The Impact of Polycyclic Aromatic Hydrocarbons and Fine Particles on Pregnancy Outcome

Jan Dejmek,[1] Ivo Solanský,[1] Ivan Beneš,[2] Jan Leníček,[3] Radim J. Šrám[1]

[1]Laboratory of Genetic Ecotoxicology, Regional Institute of Hygiene of Central Bohemia and Institute of Experimental Medicine, Academy of Science of the Czech Republic, Prague, Czech Republic; [2]District Institute of Hygiene, Teplice, Czech Republic; [3]Regional Institute of Hygiene, Ústí nad Labem, Czech Republic

The relationship between intrauterine growth retardation (IUGR) and exposure to particulate matter ≤ 10 μm (PM_{10}) and particulate matter ≤ 2.5 μm ($PM_{2.5}$) in early pregnancy was recently studied in the highly polluted district of Teplice (Northern Bohemia). From this observation rose the question about the possible role of the carcinogenic fraction of polycyclic aromatic hydrocarbons (c-PAHs), which are usually bound to fine particles. The impact of c-PAHs and fine particles on IUGR was analyzed in Teplice and in Prachatice, a region with similarly high c-PAH but low particle levels. All European, single live births occurring in a 4-year period in Teplice (n = 3,378) and Prachatice (n = 1,505) were included. Detailed personal data were obtained via questionnaires and medical records. Mean PM_{10}, $PM_{2.5}$, and c-PAHs levels during the 9 gestational months (GM) were estimated for each mother. Adjusted odds ratios (AORs) of IUGR for three levels of c-PAHs (low, medium, and high) and for continuous data were estimated after adjustment for a range of covariates using logistic regression models. In the present 4-year sample from Teplice, previously published results about increasing IUGR risk after exposure to particles in the first GM were fully confirmed, but no such effects were found in Prachatice. The AOR of IUGR for fetuses from Teplice exposed to medium levels of c-PAHs in the first GM was 1.60 [confidence interval (CI), 1.06–2.15], and to high levels 2.15 (CI, 27–3.63). An exposure–response relationship was established by analyzing the continuous data. For each 10 ng increase of c-PAHs in the first GM, the AOR was 1.22 (CI, 1.07–1.39). About the same relationship was observed in Prachatice in spite of the low particle levels. The results prove that exposure to c-PAHs in early gestation may influence fetal growth. The particulate matter–IUGR association observed earlier may be at least partly explained by the presence of c-PAHs on particle surfaces. *Key words:* air pollution, fetal growth, intrauterine growth retardation, particulate matter, polycyclic aromatic hydrocarbons, reproductive effects. *Environ Health Perspect* 108:1159–1164 (2000). [Online 7 November 2000]
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There is widespread concern over the health effects of ambient air pollution (*1,2*). There is growing support for the idea that adverse pregnancy outcomes may result from maternal (parental) exposures to airborne pollution (*3–8*). A consistent relationship between maternal exposure to fine particles in early gestation and intrauterine growth retardation (IUGR) was recently observed in Teplice, a highly polluted district of northern Bohemia (*9*). One possible explanation for this finding is that rather than particles, some associated copollutant such as polycyclic aromatic hydrocarbons (PAHs) may interfere with fetal development. Most of these compounds are usually adsorbed on the surface of fine particles (*10,11*).

The genotoxicity and embryotoxicity of particulate matter in the ambient air of the Teplice district were also investigated (*12*). Extracts eluted from fine particles were examined by an *in vitro* acellular assay coupled with ^{32}P-postlabeling of DNA adducts and a chick embryotoxicity screening test. The extracts were able to preferentially produce DNA–PAH adducts in calf thymus DNA and were also embryotoxic. The highest activity was found for fractions containing mainly PAHs (*12*). In addition, another study using the same population suggested that IUGR was positively related to the level of DNA–PAH adducts in placentae (*13*). These results indicate that PAHs are a major source of the genotoxic and embryotoxic activities of organic mixtures associated with the air pollution in the Teplice district.

This study examined the impact of PAHs on fetal growth in a larger data set from the same population. To enable a valid comparison, the effect of particulate matter on IUGR [examined recently in a 2-year data set (*9*)] was reanalyzed in this more complete (4-year) data set.

It is important to determine the real contribution (if any) of particulate matter and PAHs to the risk of IUGR; but this may not be an easy task. Dockery (*14*) discussed the methodologic problems in these types of studies: he suggested that such studies are intrinsically difficult because exposure is common, the expected effects are weak, the random misclassification of exposure is frequent, and the health indicators have multiple etiology. In addition, ambient air frequently represents a complex mixture of pollutants (*14*). We attempted to overcome or minimize these problems by using a specific study design.

