

Teplice Program—The Impact of Air Pollution on Human Health

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The aim of the Teplice Program is to investigate and assess the impact of air pollution on the health of the population in the district of Teplice, Czech Republic. Characterization of the air pollutants demonstrated unusually high concentrations during winter inversions of fine particles dominated by acidic sulfates, genotoxic organic compounds, and toxic trace elements. The major source of airborne fine particles is the burning of coal for heating and power. Human exposure and biomarker studies demonstrated large seasonal variations in air pollution within the Teplice District and higher seasonal average pollution levels than the comparative district, Prachatice. Personal exposures to fine particles and organic carcinogens [e.g., polycyclic aromatic hydrocarbons (PAH)] were correlated with excretion of PAH metabolites in urine, several trace metals in blood, and DNA adducts in white blood cells. Respiratory and neurobehavioral studies of school children were conducted using questionnaires and clinical measures. A significantly higher prevalence of adverse respiratory symptoms and decreased lung function were found in the Teplice district than in Prachatice. The neurobehavioral studies indicated significantly higher teacher referrals for clinical assessment in Teplice, but the majority of objective performance measures did not differ. Reproductive studies were conducted in both males and females. A study of the effects of exposure on pregnancy and birth found an excess prevalence of low birth weight and premature births in Teplice; these adverse effects were more common in infants conceived in the winter and whose mothers were smokers. Based on questionnaires and medical examination, the reproductive development of young men was not different between districts and seasons, however, measures of semen quality suggest that exposure to high levels of air pollution are associated with transient decrements in semen quality. — *Environ Health Perspect* 104(Suppl 4):699–714 (1996)

Key words: air pollution, air toxics, particulate matter, coal combustion, personal exposure, biomarkers, respiratory effects, lung function, neurobehavioral effects, reproductive effects, pregnancy outcome, semen quality, Eastern Europe, Czech Republic

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Abbreviations used: B[a]P, benzo[a]pyrene; CASA, computer-assisted sperm analysis; CMB, chemical mass balance; EDX, energy dispersive X-ray fluorescence analysis; FEF, forced expiratory flow; FEV, forced expiratory volume; FVC, forced vital capacity; MAC, maximal admissible concentration; NES2, Neurobehavioral Evaluation System; PAHs, polycyclic aromatic hydrocarbons; PAH_{car}, carcinogenic PAH; PCR, polymerase chain reaction; PEM, personal exposure monitor; PM, particulate matter; SCSA, sperm chromatin structure assay; SEM, scanning electron microscopy; TSP, total suspended particles; U.S. EPA, U.S. Environmental Protection Agency; VAPS, versatile air pollution sampler; VLBW, very low birth weight; WBC, white blood cell; WHO, World Health Organization.

Introduction

The Northern Bohemia brown coal basin comprises four mining districts located in the northwestern region of the Czech Republic (Figure 1). The coal in this region is low quality and generally contains 1 to 3% sulfur and in some localities as much as 5% sulfur (1). This coal is referred to as brown coal or lignite and is typically surface-mined from open pits. It is primarily used to produce steam and power for the heavy industrialization in this region. Coal-fired power plants in this region produced 35% of the electricity used in the former Czechoslovakia. The combustion of this coal combined with the heavy industrialization of this region over the past few decades has resulted in some of the worst environmental pollution in Europe (1). This high-sulfur coal has also been used extensively for local area heating (e.g., homes, apartments, offices) and local industries (e.g., glass production, chemical manufacturing, and petrochemical industries). Over the past 25 to 30 years, air pollution from these sources has caused extensive deforestation. Conifers in the Krušné Hory (Ore Mountains) forming the northern border of this region have essentially been destroyed. Moldan and Schnoor (1) published an in-depth review of these environmental problems in the Czech Republic.

The health consequences of environmental pollution in this region have been a major concern of the public. Although exploratory analysis of data collected prior to 1989 suggested a higher incidence of cancer, and reproductive and behavioral effects in this region (2,3), no research had been conducted to address these hypotheses. After major political changes in the Czech Republic in November 1989, a new research program, the Teplice Program, was developed to evaluate the short-term and long-term health impact of air pollution on the population (4). Teplice, one of the mining districts in Northern Bohemia, was designated as a model district for investigation of the health effects of air pollution. The district of Prachatice, which has some of the cleanest air in the Czech Republic, was selected as a comparison district. These two model districts are characterized in the next section.

The Teplice Program was initiated by the Czech Ministry of Environment in cooperation with the Czech Ministry of Health in the latter part of 1990 to provide scientifically valid information needed to

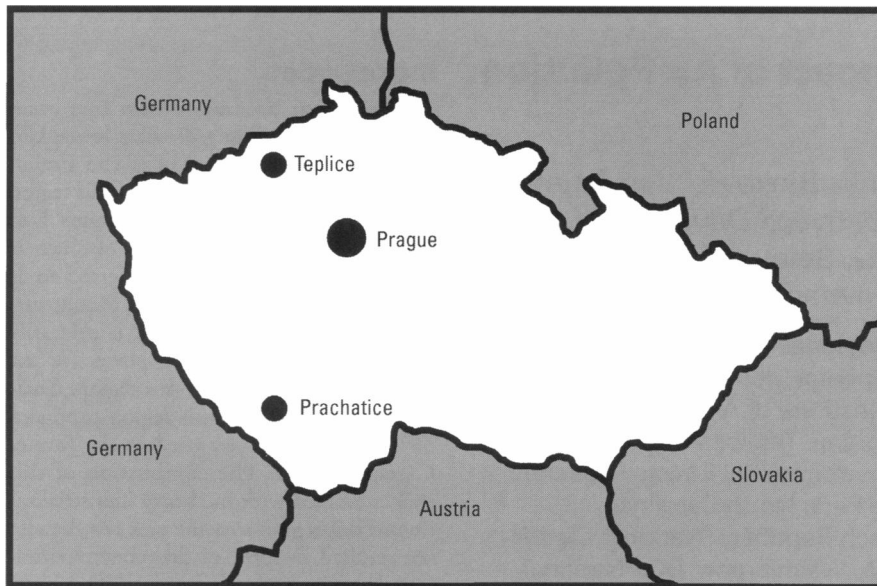


Figure 1. Map of the Czech Republic and the location of the districts of Teplice and Prachaticce.

assess environmental health problems in the Northern Bohemian basin area (4). In collaboration with the U.S. Environmental Protection Agency (U.S. EPA), the program was peer reviewed in early 1991. A collaborative research program, developed between the U.S. EPA and the Czech government, included the air pollution monitoring, human exposure, biomarker, and health effects studies reviewed here. This program has succeeded in bringing together many different research organizations and government laboratories in both the Czech Republic and the United States to accomplish the multidisciplinary program. The Commission of European Communities (DG XII) was consulted in the development of this research program that was, in part, incorporated into PHARE II, as EC/HEA/18-CZ, "Impact of Environmental Pollution on the Health of Population (Teplice Program)." The organization of the Teplice Program includes an international Board of Directors and a Scientific Review Board as well as scientific program and project managers. Several of the project areas that do not involve collaboration with the U.S. EPA (e.g., Czech studies of mortality and nutritional status; Norwegian collaborative exposure modeling studies) are not reviewed here.

The aim of the Teplice program is to evaluate the health impact of air pollution on the exposed population. In addition, as the exposures in the region were expected to decrease, longitudinal studies were planned to assess the impact of decreasing

exposure on human health. Although this review focuses on the primary source of exposures, through air pollution, the Czech government has also monitored the quality of drinking water and food (unpublished data). The central hypothesis in the Teplice Program is that the polluted air in the Teplice District adversely affected the health of the population. We review here the results of collaborative studies between scientists at the U.S. EPA, Office of Research and Development, and Czech scientists on the impact of air pollution on human exposure, biomarkers of dose and genetic damage, and reproductive, respiratory, and neurobehavioral effects. A listing of the health effects studies conducted since 1992 and continuing into 1999 is shown in Table 1.

