollution: an ecological study in districts of the Czech Republic 1986–8. Occup Environ Med 56:539–543 (1999).
12. Perera FP, Hemminki K, Grzybowska E, Motykiewicz G, Michalska J, Santella RM, Young TL, Dickey C, Brandt-Rauf P, DeVivo I, et al. Molecular and genetic damage from environmental pollution in Poland. Nature 360:256–258 (1992).
13. Perera FP, Whyatt RM, Jedrychowski W, Raugh V, Manchester D, Santella RM, Ottman R. Recent developments in molecular epidemiology. A study of the effects of environmental polycyclic aromatic hydrocarbons on birth outcomes in Poland. Am J Epidemiol 147:309–314 (1998).
14. Topinka J, Binkova B, Mrackova G, Stavkova Z, Peterka V, Benes I, Dejmek J, Lenicek J, Pilcik T, Sram RJ. Influence of GSTM1 and NAT2 genotypes on placental DNA adducts in an environmentally exposed population. Environ Mol Mutagen 30:184–195 (1997).
15. Sram RJ, Binkova B, Rossner P, Rubes J, Topinka J, Dejmek J. Adverse reproductive outcomes from exposure to environmental mutagens. Mutat Res 428:203–215 (1999).
16. Dejmek J, Selevan SG, Beneš B, Solansky I, Šrám RJ. Fetal growth and maternal exposure to particulate matter during pregnancy. Environ Health Perspect 107:475–480 (1999).
17. Barker DJP. The fetal and infant origins of disease. Eur J Clin Invest 25:457–463 (1995).
18. Dejmek J, Selevan SG, Solansky I, Benes I, Lenicek J, Sram RJ. Exposure to carcinogenic PAHs in utero and fetal growth. Epidemiology 10:S126 (1999).
19. Binkova B, Vesely D, Vesela D, Jelinek R, Sram RJ. Genotoxicity and embryotoxicity of urban air particulate matter collected during winter and summer period in two different districts of the Czech Republic. Mutat Res 440:45–58 (1999).
20. DeMarini DM, Shelton ML, Bell DA. Mutation spectra in *Salmonella* of complex mixtures: comparioson of urban air to benzo(a)pyrene. Environ Mol Mutagen 24:262–275 (1994).
21. DeMarini DM. Mutation spectra of complex mixtures. Mutat Res 411:11–18 (1998).
22. Wyrobek AJ. Methods and concepts in detecting abnormal reproductive outcomes of paternal origin. In: Male-Mediated Developmental Toxicity (Olsen AF, Mattisson DR, eds.). New York:Plenum Press 1994;1–21.
23. Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJ Jr, Jegou B, Jensen TK, Jouannet P, Keiding N, et al. Male reproductive health and environmental xenoestrogens. Environ Health Perspect 104(suppl 4):741–803 (1996).
24. Selevan SG, Borkovec L, Zudova Z, Slott V, Hajnova R, Rubes J, Perrault SD. Semen quality in young men and air pollution in two Czech communities. Epidemiology 6(suppl):S85 (1995).
25. Robbins WA, Rubes J, Selevan SG, Perrault SD. Air pollution and sperm aneuploidy in healthy young men. Environ Epidemiol Toxicol 1:125–131 (1999).
26. Rubes J, Lowe X, Moore D, Perrault SD, Slott V, Evenson D, Selevan SG, Wyrobek AJ. Smoking cigarettes is associated with increased sperm disomy in teenage men. Fertil Steril 70:715–723 (1998).