Exposure to ambient air pollution is common for populations living in a particular area. Because the heterogeneity of exposure is low, the distribution of possible health effects is hard to distinguish. In this respect, studies of the reproductive effects of common contaminants have a distinct advantage over studies of respiratory health effects or daily mortality. The probability of inducing some reproductive end points depends on the stage of the reproductive cycle (e.g., germinal mutations originate only before or during conception, and major birth defects invariably arise during the first 8 weeks of gestation). Thus, the induction of such effects is possible only in a relatively narrow sensitivity window. Some other reproductive outcomes may be less stage specific (e.g., spontaneous abortion, low birth weight). Air pollution levels vary considerably over the course of a year, whereas conceptions in the human population occur more or less continually. Therefore, it is possible to compare the prevalence of a particular stage-specific outcome in the offspring of parental groups that differ considerably in their exposure to certain pollutants during the same gestational stage.

Address correspondence to J. Dejmek, Laboratory of Genetic Ecotoxicology, Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, 142 20 Prague 4, Vídeňská 1083, Czech Republic. Telephone: +420-2-4724756. Fax: +420-2-4752785. E-mail: dejmekj@ms.anet.cz

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It has been suggested that IUGR is triggered by the abnormal reaction of the trophoblast with uterine tissues during the implantation period (*15*). If so, IUGR is a typical outcome with a sensitivity window that can be used to assess the effects of ambient air pollution.

Another methodologic difficulty results from the complex nature of air pollution. One possible way to distinguish between the effects of different components of a complex mixture that are closely related is to examine a group from a population with limited exposure to one of the pollutants (*14*). A population living in a relatively clean region of southern Bohemia was examined for this purpose. This region, the district of Prachatice, is known to have relatively low levels of particulate matter compared to the Teplice district.

The main aim of the present study was to examine the impact of carcinogenic PAHs (c-PAHs) and particulate matter on fetal growth in Teplice, a severely polluted district of northern Bohemia. Data from the Prachatice region were used to evaluate the respective roles of c-PAHs and fine particles in the retardation of fetal growth.

Material and Methods

The background sample included all single births that occurred in the Teplice and Prachatice districts during the 4-year period from April 1994 through March 1998 [the previously mentioned study about PM/IUGR was based on 2-year data from the Teplice district only (*9*)]. The district of Teplice, with approximately 120,000 inhabitants and 1,100 births per year, lies in the brown-coal basin of northern Bohemia, along with chemical industry, surface mining, and large coal power plants. Prachatice, with approximately 50,000 inhabitants and about 450 births per year, is a mostly agricultural district in southern Bohemia without heavy industry; there are many forests in this mountainous region and only moderate levels of air pollution.

We restricted the study samples to full-term births of European origin. Preterm births (< 37 weeks gestation) were excluded from analysis because of the differences in factors affecting fetal growth (*16*). Pregnancies of women of non-European origin were excluded to avoid additional variability related to ethnic and cultural differences (*9*). Detailed health, personal, and lifestyle data were obtained via self-administered maternal questionnaires and medical records. We defined an IUGR birth as birth weight below the 10th percentile, by sex and gestational week, in the general Czech population. We estimated gestational age using each woman's last menstrual period date (LMP), decreasing the gestational age by 2 weeks [for more details, see Dejmek et al. (*9*)].

The concentrations of particulate matter ≤ 10 μm (PM_{10}) and particulate matter ≤ 2.5 μm ($PM_{2.5}$) were measured continuously using a Versatile Air Pollution Sampler (VAPS) (*17*); this modified dichotomous sampler collects ambient aerosol in two fine particle samples and one coarse particle sample. We obtained 24-hr values directly from an analysis of the filters. PAHs were obtained by extraction of the polyurethane foam trap and quartz filters of VAPS (*17*). Twelve PAHs were extracted from the filters and analyzed by HPLC with fluorometric detection according to U.S. Environmental Protection Agency methods (*18*); seven of them, potentially carcinogenic to humans according to the International Agency for Research on Cancer (*19*), were evaluated separately as the sum of c-PAHs: chrysene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, and indeno[1,2,3-*c*,*d*]pyrene.