Characterization of Model Districts

Teplice

The district of Teplice is situated in the middle of the North Bohemian brown coal basin, which stretches from east to west and borders on the Krušné Hory (Ore Mountains) in the north and on the České Středoohoří (Central Bohemian Highlands) in the south (Figure 1). The geographical features of the area create a basin in which the air pollution emissions are trapped during meteorological conditions that result in inversions leading to high concentrations of air pollutants, particularly in the winter. Elevations in the district range from

160 m in the valley to 910 m in the Ore Mountains and average 200 m. Average daily temperatures fluctuate by about 10°C and range from as low as -20°C in the winter to +35°C in the summer.

The total area of the district is 469 km². It consists of 170 km² of agricultural land and 165 km² of forests. Coniferous trees have become virtually extinct because of emissions, while the deciduous trees have sustained damage. A large part of the area has been devastated by the strip-mining of coal and associated industrialization. In the Krušné Mountains, there are rich deposits of fluorite, which is extracted and processed locally. Most (75%) of the brown coal extracted from the Czech Republic comes from the four districts of Northern Bohemia, including Teplice. Extraction is almost entirely (91%) from open pits. In addition, half of the coal extracted is processed locally. Consequently, half of all the sulfur dioxide and nitrogen oxides emitted in the Czech Republic originates from the mining districts of Northern Bohemia. Similarly, this region contributes about one-quarter of the total air particulate matter emissions in the country. Prior to 1996, most of the home heating systems used lignite coal as fuel. As discussed below, this study has shown that 30 to 40% of the fine particles contributing to human exposure in Northern Bohemia are due to home heating.

Teplice has a population of approximately 132,000 people, with an average density of about 285 people per km². Its density is the highest in Northern Bohemia and, with the exception of large cities, is one of the highest in the Czech Republic. About 50% of the employed population work in industrial jobs and less than 3% in agriculture.

Prachaticce

The district of Prachaticce is situated in the southwestern part of the Southern Bohemia region. The border with Austria in the south is rather short, while the ridges of the Šumava (Bohemian Forest) Mountains in the southwest form the natural border with the Federal Republic of Germany.

The area of the district of Prachaticce (1375 km²) ranks fourth in the region of Southern Bohemia (covering 12% of the region's area). This area has the lowest number of inhabitants of Southern Bohemia (50,740) and its population density (39.6 per km²) is one of the lowest in the Czech Republic. There is a slight predominance of urban population (51.4%) living in the four towns of the district.

Table 1. Summary of studies by season and year.

Year	Winter ^a High pollution period	Spring/Summer ^b Low pollution	Fall ^c Post-low pollution
1992	Studies initiated for personal exposure and biomarkers Respiratory study of 8th-grade students Neurobehavioral study of 8th-grade students	Respiratory study—continuation	Respiratory cross-sectional study of 2nd-, 5th- and 8th-grade students Neurobehavioral study of 8th-grade students Semen study (18-year-old men)
1993	Personal exposure and biomarker Repeated measures study initiated Respiratory cross-sectional study of 2nd-, 5th-, and 8th-grade students Semen study (18-year-old men)		Neurobehavioral study of 2nd-grade students Semen study (18-year-old-men)
1994	Pregnancy outcome study Initiated to continue through 1997 Semen study (18-year-old men)		Neurobehavioral study of 4th-grade students
1995	Personal exposure and biomarker repeated measures study continues		Longitudinal semen study (baseline sample) Longitudinal respiratory study of 2nd-grade students from 1992
1996	Longitudinal semen study (3 monthly samples)		Longitudinal semen study (final sample)
1997			Pregnancy outcome study ends
1998			Longitudinal respiratory study of 2nd-grade students from 1992
1999	Respiratory study of 2nd-grade students born in 1992		

^aJanuary, February, March. ^bApril–August. ^cSeptember, October, November.

Climatic conditions vary greatly because of the varied elevation and the character of the terrain. At elevations over 800 m, the climate is mildly cold, in those under 800 m it is mildly warm. Mountainous areas of the district are covered with snow for more than 130 days on the average. The long-term yearly averages of air temperature depend on the elevation. There are also frequent fogs and inversions in this district.

The district has vast, predominantly spruce forests covering the area of 71 thousand hectares (52% of its area). These forests contribute to the natural beauty of the district and help make it attractive to tourists. To preserve the Šumava Mountain region, its water resources, and tourist attractions, the Czech government declared 1630 km² of the range a protected landscape area in 1963. The largest part of the Šumava protected area is in the district of Prachatice. Aside from several stone quarries, there is no mining in Prachatice.

Air Pollution Concentrations and Source Contributions

Particle- and gas-phase air pollutant measurements were made at two primary

locations during this study (5), using the versatile air pollution sampler (VAPS) (6). The main monitoring site was located in the city of Teplice, and the second was located in the city of Prachatice. Ambient SO₂ and particulate matter (< 10 μm or PM₁₀) was monitored daily in both districts during the health studies (Figure 2). The fine or respirable particle (< 2.5 μm or PM_{2.5}) composition and coarse particle (2.5–10 μm) composition [anions by ion chromatography and toxic metals by X-ray fluorescence (7)] as well as concentrations of polycyclic aromatic hydrocarbons (PAHs) were measured daily in the winter and periodically during the other seasons. These data were used to characterize the ambient population exposures to air pollutants for the health studies and were also used in receptor models to determine the relative contribution of the major air pollution sources in the northwestern region of the Czech Republic. The composition of emissions from power plants, glass factories, incinerators, motor vehicles, residential space heating, and soils was also determined by using a dilution sampling probe. Scanning electron microscopy (SEM) was

performed on individual particles to provide size, morphology, chemistry, and particle category.

Mass and elemental compositions of particles were measured in Teplice and Prachatice beginning in 1992. The concentrations of all pollutants measured, including particles and sulfur dioxide, were significantly higher in the winter than the spring and summer in both districts. The Teplice District, however, had more severe pollution episodes (Figure 2) and higher winter average pollutant concentrations. During the winter of 1993 the average fine particle mass in Teplice was 122 μg/m³ compared to 44 μg/m³ in Prachatice, and the spring and summer average fine particle concentrations for Teplice and Prachatice were 28.7 and 17.9 μg/m³, respectively (Tables 2, 3). While the fine particle mass, trace elements, and PAHs in Teplice were on average 2- to 3-fold higher than those in Prachatice, the average sulfur dioxide concentrations were generally at least 5-fold higher in Teplice than in Prachatice. Seasonal differences in air pollutant concentrations, even within the same district, were even greater than the district differences.

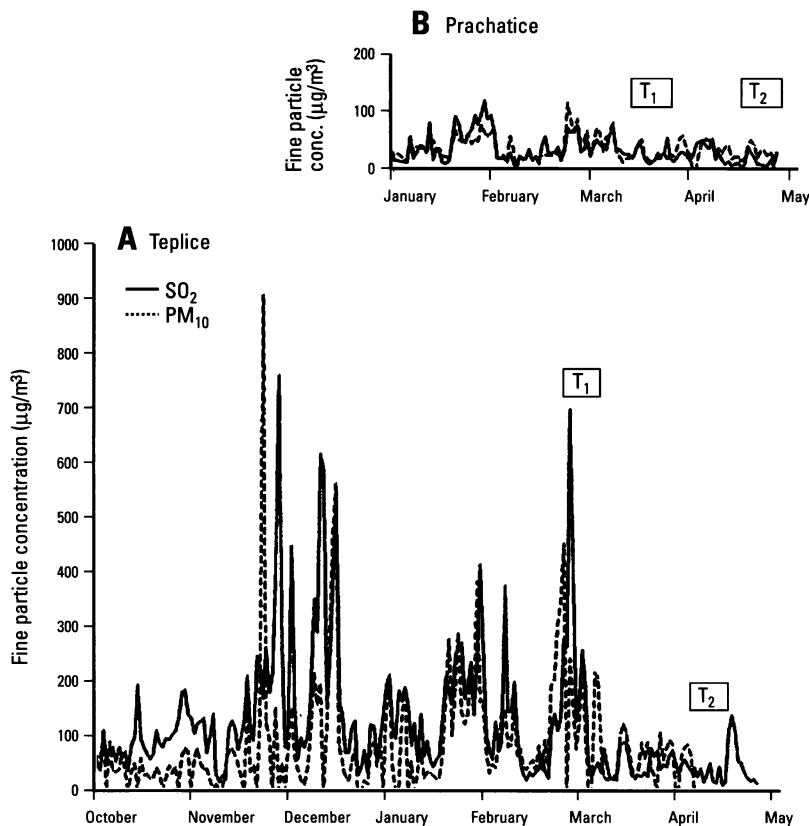


Figure 2. Daily 24-hr average concentrations of SO₂ and PM₁₀ from October 1991 through April 1992 in Teplice (A) and from January through April 1992 in Prachaticce (B) during the initial collaborative health studies. T₁ and T₂ are times of first and second respiratory testing periods, respectively.