We determined each mother's PM_{10}, $PM_{2.5}$, and c-PAHs levels using averages for each of nine consecutive 30-day periods after the estimated date of conception (EDC); these periods correspond roughly to the 9 months of gestation. For each month, the pollutant data were divided into three categories for analysis: low (L), medium (M), and high (H). For PM_{10} these cutoffs were L = < 40 μg/m^3, M = 40 to < 50 μg/m^3, and H = 50 μg/m^3 or higher; for $PM_{2.5,}$ L = < 27 μg/m^3, M = 27–37 μg/m^3, and H = 37 μg/m^3 or higher; for c-PAHs, L = < 15 ng/m^3, M = 15 to < 30 ng/m^3, and H = 30 ng/m^3 or higher. In the analyses, these categories were examined as dummy variables (medium vs. low, high vs. low). Finally, we analyzed continuous data to test for the possibility of a dose–response relationship.

We estimated adjusted odds ratios (AORs) and their 95% confidence intervals (CI) using logistic regression models (*20*). First, the crude odds ratios were estimated. A continuum of covariates associated with fetal growth or exposure was screened for inclusion into logistic models. These were maternal age (< 19, 19–34, > 34 years), maternal and paternal education (basic, high school, university), marital status (currently married, other), parity (first, second, third, higher), spontaneous abortion (< 2, > 1), induced abortion (< 2, > 1), alcohol habits of mother and father (< 1 drinks in a week, other), maternal smoking before conception (0, 1–9, >9 cigarettes per day), passive smoking (0, 1–9, > 9 cigarettes per day), paternal smoking (0, 1–9, > 9 cigarettes per day), and employment of mother and father (employed or not).

Long-term and/or seasonal rhythms of conceptions in the Czech population were estimated on the basis of 10-year data from official statistics; a parameter "rhythm" was introduced weighting each calendar month by its relative contribution in long-term yearly totals. Each month of pregnancy was analyzed separately, allowing some factors to vary over time (e.g., pollutant levels, maternal smoking, alcohol consumption, and season). Although pollution levels were associated with season, season alone might also be a surrogate for other factors such as weather patterns or diet. "Summer" was defined as the months from April through September and "winter" as October through March. Some variables considered for analysis were correlated (e.g., smoking variables, maternal weight and height), and thus we used a stepwise approach to develop the final models. Finally, we examined other factors for potential confounding by comparing the AORs of IUGR for exposure to determine how much change resulted from inclusion versus exclusion of the potential confounder. A change in β of > 15% was used as a reasonable criterion for inclusion (*20*). We used SAS version 6.12 for statistical analysis (*21*).

Results

The mean monthly concentrations of pollutants fluctuate, with the highest values observed during winter and the lowest during summer (Figures 1 and 2). The mean annual levels of PM_{10} in the Teplice district (μg/m^3) also changed during the 4 years of the study (Table 1). The mean annual concentrations of PM_{10} were always much lower in the Prachatice district. In contrast, the mean yearly concentrations of c-PAHs were about the same. The mean monthly levels of c-PAHs were highly correlated with the concentration of particles in Teplice (r = 0.74, p < 0.0001 for $PM_{10;}$ r = 0.78, p < 0.0001 for $PM_{2.5}$). Yearly correlations remained stable around 0.8 in all 4 years of the study in the Teplice region. A much lower PAH–particles correlation was observed in the Prachatice district. The overall correlation of c-PAHs in Prachatice was r = 0.58 for PM_{10} and r = 0.53 for $PM_{2.5}$ (both p < 0.0001). In addition, the yearly correlation values of c-PAHs fluctuated for both PM classes between r = 0.35 in the second year and r = 0.85 in the third. The correlations between annual as well as total mean levels of the two overlapping classes of particles were invariably high in both districts (between 0.92 and 0.99).

A total of 3,349 pregnancies were included in the Teplice study sample; 322 (9.6%) of them exhibited IUGR. In the Prachatice sample 1,505 pregnancies were enrolled, 124 (8.2%) of which were IUGR. Ten covariates were included in the final models for analysis of all three pollutants studied: parity, maternal age and height, prepregnancy weight, education, marital status, month-specific maternal smoking, season, rhythm, and year

of the study. We tested season and year for confounding, given their association with exposure and potential association with IUGR. Year, added alone to the final model, did not appreciably affect the results; season, however, did. When both season and year were added, confounding was noted, especially in the high exposure groups. Therefore, all subsequent analyses include season and year.

As a first step, we estimated the relative risk of IUGR for mothers exposed to low, medium, and high (L, M, H) levels of PM_{10} and $PM_{2.5;}$ as in the previous analysis of 2-year data (*9*), a consistent association was found only in the first month of gestation. In Teplice, sample AORs of IUGR for PM_{10} were M = 1.44 (CI, 1.03–1.2.02) and H = 2.14 (CI, 1.42–3.23). Corresponding values for $PM_{2.5}$ were M = 1.38 (CI, 0.95–1.92, $p <$ 0.15) and H = 1.96 (CI, 1.02–3.11, $p <$ 0.002). In the district of Prachatice, the only significant association was also observed in the first gestational month, but the results were not consistent: AORs of IUGR for PM_{10} were M = 2.11 (CI, 1.03–4.33) and H = 1.09 (CI, 0.49–2.46; Table 2). No statistically significant association at any stage of gestation was found for $PM_{2.5}$ in the Prachatice sample. The analysis using dummy variables indicated the exposure–response relationships; this suggestion was tested by analyzing the continuous data. For each 10 µg increase of PM_{10} in the first gestational month, the AORs of IUGR in Teplice increased significantly by 1.19 (CI 1.06–1.33, $p < 0.003$); this value was only 1.04 (CI, 0.86–1.27, $p < 0.7$) for the Prachatice region (Table 3).

The crude odds ratios of IUGR increased exclusively for c-PAH levels in the first gestational month in Teplice (M = 1.17, CI, 0.90–1.52, $p < 0.24$; H = 1.32, CI, 0.94–1.87, $p < 0.10$). AORs of IUGR for c-PAHs in the Teplice district are presented in Figure 3. A consistent and significant association of IUGR was observed again in the first month of pregnancy, when M = 1.59 (CI, 1.06–2.39, $p < 0.025$) and H = 2.15, (CI, 1.27–3.63, $p < 0.0043$; Table 2 and Figure 3). However, in contrast to our expectation about particulate matter, IUGR risk seemed to be related to exposure during late pregnancy. AORs of IUGR for exposure to medium and high c-PAHs levels during the eighth gestational month were M = 1.52 (CI, 1.02–2.27, $p < 0.05$) and H = 1.51 (CI, 0.92–2.47, $p < 0.10$; Figure 3).

Using the same cutoffs for Prachatice, IUGR showed no association with c-PAH levels in the first gestational month (AOR for M = 1.49, CI, 0.81–2.73, $p < 0.19$; H = 1.26, CI, 0.60–2.63, $p < 0.54$; Table 2). However, for mothers living less than 7.5 km from the monitoring station, the values of AOR were M = 1.89 (CI, 0.56–6.29) and H = 2.44 (CI, 0.60–9.83; Table 2). Thus, in this area the AORs were close to those for Teplice, though not significant (mostly due to the smaller sample size; n = 551). It may be expected that the accuracy of the exposure estimates would be higher for mothers living nearer to the monitor (*22*). When other concentration intervals were checked, the cutoffs L < 2 ng/m^3, M 2–<20, and H ≥ 20 gave a good dose–effect scale. Using these lower cutoffs and the whole Prachatice data set, the results were similar to those for the Teplice district. Again, the only consistent c-PAH/IUGR association was observed in the first gestational month (AOR for M = 1.63, CI, 0.87–3.06, $p < 0.13$; H = 2.39, CI, 1.01–5.65, $p < 0.045$; Table 2).

The analysis of continuous data revealed an exposure–response relationship between c-PAH levels in the first month of gestation and IUGR even in the crude ORs (Table 3). This relationship was highly significant, especially after adjustment: each 10-ng increase in c-PAH levels in the Teplice district resulted in an increase of AORs by 1.22 (CI, 1.07–1.39, $p < 0.004$; Table 3); a similar, but nonsignificant increase was found for Prachatice (1.17, CI, 0.92–1.89). No significant difference of AORs from 1.0 was observed in any gestational month other than the first. This was also valid for the eighth month of pregnancy in Teplice (AOR = 1.06, CI, 0.93–1.22), for which a weak association was found using the analysis of dummy variables.