The most dramatic pollution changes occurred in Teplice during winter inversions. During an inversion episode, between January 29 and February 6, 1993, the SO₄²⁻ and the organic carbon peak concentrations were 400 and 140 µg/m³, respectively. The presence of acidic particles was indicated by the pH level; for example, one sample collected at the peak of the episode had a pH of 4.1. Fine particles collected during the winter season were dominated by sulfates, organic carbon, and trace metals. The fine particle mass observed in Prachaticce was one-third of the levels in Teplice during the winter episodes (Tables 2, 3). Particle concentrations in spring and summer were substantially lower. These findings reflect the absence of home heating emissions and more favorable meteorological conditions.

VAPS samples were also analyzed for a series of carcinogenic and other PAHs (Tables 2, 3). Total PAH concentrations in Teplice in the winter were approximately twice the average winter concentrations of PAH in Prachaticce. The most dramatic differences (10 times) were observed between

the winter and spring/summer averages in Teplice. Evaluation of the benzo[*a*]pyrene (B[*a*]P) to lead (Pb) ratio in Teplice over time indicates the presence of at least two sources of PAHs (5). During the summer, when mobile sources are the major contributor to B[*a*]P, the ratio of B[*a*]P to Pb is about 0.01. During the winter, when the ratio is 0.05 to 0.15, emissions from inefficient combustion of brown coal (lignite) in home heating systems is the most probable source of PAH.

Subsets of ambient and source samples (home heating, power plant, traffic tunnel) were analyzed manually by SEM using an SEM/EDX (energy dispersive X-ray fluorescence) system. Coarse particles comprise mainly minerals and fly ash particles. The fine fraction was dominated by sulfates and carbonaceous particles. Fly ash particles were also present in large numbers. In addition to soil, power plants, fine sulfates, and carbonaceous particles, SEM analysis of individual particles provided information on coal, steel, and motor vehicles particles.

Estimates of the contributions of emissions from power plants, incinerators,

automobiles, and home heating to the total fine particle mass measured at the main monitoring site in Teplice were made by using the chemical mass balance (CMB) receptor model. CMB models are not suitable for apportioning sources of secondary SO₄²⁻, which is formed in the atmosphere by oxidation of SO₂. Primary sulfate represents at most only a small percentage of the fine particle emissions from all the sources considered in this study, whereas secondary sulfate constitutes a much larger fraction of the ambient samples.

Two methods were applied to apportion the secondary sulfate. In the first method, the contributions of power plants and home heating to ambient sulfate were scaled according to their total SO₂ emissions in the region, estimated from emission inventories. In the second method the receptor model results for trace metals were used to apportion secondary sulfate. According to emission inventories, 95% of SO₂ in the Teplice region during the winter was emitted from power plants, and only 5% were from home heating. Power plant emissions on the Teplice air shed emissions are scaled by a factor of 1/R where R is the distance of the power plant to Teplice. Finally, home heating emissions from districts outside Teplice and Usti (a neighboring city) were also scaled accordingly. With these assumptions, 77% of the SO₄²⁻ measured at the receptor site arises from power plant emissions, and 23% is from local home heating systems.

In the second method, the power plant and home heating contributions to ambient sulfate are calculated by scaling to the receptor modeling results for nonsulfate particles, i.e., trace metals and organic carbon. The results obtained in this case were 66% for power plants and 34% for home heating. The two methods (emissions scaling and receptor model scaling) place brackets of 66 to 77% on the power plant contribution to ambient sulfate, and 23 to 34%, on the home heating contribution to ambient SO₄²⁻ for the 1993 winter average.

CMB modeling results for the Teplice fine mass data of the 1993 winter are shown in Figure 3. It can be seen that most of the fine particle mass is produced by home heating and power plants. Mobile sources account for only a few percent of the total mass. Two major air pollution episodes occurred during the winter of 1993. The results of CMB modeling for the first episode of February 1993 showed that power plant contribution was 29 ± 13% and home heating (and small

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Table 2. Mean aerosol composition in Teplice in the winter and summer of 1993.^a

Species	January–March		PM (µg/m ³)	May–August	
	Fine (<2.5 µ)	Coarse (2.5–10 µ)		Fine (<2.5 µ)	Coarse (2.5–10 µ)
Total mass	122 (3.0)	18.5 (4.9)		28.7 (1.2)	10.6 (1.1)
Organic carbon	33.8 (5.0)	NA		NA	NA
Elemental carbon	2.3 (0.3)	NA		NA	NA
Metal oxides	6.5 (0.5)	14.0 (2.0)		1.88 (0.14)	7.3 (1.0)
Sulfate ^b	41.9 (5.7)	ND		10.2 (1.3)	ND
Sum of PAHs	0.278 (0.012)	NA		110.027 (0.001)	NA
Benzo[<i>a</i>]pyrene	0.008 (0.00004)	NA		0.0005 (0.0004)	NA
Trace elements (ng/m ³)					
Al	830 (130)	2020 (544)		264 (500)	850 (210)
Si	940 (180)	3240 (770)		296 (47)	1660 (400)
S	10140 (920)	ND		2460 (190)	ND
Cl	430 (27)	95.1 (18.3)		31.2 (3.9)	78.3 (6.2)
K	300 (24)	204 (23)		96.8 (7.6)	124 (7.1)
Ca	145 (12)	574 (45)		75.3 (6.3)	435 (33)
Ti	71.3 (7.4)	153 (22)		27.0 (4.4)	73.4 (11.0)
V	11.7 (1.8)	ND		ND	ND
Cr	5.9 (1.00)	ND		ND	ND
Mn	18.9 (1.7)	12.3 (1.5)		10.2 (1.0)	10.8 (1.1)
Fe	380 (42)	820 (84)		150 (14)	480 (48)
Cu	14.1 (1.7)	6.6 (1.2)		ND	4.1 (0.7)
Zn	160 (16)	17.6 (4.0)		33.6 (3.6)	10.2 (1.6)
Ga	1.6 (0.2)	ND		ND	ND
As	44.5 (4.5)	ND		ND	ND
Se	8.1 (1.0)	ND		ND	ND
Br	18.5 (2.1)	4.4 (0.7)		5.1 (0.9)	2.0 (0.3)
Pb	108 (11)	6.8 (2.1)		39.8 (4.4)	ND
SO ₂ (µg/m ³)	153 (60)			NA	

Abbreviations: NA, measurements not available; ND, detected at the 3 standard deviation level in fewer than half the samples. ^aEstimated uncertainty in parentheses as determined statistically (11). ^bSulfur expressed as ammonium sulfate.