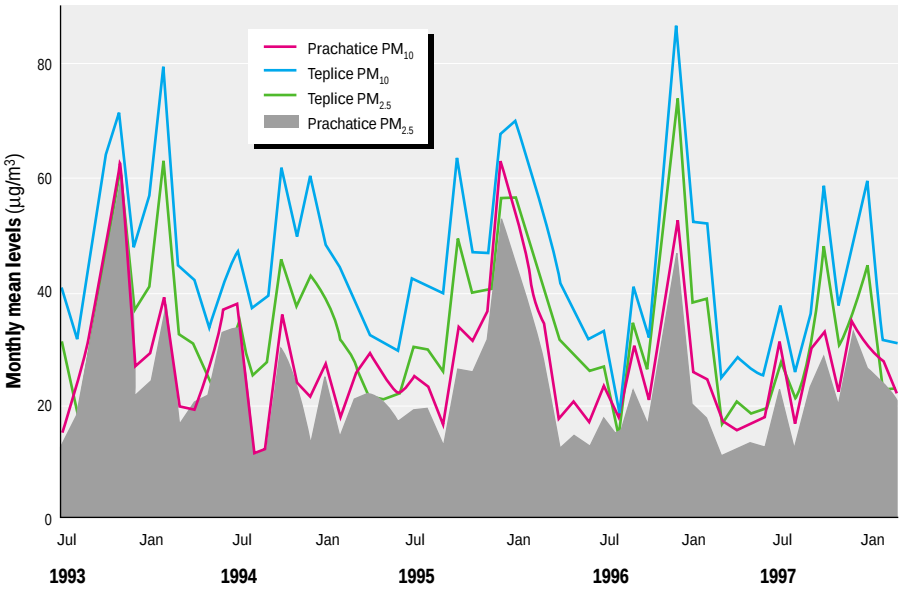

Figure 1. Monthly mean levels of PM_{10} and $PM_{2.5}$ during the study period in the two districts.

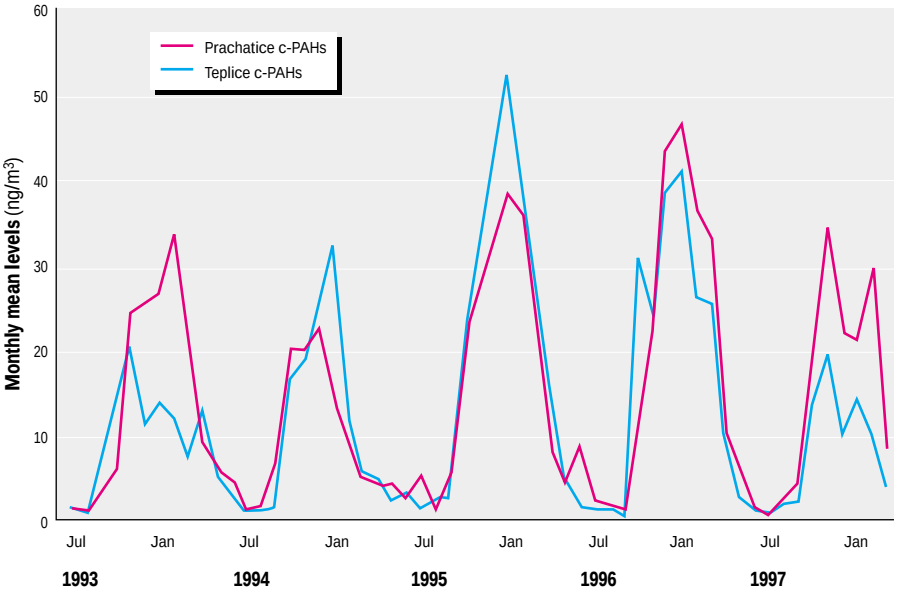

Figure 2. Monthly mean levels of c-PAHs during the study period in the two districts.

Discussion

Air pollution levels were changing during the study period in Teplice as well as in Prachatice (Figures 1 and 2). The annual averages of particulate matter decreased in the second and fourth years in both districts without any general tendency. In contrast, c-PAH levels were low in the first 2 years (approximately 10 ng/m^3) and increased considerably in the last 2 years in both localities (Table 1). The most important phenomena were that levels of particulate matter were always higher by 60–70% in the Teplice region than in Prachatice, and c-PAH levels were rather high and similar in both districts (Table 1). This situation enabled us to evaluate the influence of particles and PAHs more or less independently. Such an evaluation would not be possible using the Teplice data set alone.

Despite the similar annual averages, there were differences between the c-PAH pollution patterns in the two districts. The c-PAH/PM correlations were high in the Teplice region (around 0.8), and the correlation matrix there was relatively stable through all the 4 years studied. The mean total c-PAHs/PM correlation in Prachatice was much lower and fluctuated considerably between 0.34 and 0.87 in individual years. This may be connected with a particular phenomenon discovered in 1998 during Teplice program research activities (*23*): There is one extremely potent point-source of PAHs in Prachatice town (close to the monitoring station): emissions from the chimney of a small furniture factory. These emissions seem to be a significant source of PAHs throughout the whole district. However, it is unlikely that these emissions are dispersed uniformly in the area's broken, mountainous terrain. It is possible that the differences in the dose–effect association course between the two districts may be connected with emissions from the furniture factory. Confirming this association in Prachatice will require using either a smaller area with more reliable exposure estimates or lower cutoffs. However, the results of the continuous data analysis demonstrated that the IUGR/c-PAHs association is as real a phenomenon in Prachatice as it is in the Teplice region.

A possible relationship between the two pollutants and fetal growth was examined in 9 monthly exposure windows from conception until delivery to evaluate potentially sensitive stages of prenatal development. Initially the association of IUGR with exposure to two overlapping classes of particles, PM_{10} and $PM_{2.5}$, was evaluated. This relationship was previously observed in the same population using a smaller, 2-year Teplice data set (*9*). The results of the present analysis for the Teplice district are similar to those of our recently published study. IUGR risk was associated with the concentration of particles exclusively in the first month of gestation. The AORs for PM_{10} in the previous and the present study were 1.62 and 1.42 for medium levels and 2.64 and 2.10 for high levels, respectively. The AOR values seem to be slightly lower in the present study, though at the same levels of significance. As in the previous 2-year study, the association of IUGR with $PM_{2.5}$ in early gestation is weaker than the association with PM_{10}: the AORs are lower than for PM_{10} and they are significant for high levels only.

In Prachatice the association of IUGR risk with PM_{10} levels was not significant either in the analysis using concentration categories or in analysis of continuous data (Tables 2 and 3). Thus, the significant elevation of IUGR risk found in Prachatice (*9*) for those exposed to PM_{10} = 40 µg/m^3 during preliminary analysis was not confirmed in the present larger data set. The results for the $PM_{2.5}$ fraction in the Prachatice region also provided no significant information.

A sensitive period for retardation of fetal growth in early gestation observed for particulate matter in Teplice was also seen for c-PAHs. The AORs of IUGR were consistently rising with the levels of c-PAHs in the first month, and this relationship was highly significant; the same time trend, though not always significant, held true for Prachatice (Tables 2 and 3, Figure 3).

Thus, the first gestational month seems to be the critical period for the effects of pollution on fetal growth. The timing of this association is in agreement with the current hypothesis that IUGR pathogenesis is triggered by an abnormal reaction between the trophoblast and uterine tissues in the first weeks of pregnancy (*24*). The altered growth may arise from defective trophoblast invasion, resulting in suboptimal placentation and maternal hemodynamic maladaptation (*15*,*25*).

The ability of PAHs to reach placental tissue has been suggested in many experimental and human studies of aryl hydrocarbon hydroxylase (AHH) activities in placenta and cord blood (*26–29*). Hatch at al. (*30*) suggested that the placenta and the fetus are targets for DNA damage: PAH–DNA adducts were found in the placenta and various fetal organs from spontaneously aborted fetal tissue from nonsmoking women. This idea was confirmed in many later studies observing PAH–DNA adducts in term placentas and cord blood (*13*,*31*,*32*). Using

Table 1. Yearly mean concentrations of pollutants in the two districts during the study period.[a]

Pollutant	District	1st year	2nd year	3rd year	4th year	Total
PM_{10}	Teplice	48.7 ± 13.1	42.7 ± 10.2	49.4 ± 12.5	37.7 ± 17.9	44.9 ± 14.3
(µg/m^3)	Prachatice	31.9 ± 11.1	21.8 ± 7.9	30.9 ± 14.0	25.0 ± 10.9	27.5 ± 11.8
$PM_{2.5}$	Teplice	36.1 ± 12.9	30.8 ± 10.2	38.6 ± 11.0	29.9 ± 16.7	33.9 ± 12.8
(µg/m^3)	Prachatice	28.7 ± 11.1	18.4 ± 6.7	24.9 ± 12.7	19.5 ± 10.2	23.1 ± 11.1
c-PAH	Teplice	13.3 ± 11.0	9.5 ± 7.6	17.4 ± 13.0	16.9 ± 18.0	14.0 ± 13.0
(ng/m^3)	Prachatice	8.2 ± 5.8	9.9 ± 9.8	19.6 ± 18.5	17.3 ± 16.7	13.1 ± 13.8

[a]Values shown are arithmetic means ± SDs.

Table 2. AORs of IUGR by c-PAHs and PM_{10} in the first gestational month in Teplice and Prachatice.