Table 3. Mean aerosol composition in Prachatice in the winter and summer of 1993.^a

Species	January–March		PM (µg/m ³)	May–August	
	Fine (<2.5 µ)	Coarse (2.5–10 µ)		Fine (<2.5 µ)	Coarse (2.5–10 µ)
Total mass	44.0 (0.8)	8.0 (0.3)		17.9 (0.4)	5.7 (0.2)
Organic carbon	NA	NA		NA	NA
Elemental carbon	NA	NA		NA	NA
Metal oxides	1.84 (0.09)	4.35 (0.52)		1.12 (0.07)	2.78 (0.35)
Sulfate ^b	9.5 (1.2)	1.0 (0.13)		6.7 (0.9)	ND
Sum of PAHs	0.163 (0.008)	NA		0.024 (0.001)	NA
Benzo[<i>a</i>]pyrene	0.00470 (0.00024)	NA		0.00014 (0.00001)	NA
Trace elements (ng/m ³)					
Al	ND (33)	595 (137)		128 (20)	295 (75)
Si	240 (36)	890 (210)		165 (24)	640 (150)
S	2310 (160)	240 (70)		1630 (110)	ND (47.3)
Cl	197 (14)	63.0 (8.3)		21.4 (2.2)	8.4 (1.4)
K	215 (14)	91.9 (11.0)		89.9 (5.9)	82. (8.5)
Ca	64.6 (4.9)	240 (18)		49.9 (3.5)	165 (12)
Ti	17.0 (2.7)	50.8 (7.6)		10.3 (1.6)	25. (3.7)
V	4.2 (0.9)	ND (0.8)		1.8 (0.5)	ND (0.4)
Cr	0.8 (0.4)	ND (0.3)		0.6 (0.2)	ND (0.2)
Mn	5.5 (0.6)	5.2 (0.6)		3.6 (0.4)	3.9 (0.4)
Fe	104 (9.5)	235 (22)		85.7 (7.5)	169 (16)
Cu	15.6 (1.6)	5.6 (0.8)		21.8 (2.0)	7.4 (1.0)
Zn	69.2 (6.2)	9.8 (1.8)		23.9 (2.1)	6.1 (0.9)
Ga	ND (0.5)	ND (0.2)		ND (0.2)	ND (0.1)
As	26.2 (2.6)	3.5 (0.7)		3.2 (0.6)	ND (0.2)
Se	2.4 (0.4)	ND (0.1)		ND (0.2)	ND (0.0)
Br	10.1 (1.1)	2.3 (0.4)		4.3 (0.5)	1.6 (0.2)
Pb	48.7 (4.6)	6.3 (1.1)		24.3 (2.3)	3.5 (0.6)
SO ₂ (µg/m ³)	29.0 (11)			4.4 (1.6)	

Abbreviations: NA, measurements not available; ND, detected at the 3 standard deviation level in fewer than half the samples. ^aEstimated uncertainty in parentheses as determined statistically (11). ^bSulfur expressed as ammonium sulfate.

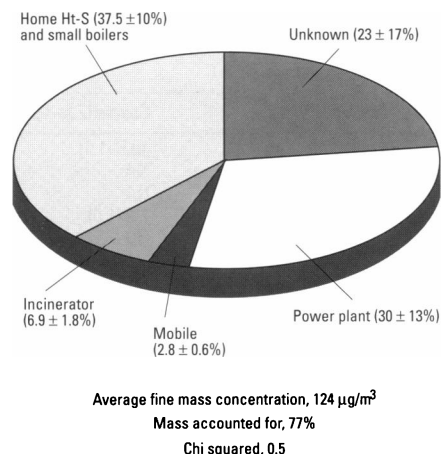


Figure 3. Sources of fine particle mass in Teplice as determined by chemical mass balance (CMB) modeling for the winter (February–March) of 1993.

industrial boilers) contributed $36 \pm 10\%$. Most of the organic carbon was contributed by home heating emissions. CMB results for the second episode showed that the power plants contributed $55 \pm 22\%$ (mainly sulfate) and home heating contributed $29 \pm 8\%$ (mainly organic carbon) to the average fine particle mass.

Significant features of the two winter pollution episodes of 1993 were the differences between SO_2 and fine particle mass concentrations, and the ratios of $\text{SO}_4^{2-}/(\text{SO}_2 + \text{SO}_4^{2-})$ provide an indication of the conversion of SO_2 to SO_4^{2-} . SEM analysis of the fine fraction collected during the 1993 winter episode showed that the fine particles containing sulfur (sulfates) were typically in the size range of 0.5 to 1 μm in diameter. Meng and Seinfeld (8) theorized that SO_4^{2-} particles formed from heterogeneous aqueous reactions would result in particles from 0.7 to 1.5 μm in diameter. This particle size information and the $\text{SO}_4^{2-}/(\text{SO}_2 + \text{SO}_4^{2-})$ ratios suggest fog-assisted particle growth and SO_2 to SO_4^{2-} conversion by a heterogeneous mechanism. Studies of Bizjak et al. (9) in Yugoslavia support these conclusions.

During the winter episode of 1993 in Teplice, the meteorological conditions were similar to those in London, December 5–9, 1952 (10). The average particulate matter concentrations (measured by the blackness of the filter, “smoke”) and SO_2 (measured by the hydrogen peroxide method) concentrations in London were about $1600 \mu\text{g}/\text{m}^3$ and $1800 \mu\text{g}/\text{m}^3$ (0.7 ppm), respectively. Figure 4 is a time series plot of PM_{10} and SO_2 concentrations measured in Teplice

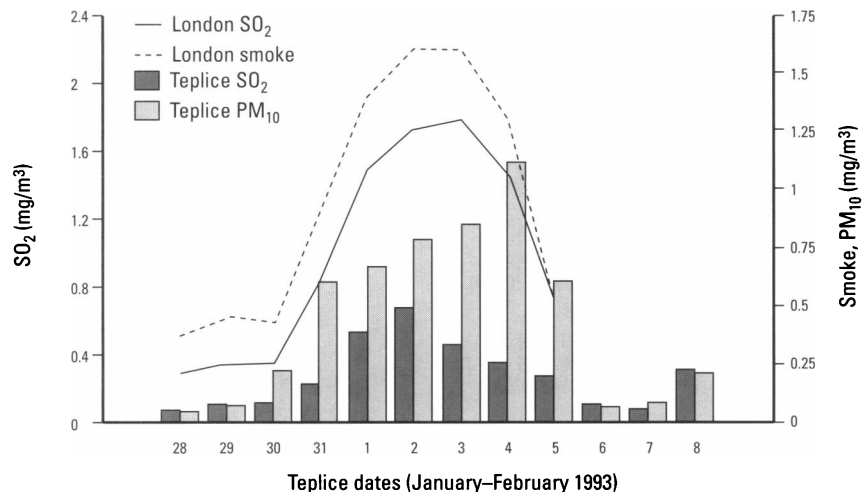


Figure 4. Time series plot of PM_{10} and SO_2 concentrations measured in Teplice during 1993 compared to data for the 1952 London episode (December 5–9).

compared to data for the 1952 London episode (December 5–9). In London, the number of deaths per day reached approximately 900 compared to 250 before the episode (10) and the norm was even less. Although the Czech government and the Teplice Program have studied the mortality in Teplice compared to that in Prachatic, the populations are too small to detect the mortality changes observed in London.

Biomarkers and Personal Exposure

Field investigations were initially conducted to compare the ambient concentrations and personal air exposures to particles, PAHs, and organic mutagens. A personal exposure monitor (PEM) designed for such studies (12) was used to measure the personal air of policemen, coal miners, and other workers in the Teplice district. Stationary medium-volume (PM_{10}) and high-volume ($\text{PM}_{2.5}$) and total suspended particles (TSP) samplers were also used to collect ambient air samples. The initial studies conducted in the winter of 1992 with a group of Teplice policemen showed that exposures to B[a]P averaged $40 \text{ ng}/\text{m}^3$. Ambient high-volume (HiVol) air sampling results from 12-hr nighttime samples collected in Teplice between February 17 and March 27, 1992, showed particle-associated B[a]P averaged $12 \text{ ng}/\text{m}^3$ and ranged from 2 to $34 \text{ ng}/\text{m}^3$. The 16 PAHs that were quantified averaged $131 \text{ ng}/\text{m}^3$ for the same periods. Approximately 50% of the particle-bound PAH concentrations in the Teplice air included compounds that are carcinogenic in animals. Mutagen concentrations

and potency were determined by the Ames plate incorporation assay (13). The mutagenic potency of extractable organics from ambient air particles was higher than that for U.S. residential areas that are heavily impacted by wood smoke but similar to that from U.S. cities more heavily impacted by vehicle emissions (13).