			Medium[a]		High[a]	
Pollutant	Specification	District	AOR[b]	95% CI	AOR[b]	95% CI
PM_{10}	—	Teplice	1.44	(1.03–2.02)	2.14	(1.42–3.23)
	—	Prachatice	2.11	(1.03–4.33)	1.09	(0.49–2.46)
c-PAHs	—	Teplice	1.59	(1.06–2.39)	2.15	(1.27–3.63)
	—	Prachatice	1.49	(0.81–2.73)	1.26	(0.60–2.63)
	< 7.5 km (n = 551)[c]	Prachatice	1.89	(0.56–6.29)	2.44	(0.60–9.83)
	Lower cut-offs[d]	Prachatice	1.63	(0.87–3.06)	2.39	(1.01–5.65)

[a]See text for cutoffs. [b]Adjusted for parity, maternal age and height, prepregnancy weight, education, marital status, month-specific maternal smoking, season, rhythm, and year of the study. [c]Only mothers living up to 7 km from the monitor station in Prachatice region. [d]c-PAHs: low = < 2 ng/m^3; Medium = 2 to < 20 ng/m^3 ; High = ≥ 20 ng/m^3.

Table 3. AORs of IUGR by c-PAHs and PM_{10} in the first gestational month in Teplice and Prachatice: models with continuous variables.

Pollutant /increase	District	Crude OR	95% CI	AOR[a]	95% CI
PM_{10}					
10 µg/m^3	Teplice	1.07	(0.99–1.16)	1.19	(1.06–1.33)
10 µg/m^3	Prachatice	1.07	(0.92–1.24)	1.04	(0.86–1.27)
c-PAHs					
10 ng/m^3	Teplice	1.08	(1.00–1.18)	1.22	(1.07–1.39)
10 ng/m^3	Prachatice	1.08	(0.95–1.22)	1.17	(0.92–1.89)

[a]Adjusted for parity, maternal age and height, prepregnancy weight, education, marital status, month-specific maternal smoking, season, rhythm, and year of the study.

monoclonal antibodies, Zenzes et al. (*33*) found benzo[*a*]pyrene diol epoxide DNA adducts even in preimplantation human embryos.

Little is known about a possible mechanism of the observed PAH influence on fetal growth. A direct effect of PAHs on receptors for epidermal growth factor (EGF) and insulin-like growth factors (IGF) I and II in placental cells was found by Guyda (*34*); these changes tended to influence placental growth. Benzo[*a*]pyrene, one of the carcinogenic PAHs, was the most potent PAH tested. Benzo[*a*]pyrene inhibited EGF more significantly than IGF or insulin receptor binding in early gestation placentas (*34*). This observation was recently confirmed by Zhang et al. (*35*), who showed that benzo[*a*]pyrene mediates a loss of EGF receptors and alters trophoblast proliferation as well as its endocrine function. This indicates that PAHs may directly affect early trophoblast proliferation due to their reaction with growth factor receptors. In this way feto-placental exchange and, consequently, fetal nourishment and growth, may be impaired.

The fetotoxicity of PAHs has been demonstrated in several experimental studies. Prenatal exposure to benzo[*a*]pyrene affected fetal development and viability and lowered litter weights in rats and mice (*36,37*). However, the experimental daily dose of 10 mg benzo[*a*]pyrene/kg body weight that was used is about 1 million times the mainstream dose in 100 cigarettes (*38*). Therefore, the mechanism involved and also the biological end points may be substantially different. In addition, Shum et al. (*39*) suggested a genetic predisposition to AHH induction, hypothesizing that the genotype of both parents may determine the reproductive effects of PAHs on the offspring. This idea was recently confirmed in the polluted regions of Poland (*40*) and the Czech Republic (*13*).

The question about the respective role of particles and c-PAHs should be studied further.

Examining the Teplice data, the shape and size of the association between c-PAHs and IUGR were similar to those of particles and IUGR; these results did not provide a sufficient indication of which of these two relationships is the primary one. This striking similarity may be partly due to the high correlation between PAHs and particle levels. It is also well known that PAHs, especially those with larger molecules, are mostly adsorbed on fine particles (*10,11*).

Thus, the results based on the Teplice data alone are consistent with the idea that the effects of c-PAHs and particles on IUGR could be combined, probably in an additive rather than in a synergistic manner. However, another hypothesis, that only one of the two factors—either particulate matter or c-PAHs—may be the major etiological factor, is also compatible with the results. There are many observations that support the idea of a primary role of c-PAHs and their reactive derivatives in the observed slow-down of fetal growth.

- The observed association of the IUGR risk with particulate matter could be explained due to the close correlation between both noxae, particles and PAHs, irrespective of the real effects of particles.
- Another argument follows from the analysis of the Prachatice data set: the levels of particles in this region were considerably lower during the study period (Table 1, Figure 1). It was shown that the effects of c-PAHs combined with low levels of PM in Prachatice were as strong as in Teplice, where c-PAHs occur simultaneously with much higher levels of particles (Tables 2 and 3). Also, the differences in the prevalence of IUGR between Teplice and Prachatice were relatively small (9.5 vs. 8.3, respectively), in spite of large differences in the levels of particulate matter. This shows that the influence of c-PAHs on fetal growth is more or less independent of the relative PM concentrations.
- At this time almost nothing is known about a possible mechanism of the reproductive effects of particulate matter. There is some information that may contribute to a plausible explanation of the effects of PAHs on fetal development and growth: PAHs may penetrate the human placenta and different fetal tissues (*28–30*), and c-PAHs may directly interfere with placental growth factors (*34,35*).

These arguments in favor of c-PAH influence on fetal growth do not rule out a simultaneous influence of particles. Airborne particles always originate in combustion processes together with polynuclear organic compounds. Thus, c-PAHs and airborne particulate matter always operate simultaneously. The critical point seems to be the proportion of organic compounds adsorbed on fine particles. The observed association between particles and IUGR could be at least partly explained by the presence of c-PAHs and their highly biologically active derivatives. Fine particles might influence the transport, penetration, and deposition of organic compounds. Because respiratory tract deposition of particles generally increases with size from 2.5 µm to 10 µm (*41*), PM_{10} may better represent an individual's total exposure, as supported by the results of the present study.

Conclusions

It can be concluded that the risk of delivering a growth-retarded infant increases with the level of fine particles and c-PAHs in early gestation (first month). Our results support the hypothesis that c-PAHs play a primary role in the retardation of fetal growth. According to some experimental results, c-PAHs may directly modulate the proliferation of the trophoblast due to their reaction with receptors for placental growth factors. In this way the feto-placental exchange of oxygen and nutrients may be reduced and fetal growth can be impaired. The present

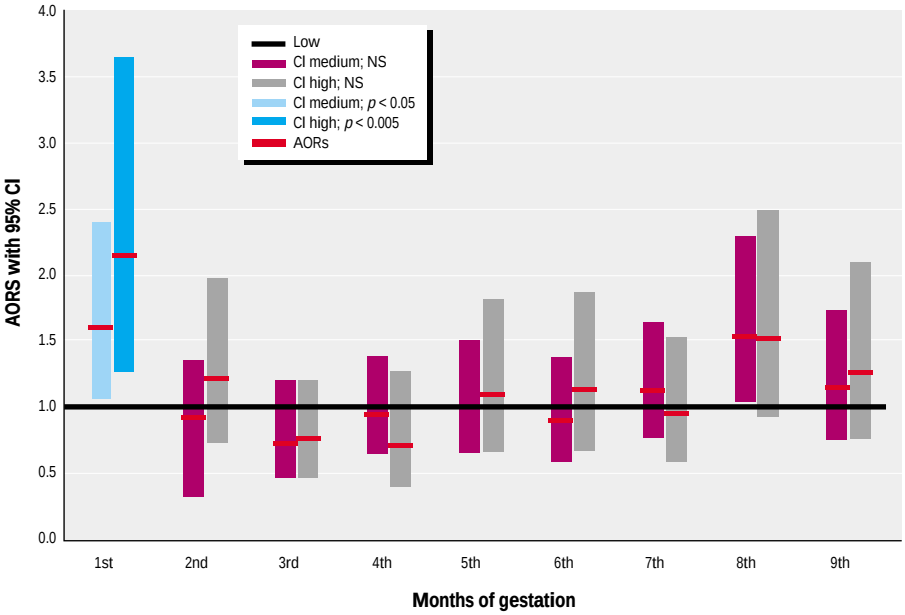

Figure 3. AORs of IUGR by exposure to c-PAH in different months of gestation. NS, not significant. See text for definition of low, medium, and high concentrations. Odds ratios were adjusted for parity, maternal age and height, prepregnancy weight, education, marital status, month-specific maternal smoking, season, rhythm, and year of the study.

results show that the effects of c-PAHs on fetal growth can be more or less independent of the concentration of associated particulate matter. In contrast, the impact of airborne particles on fetal growth may depend on the proportion of c-PAHs adsorbed on fine particles. However, the results cannot exclude other explanations, including some interaction between the effects of particles and PAHs on fetal growth. Particulate matter can influence the transport and penetration of c-PAHs from the ambient air into the maternal organism. Further investigation of the simultaneous impact of particles and PAHs on pregnancy outcome is needed. The effects of PAHs on the fetus may be affected by the genotypes of the parents. Elucidating the influence of genetic polymorphisms on pregnancy outcome and specifically on IUGR induction will certainly be a task for future research.

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