As a result of these pilot studies, a research project to evaluate the relationship between ambient concentrations of air pollutants and human exposure and dose was developed. The objectives of this project are to evaluate simultaneously personal exposure to air pollution and internal measures of exposure, dose, and genetic effects using a series of biomarkers. Ambient monitoring was conducted simultaneously as described above. PAHs were selected as the air pollutant marker for monitoring personal exposure because the major source of air pollution in this region is coal combustion. PAH and other genotoxic polycyclic aromatic compounds adsorbed onto fine soot particles have been estimated to be the greatest potential source of lung cancer risk. The biomarkers of internal exposure, dose, genetic effects, and susceptibility were selected based on the knowledge that PAH is rapidly metabolized via microsomal oxidative pathways to reactive intermediates that may bind to protein and DNA or that may be excreted as phenol and diols in urine. Biomarkers of internal exposure and dose to PAHs selected for this project included immunoassay methods for PAHs protein adducts, ^{32}P -postlabeling methods for PAH-DNA adducts analysis, single cell gel electrophoresis (comet assay) to detect

DNA damage, and chemical analytical methods to detect PAH urinary metabolites. In addition to these markers, urinary mutagenicity was measured. Cytogenetic effects were measured using sister chromatid exchanges and chromosome aberrations. Metabolic susceptibility biomarkers, such as glutathione *S*-transferase (*GSTM1*) and *N*-acetyltransferase (*NAT2*) were determined using the polymerase chain reaction (PCR) method.

A group of 30 women working outdoors in the Teplice district in Northern Bohemia was compared with a group of 30 women from the Prachatice district of Southern Bohemia. These groups were sampled over several days during the winter season of 1992. In 1993 a repeated measures follow-up study was started with a group of nonsmoking women from the town of Teplice, the polluted district. Both studies examined the influence of personal exposure and other factors that alter exposure and metabolism (e.g., *GSTM1* genotype, age, diet) on biomarkers of exposure, dose, and genetic effects in these women in both districts (14,15).

Personal exposure monitoring of individual exposure $PM_{2.5}$ was conducted for the 24-hr period prior to collection of blood and urine. Particle extracts were analyzed for carcinogenic PAH (PAH_{car}) (13). High correlations were observed between the mass of fine particles measured on the filters and the personal exposure to PAH_{car} (total) or B[a]P (Table 4). Significant correlations were also observed between the personal exposures to $PM_{2.5}$ or PAH_{car} and two of the blood metals that were measured (blood lead and blood selenium) (Table 4). Urine samples were collected for exposure marker analysis as follows: PAH metabolite analysis, urinary mutagenicity, and cotinine analysis to control for exposure to tobacco smoke as one of the confounding factors in these studies. The urine markers were all adjusted for creatinine content to control for variations in urine

volume. The urinary PAH metabolites were also significantly correlated with personal exposure to either $PM_{2.5}$ or PAH_{car} .

Within the nonsmokers from Teplice, significant correlations of personal exposure to carcinogenic PAH with DNA adduct levels in white blood cells (WBC) analyzed by ^{32}P -postlabeling using butanol enrichment procedures were found (14) (pilot study: $r=0.54$, $p=0.016$; follow-up study: $r=0.71$, $p<0.001$) (Figure 5). DNA damage as measured by the comet parameter (percentage of DNA in the comet tail) correlated with exposures to respirable particles ($r=0.304$, $p=0.015$).

Metabolic susceptibility biomarkers were determined by genotyping the DNA isolated from the WBCs. We have examined the influence of *GSTM1* genotype on the biomarkers of exposure and DNA damage. *GSTM1* genotype had a significant effect on urinary PAH metabolites ($p=0.037$), urine mutagenicity ($p=0.033$) and comet parameters ($p=0.002$) when *GSTM1* genotype was considered as a single factor affecting these biomarkers (15). Studies in progress are examining the influence of *GSTM1* and *NAT2* genotypes on the dose-response relationship between personal exposure to PAH_{car} and multiple biomarkers. Stratifying the cohort by allele-specific genotype decreased the interindividual variability and increased the correlation observed between personal exposure and several biomarkers. This suggests that metabolic susceptibility differences between individuals in the population account for some of the interindividual variability in the population.

Respiratory Effects in Children

The purpose of respiratory studies was to ascertain whether the prevalence of respiratory morbidity was greater for Czech children who were lifetime residents of Teplice compared to the children of Prachatice. Children in Teplice had substantial lifetime exposure to high levels of

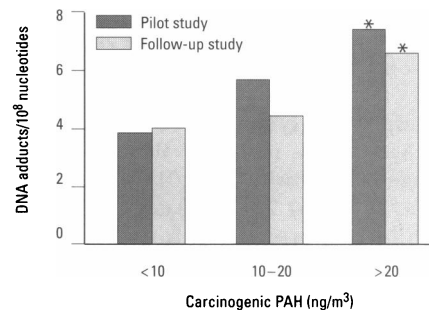


Figure 5. DNA adduct levels in nonsmoking women from Teplice district with personal exposure to carcinogenic PAH less than 10 ng/m³, between 10–20 ng/m³ and greater than 20 ng/m³. Asterisks indicate $p<0.01$ comparison of groups with exposures >20 ng/m³ and <10 ng/m³ by the Mann-Whitney rank sum U-test.

SO_2 and PM_{10} . The average of the annual SO_2 concentrations from 1977 to 1993 was 125 $\mu\text{g}/\text{m}^3$, 2.5 times the World Health Organization's (WHO) recommended annual maximal admissible concentration (MAC) of 50 $\mu\text{g}/\text{m}^3$. While only limited data are available, PM_{10} from 1991 through 1993 was about 80% SO_2 . Since very little change occurred in Teplice since 1977, it is assumed that PM_{10} averaged about 100 $\mu\text{g}/\text{m}^3$ during this period; a level also well in excess of the annual MAC recommended by WHO for PM_{10} . A definite difference in the respiratory health status was found between children living in the two districts, which was likely due to the high levels of PM_{10} and/or SO_2 that were present in Teplice.

Initial Study

During the winter and spring of 1992, a study was completed comparing responses of 8th-grade students, mean age = 14 years; the results of this study are in press (16). The objectives of this study were *a*) to compare lung functions and respiratory questionnaire responses for older children who grew up in Teplice with those who grew up in Prachatice, *b*) to provide some indication of whether observed functional decrements were acute or chronic, and *c*) to determine if these effects were influenced by factors other than outdoor air pollution.

Respiratory questionnaire responses were obtained for 90% of all 8th-grade students in both districts: $N_{\text{Teplice}} = 1207$, $N_{\text{Prachatice}} = 645$. Children in Teplice had more allergies than Prachatice, while there were no district differences for hay fever, spastic bronchitis, or asthma. However, in many countries, children with spastic

Table 4. Correlation between personal exposure monitoring of respirable particles ($PM_{2.5}$) and internal exposure and biomarker measurements for all individuals in both districts.

Exposure measure	$PM_{2.5}$	PAH_{car}
PEM carcinogenic PAH	$r=0.79^a$ $p<0.0001^b$ (60) ^c	$r=0.79^a$ $p<0.0001^b$ (60) ^c
PEM benzo[a]pyrene	$r=0.85$ $p<0.0001$ (60)	ND
Blood Pb	$r=0.39$ $p=0.003$ (58)	$r=0.35$ $p=0.009$ (58)
Blood Se	$r=0.55$ $p<0.0001$ (60)	$r=0.49$ $p=0.0002$ (58)
Urinary PAH metabolites (total)	$r=0.48$ $p=0.0002$ (58)	$r=0.40^a$ $p=0.002^b$ (60) ^c
Urinary mutagenicity (MS YG1041-S9) ^d	ND	$r=0.67$ $p=0.016$ (21) ^e
DNA adducts	ND	$r=0.54$ $p=0.016$ (21) ^e

ND, not determined. ^aSpearman rank correlation. ^bSignificance. ^cNumber of observations. ^dMicrosuspension assay. ^eTeplice nonsmokers only.

bronchitis would probably be classified as asthmatic. Combined asthma and spastic bronchitis was significantly ($p < 0.05$) higher in Teplice than in Prachatice. Respiratory symptoms (cough, phlegm, wheeze) were more prevalent in Teplice than in Prachatice ($p < 0.01$); most notable was an almost 2-fold greater frequency of children hospitalized for wheezing in Teplice. Surprisingly, a greater occurrence of some respiratory illnesses was reported for Prachatice children when they were < 2 years of age, but not during the 12 months prior to the study.

Forced expiratory spirometry was measured as forced vital capacity (FVC), forced expiratory volume in 1.0 sec (FEV_1), and forced expiratory flow between 25 and 75% FVC (FEF_{25-75}). These parameters were measured twice in 1992, once in the winter (T_1), and once in the spring (T_2) (Figure 2), so that samples could be acquired from 8th-grade students in each of the districts: $N_{Teplice} = 220$, $N_{Prachatice} = 234$ (Table 5). Analysis of variance appropriate for repeated measures was used to compare all pulmonary functions for district, gender, and season of testing, and all 2- and 3-way interactions of these variables (Table 5).

Significant district differences in the expected direction, i.e., lower lung functions in Teplice, were observed for the three indicators of respiratory flow obstruction, FEV_1 , FEF_{25-75} , and FEV_1/FVC ; FVC was not different. As expected, there were also gender differences for FVC and FEV_1 , but not for FEF_{25-75} . There were no interactive effects for district \times gender, indicating that the district differences were independent of

and valid for both genders. There were some statistically significant interactive effects; however, the magnitudes of these differences were very small and of little clinical consequence. When data from both genders and season of testing were combined, the average differences between the two districts were 3, 6, and 1.4% for FEV_1 , FEF_{25-75} , and FEV_1/FVC , respectively.

Step-forward regression analysis was performed to determine whether FEF_{25-75} , as a representative response, measured in February/March was less in Teplice than in Prachatice when controlling for other factors. Significant differences in FEF_{25-75} were only observed for district and households with smokers. FEF_{25-75} was 0.288 liters/sec less in Teplice than in Prachatice. Surprisingly, it was 0.120 liters/sec greater for children who resided with smokers in both districts.

There were four pollution episodes in the winter prior to, as well as a major episode during, our February/March pulmonary function testing (Figure 2). Average PM_{10} and SO_2 concentrations during the episodes were 5 to 7 times the annual MAC recommended by WHO. Thus, acutely induced decrements in the children's lung function were expected. For the period beginning immediately after the February/March testing (3/7), and ending with the completion of the April testing (4/15), the average PM_{10} and SO_2 concentrations were less than the annual MAC recommended by WHO. These 30+ days of breathing substantially cleaner air should have been sufficient time to bring about at least a partial recovery of acutely induced

decrements in lung function. However, in Teplice, no differences were observed between lung functions measured at the end of the high pollution/inversion season (February/March) and those measured after the children breathed much cleaner air for a 4-week period (April). This finding suggested either nonrecovery of acutely depressed or a condition of chronically depressed lung function. No differences in lung function across time were observed in Prachatice, suggesting that our measurements were reliable.

Cross-sectional Study

Since environmental conditions were expected to improve rapidly in Northern Bohemia, a cross-sectional study was undertaken to compare the responses of 2nd-, 5th-, and 8th-grade students during the fall of 1992 and the winter of 1993. The primary purpose of this study was to ascertain at what age decreased pulmonary function and other indices of respiratory morbidity of children living in Teplice were discernible from children living in Prachatice. A second objective was to definitively determine if additional decrements in lung function result from exposure during the winter inversion/high pollution episode season.

The data from this study have not been completely analyzed. Currently, additional factors that could influence the interpretation of results are being considered. However, preliminary analyses do indicate some robust differences between Teplice and Prachatice. The prevalence of spastic bronchitis for Teplice children, all grades combined, was more than twice that of Prachatice children ($p < 0.001$), while that of asthma and hay fever was not different between the two districts ($p > 0.10$). Respiratory symptoms (cough, phlegm, wheeze) were increasingly more prevalent as school grade decreased and significantly more prevalent in all three grades in Teplice than in Prachatice ($p < 0.001$).

PM_{10} and SO_2 levels from April through September 1992 averaged about $20 \mu\text{g}/\text{m}^3$. Forced expiratory spirometry was initially measured in October 1992. This 6-month period of perhaps the cleanest air in Teplice in two decades was sufficient time for the recovery of any acutely induced decrements in pulmonary functions. Both height-adjusted FVC and FEV_1 were significantly lower in all three grades in Teplice than in Prachatice ($p < 0.01$).

Spirometry measurements were repeated in March 1993, following the winter

Table 5. Effect of district, season, and gender on pulmonary function in 8th-grade students.

Measures of pulmonary function		Winter		Spring			
		February–March 1993		April 1993			
		Prachatice	Teplice	Prachatice	Teplice		
Mean \pm SE for height-corrected pulmonary function measures							
FVC (liters)	Boys	3.93 \pm 0.05 ^a <i>n</i> =110	3.90 \pm 0.05 <i>n</i> =109	3.94 \pm 0.05	3.88 \pm 0.05		
	Girls	3.55 \pm 0.03 <i>n</i> =124	3.51 \pm 0.04 <i>n</i> =128	3.52 \pm 0.04	3.45 \pm 0.04		
FEV_1 (liters)	Boys	3.42 \pm 0.05	3.32 \pm 0.04	3.40 \pm 0.04	3.30 \pm 0.04		
	Girls	3.21 \pm 0.03	3.13 \pm 0.03	3.18 \pm 0.03	3.05 \pm 0.03		
FEV_1/FVC (%)	Boys	87.1 \pm 0.6	85.4 \pm 0.6	86.6 \pm 0.6	85.6 \pm 0.7		
	Girls	90.7 \pm 0.5	89.4 \pm 0.5	90.8 \pm 0.5	88.7 \pm 0.6		
FEF_{25-75} (liters/sec)	Boys	3.93 \pm 0.08	3.65 \pm 0.08	3.87 \pm 0.08	3.65 \pm 0.08		
	Girls	3.93 \pm 0.06	3.73 \pm 0.06	3.91 \pm 0.06	3.62 \pm 0.07		
Analysis of variance (<i>p</i> -values)							
	District (D)	Gender (G)	D \times G	Season (S)	G \times S	D \times S	D \times S \times G
FVC	0.26	<0.001	0.87	0.003	0.02	0.17	0.85
FEV_1	0.006	<0.001	0.88	<0.001	0.02	0.21	0.06
FEV_1/FVC	0.006	<0.001	0.75	0.22	0.70	0.99	0.03
FEF_{25-75}	<0.001	0.77	0.99	0.02	0.44	0.54	0.08

pollution season. There were three pollution episodes of < 10 days in November and December, while from late January to mid-February average PM₁₀ and SO₂ levels were well above 200 µg/m³ with some 24-hr averages in excess of 800 µg/m³. For comparison, PM₁₀ and SO₂ in Prachatice averaged about 30 µg/m³ for November through January, while the average for February, was about 50 µg/m³. Once again, height-adjusted FVC and FEV₁ were significantly lower in Teplice than in Prachatice (*p* < 0.01). Height-adjusted FVC and FEV₁ were slightly higher than when measured in October 1992, suggesting possible lung growth over the 6-month period between testing. The absence of an acute effect suggests Teplice children had chronically depressed lung function.

Elevated particulate and SO₂ have been shown to alter the respiratory health status of school children from both the United States and Europe. While acute effects were not observed in this study, increased school absenteeism and respiratory symptoms (17), as well as decrements in both pulmonary volume and flow (18,19) were observed after episodes of elevated particles and SO₂. There is unequivocal evidence from a substantial number of controlled exposure (chamber) studies that as little as 2 min of exposure to moderate concentrations of SO₂ induces symptomatic bronchoconstriction (asthma attack) in sensitive young asthmatics performing moderate exercise (20).

The findings in this Teplice study regarding chronic effects of air pollution in children do concur with results from previous studies. For chronic exposures, increased respiratory morbidity has been reported in studies restricted to small geographical areas, as well as those with a widespread population base, including multi-city and state and national and multinational surveys. In the former case, upper and lower respiratory illness (21,22), decrements in lung function (23), and increased prevalence of respiratory symptoms (24,25) were observed for children living in those areas with elevated particles and/or SO₂. In the case of the larger population bases, a greater prevalence of upper respiratory illness (26), decrements in lung function (26,27), chronic nonspecific lung disease (28), as well as chronic cough, bronchitis and lower respiratory illness (29) were observed for children living in communities with elevated levels of particles and/or SO₂.

The studies of the 2nd-grade students from the cross-sectional study will be

repeated at 3-year intervals from their first testing to evaluate the effectiveness of air pollution cleanup in improving respiratory health status in these children in a longitudinal design. The initial 3-year interval testing was performed in the fall of 1995, together with a new cohort of 2nd-grade students. The final 3-year interval testing is planned for the fall of 1998. Studies are planned for 1999 to determine the respiratory health status of 2nd-grade students who were born in 1992, which is the beginning of reduced air pollution in Teplice.

Neurobehavioral Studies

Although the high SO₂ pollution found in the district of Teplice is not generally associated with neurobehavioral deficits, toxic trace metals adsorbed to the high concentrations of respirable particles may be neurotoxic, and children living in this region have been considered to be at greater risk for learning disorders than are other children in the Czech Republic (30). Šrám (3) hypothesized that *in utero* exposure to environmental chemicals causes functional changes in the nervous system expressed as developmental disorders or other behavioral dysfunctions. Air monitoring studies in the district of Teplice (5) indicate that trace metals including arsenic (As), mercury (Hg), and cadmium (Cd) occur as byproducts of the combustion of brown coal. Exposure to one or more of these metals could produce neurotoxicity in humans (31). Children are known to be particularly susceptible to heavy metal poisoning (32).

We initially chose to measure Hg and As levels based, in part, on the results of preliminary air monitoring studies (6), which suggested that Hg and As levels were elevated in the Teplice District. Bencko et al. (33) have also reported impaired hearing in children (from nearby Slovakia) associated with As from the combustion of coal. Dahl et al. (34) have more recently reported a significant association of umbilical cord methylmercury levels and neurobehavioral measures obtained in Faroese children when they reached 7 years of age.

Another metal with well-known neurobehavioral sequelae is Pb. Pb exposure has been associated with a broad spectrum

of neurobehavioral effects [see review (32)] including the performance of East German children on tests similar to those used in the current project (35). We have not obtained measures of Pb exposure in Czech children evaluated thus far because of a limitation to noninvasive biological sampling methods such as urine and hair. Since venipuncture sampling is the recommended method to determine Pb exposure (measures of Pb in hair are not acceptable to the U.S. EPA or WHO), we have not attempted to measure Pb levels in children participating in the study.

A series of studies of neurobehavioral function in Czech school children has been conducted in conjunction with the Teplice Program (36–38). Basic objectives of these studies were to compare neurobehavioral performance and the prevalence of learning disabilities in children living in districts of Bohemia with varying levels of air pollution. Results of these studies are summarized below.

Three cohorts of school children were assessed, including 2nd-, 4th- and 8th-grade students and are summarized as follows: *a*) 2nd-grade cohort of 7- to 8-year-old children included 189 males and 161 females from Teplice and 241 males and 181 females from Prachatice for a total of 772 children, *b*) 4th-grade cohort of 9- to 10-year-old children from three districts (Table 6), and *c*) 8th-grade cohort of 14- to 15-year-old children that included 100 males and 136 females from Teplice and 119 males and 115 females from Prachatice for a total of 470 children. Eighth-grade students were tested first to take advantage of extended exposure histories and less variability in the performance of older children. Second-grade children, the youngest group that can adequately perform an extended set of computerized neurobehavioral tests, were evaluated next. The third cohort consisted of an intermediate group of 4th-grade children. In each cohort, boys and girls from representative urban and rural schools were studied.

The methods used in these studies included the Neurobehavioral Evaluation System (NES2) and questionnaires administered to both the children's teachers and

Table 6. District differences in the prevalence of 4th-grade students referred for assessment of learning disabilities.

Districts (males, females, and total cohort)	Referred	Not referred	% Referred
Teplice (91 males, 70 females, 161 total)	44	117	27.3
Prachatice (75 males, 86 females, 161 total)	21	140	13.0
Znojmo (93 males, 80 females, 173 total)	22	151	12.7
Total (259 males, 236 females, 495 total)	87	408	21.3

parents. The NES2, a computerized assessment battery developed by Baker and Letz (39) for neurotoxicity field testing, was used to evaluate sensorimotor and cognitive function in children. Tests used were finger tapping, visual digit span, continuous performance, symbol-digit substitution, pattern comparison, hand-eye coordination, switching attention, and vocabulary. Teachers of children participating in the studies completed a questionnaire, adapted from Matejcek (40), to assess symptoms of learning disabilities. Ratings of speech, writing, and motor coordination skills, attention, and conduct were obtained. Teachers were specifically asked if children had ever been referred for clinical assessment of learning or behavioral problems. A questionnaire was administered to parents of participating children to assess family demographics (e.g., parental education, occupations, type of housing, family income); health history of children and parents; drug, tobacco, and alcohol consumption of parents; and chemical exposure history of parents. These data were used in statistical analyses to control for possible confounding effects of socioeconomic, neonatal health, and other factors.

In 1992, a pilot study of 8th-grade children from the districts of Teplice and Prachatice was completed using the questionnaires and five NES2 tests. Teacher ratings on manual dexterity, drawing ability, and parental caregiving, but not on ability to concentrate, were significantly worse for children from Teplice. Children from Teplice also performed significantly worse than children from Prachatice on finger tapping. The observed differences could not be accounted for by possible confounding effects of socioeconomic status, neonatal health or other factors. This study has been reported in detail elsewhere (36).

In 1993, 2nd-grade children from the same districts were assessed. Seven NES2 tests and parent-teacher questionnaires were again administered to the children. The possible confounding effects of other variables were controlled in statistical analyses. Hair and urine samples were also obtained from 600 2nd-grade children to determine the association of neurobehavioral performance and biological measures of As and Hg exposure. Levels of As and Hg observed in children from both districts were surprisingly low. The average Hg level in hair in Teplice children was 0.27 ppm and the average in Prachatice children was 0.89 ppm. Hg levels in urine were similarly low.

Teachers reported that significantly more 2nd-grade children from Teplice (26.6%) than from Prachatice (12.9%) had been referred for clinical assessment of learning or behavioral problems. On the other hand, district comparisons showed poorer performance in Prachatice children on several neurobehavioral tests, consistent with higher levels of Hg in hair. Regression analyses, controlling for possible confounding factors of socioeconomic status, neonatal health, and other factors, indicated significant, but weak associations of hair Hg and finger tapping, hand-eye coordination, and coding performance. No meaningful associations of any neurobehavioral measures with hair As or urinary measures of either metal were found.

In 1994, 4th-grade children from Teplice, Prachatice, and Znojmo (a second comparison district that uses natural gas for home heating) were similarly assessed. Consistent with 2nd-grade results, significantly more children from Teplice (27.3%) than from Prachatice (13.0%) or Znojmo (12.7%) had been referred for clinical assessment of learning or behavioral problems (Table 6). Neurobehavioral performance on four of seven tests was significantly poorer in children referred for assessment of learning disabilities (Table 7) after adjustment for confounding variables. Significant district differences in performance of digit span and symbol-digit were also found, i.e., children from the mining district performed more poorly than children from the comparison districts. When scores were adjusted for parental education, however, the district differences were no longer significant.

In summary, selective differences were found in teacher ratings and neurobehavioral performance of 2nd-, 4th-, and 8th-grade Czech children living in districts with varying levels of air pollution. Possible confounding effects of socioeconomic, neonatal health, smoking in the home, and

other factors were assessed. Evidence of the higher prevalence of learning disabilities in 2nd- and 4th-grade children from the highly polluted mining district of Teplice was found in teacher reports of children referred for clinical assessment of learning and behavioral problems. Results of computerized tests were less consistent. District differences in finger-tapping performance in 8th-grade children remained after controlling for possible confounders. In 2nd-grade children, weak but significant associations were found in the performance of three neurobehavioral tests and hair Hg levels. These associations also remained after controlling for possible confounders. On the other hand, no significant district differences in the neurobehavioral performance of 4th-grade children were found when parental education and other covariates were controlled. Nor were any significant associations of neurobehavioral measures and metal levels found in these children. Further studies are planned to clarify the association of air pollutants and neurobehavioral function in Czech children.

For assessment of Pb exposure in children living in the study area, blood samples were obtained from 200 children that were referred to hospitals in the Teplice and Prachatice Districts for blood work not related to our studies. These samples indicated blood Pb means of 5.0 µg/dl (range 1.0–17.6) in children from Teplice and 3.8 µg/dl (range 0.9–14.0) in children from Prachatice. While these levels do not suggest that children in either district are at serious risk for lead poisoning, Winneke et al. (35) have recently shown an association of comparable blood Pb levels and performance on similar neurobehavioral tests in 6-year-old East German children. District differences in performance observed in 2nd- and 8th-grade children in this project could be due to Pb exposure or other neurotoxicants. We hope to clarify this question in later studies.

Table 7. Neurobehavioral differences in the performance of 4th-grade students referred and not referred for assessment of learning disabilities.^a

Neurobehavioral measures	Referred mean (SD)	Not-referred mean (SD)	p-Values
Finger tapping (alternating/# taps)	157 (27)	153 (27)	NS
Hand-eye coordination (2 best trials, rms error)	2.48 (0.28)	2.44 (0.31)	0.04
Visual digit span (forward + backward)	8.6 (1.7)	9.6 (1.8)	0.001
Pattern comparison (mean RT - correct, sec)	7.07 (1.6)	6.6 (1.9)	NS
Continuous performance (mean RT, msec)	436.9 (65)	435.7 (60)	NS
Switching attention (errors, side)	1.05 (1.6)	0.52 (1.0)	0.002
Symbol-digit substitution (mean latency per digit, 2-best trials, sec)	3.91 (0.81)	3.50 (0.65)	<0.0001

Abbreviations: SD, standard deviation; RT, reaction time; NS, not significant. ^aNeurobehavioral performance as measured by NES battery.

Pregnancy Outcome

The main aim of this study was to evaluate the impact of air pollution and lifestyle variables on all hospitalized pregnancies in two districts (Teplice and Prachatice) using biomarkers as a measure of exposure. The hypothesis was that the pregnancy outcomes would be generally worse in the district with higher pollution level (Teplice). Exposure is estimated by air pollution monitoring and modeling, questionnaire information and selected biomarkers. Based on the power analysis, the main reproductive effects were chosen, namely, low birth weight (below 2500 g), premature births (below 37 weeks), and intrauterine growth retardation (small for gestational age).

Two basic approaches are combined:

a) In the prospective cohort study, all hospitalized pregnancies terminated in Teplice and Prachatice districts during a 3-year period (1994–1997) will be involved. Personal and lifestyle data are obtained via self-administered questionnaires, together with information about reproductive history, work and environmental exposures, health status, diseases, and medications.

b) Selected biomarkers are analyzed in venous blood, cord blood, and placenta of the women enrolled in a nested case-control study. Women with pregnancies less than 37 weeks or with babies weighing less than 2500 g are classified as cases. An equal number of women giving birth to babies with normal parameters are selected systematically from the whole cohort as controls (each fifth noncase birth was selected). Biomarkers such as DNA adducts, Comet assay, cell and humoral immunity markers, and vitamins A, E, and C are analyzed in blood and placenta obtained from cases and controls. Metabolic genotypes (*GSTM1* and *NAT2*) are determined. Isolated DNA and other biological materials are stored for possible analysis of other biomarkers in future.

In the final data analysis, logistic regression will be used for the analysis of dichotomous outcomes (low birth weight, premature births, fetal loss, etc.). For continuous outcomes (birth weight, gestation length, etc.), regression analysis and other procedures of parametric statistics will be applied. Critical windows will be considered for the various outcomes based on biological plausibility for the exposures observed. At present, after 15 months of data collection, only some general statistical procedures were used for the analysis of descriptive data. The preliminary analyses described here include univariate analysis

Table 8. Prevalence of low birth weight and premature births in the whole cohort and in European births only.

Population	District	Births	<i>n</i> < 2500 g	% of births < 2500 g	<i>n</i> < 37 weeks	% of births < 37 weeks
All births	Teplice	1626	143	8.8	101	6.2
	Prachatice	644	21	3.3	22	3.4
				<i>p</i> < 0.0001		<i>p</i> < 0.01
European births	Teplice	1380	88	6.4	69	5.0
	Prachatice	623	18	2.9	19	3.1
				<i>p</i> < 0.002		<i>p</i> < 0.05

of relative risk and linear risk trends for analysis of dose–response relationships.

Preliminary analyses were done on roughly 2500 pregnancies collected during the first 15 months of the study (41). As expected, the prevalence of low birth weight infants in the district of Teplice (8.8%) was significantly higher (*p* < 0.0001) than in the district of Prachatice (3.3%). Similarly, the prevalence of premature births is 6.2% in Teplice and 3.4% in the Prachatice district (*p* < 0.01) (Table 8). However, the populations of the two districts differ significantly in their ethnic composition. About 14.1% of births in Teplice, but only 2.9% in the Prachatice district, were of Gypsy ethnicity. Gypsies, with origins in India, differ from other inhabitants (mostly of European origin) in many biological and social characteristics (42). Differences were observed in their pregnancy outcomes: 13.4% of Gypsy births were premature and about 23.6% of infants weighed less than 2500 g at births. Thus, the difference observed in the two districts fell substantially after exclusion of Gypsy births; even after this exclusion the difference remains statistically significant (Table 8).

As expected, a clear-cut relationship was found between low birth weight and maternal active cigarette smoking (43,44). A highly significant linear relationship was observed between the risk of low birth weight and the number of cigarettes smoked per day for all births of European as well as Gypsy origin (Figure 6). This relationship was also observed for premature births. Women living in the two districts differ significantly in their smoking habits in early pregnancy. Women from Teplice smoke more during early pregnancy (42%) than do women in Prachatice (33%). The smoking patterns are more similar after the exclusion of Gypsy women (38 vs 32%). The smoking habits of Gypsy women are quite different from other women: 67% of Gypsy woman reported smoking in the first trimester with two-thirds of these smoking more than 10 cigarettes per day. Thus, in addition to ethnic

specificity and lower socioeconomic status of Gypsy mothers, more smoking may contribute to lower birth weight of Gypsy infants. The interdistrict difference in smoking habits is lower, but still highly significant (*p* < 0.0001) after exclusion of Gypsy women.

Various critical windows have been considered preliminarily for seasonal exposure variation. The only interesting relationship was found between prevalence of premature births and season of conception. The prevalence of premature births for babies conceived during winter (6 months from October to March) in the district of Teplice was considerably higher than for those conceived in summer (April to September). A less pronounced but significant tendency was also seen for low birth weight. However, this effect was limited only to babies of mothers who smoked (Table 9). This pattern is expressed consistently in European and Gypsy births; if the mother is a smoker, the relative risk of premature births for European infants conceived in winter is 2.5 times greater, and for Gypsy infants 6.5 times the risk for babies conceived during summer months (Figure 7). A synergistic effect of the environmental load (increased level of pollutants in winter period) and smoking could

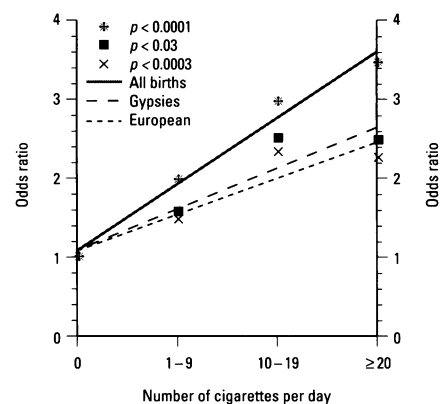


Figure 6. Linear trend of odds ratios for low birth weight births and maternal smoking habits.

be only one from many possible explanations; seasonal variation in diet, vitamin levels, and other factors could be hypothesized (45). Each attempt at interpretation is premature at this stage; this question will be addressed as more data are collected.

Significant differences in birth weight distribution between the two groups of infants conceived in summer and winter were found. A considerably higher portion of babies with very low birth weight ([VLBW] below 1500 g) was found among infants conceived in winter by women who smoked. About 26% of low birth weight babies conceived in winter by women who smoked were below 1500 g (32% for mothers who were light smokers and 24% for those who were heavy smokers). The percentage is only 6 to 8% for others.

Monitoring of many air pollutants continues from 1993. Pollution levels vary in both communities, with the highest values in the winter; the high levels in Teplice are considerably higher than in Prachatice. Air pollution exposures will be determined for each trimester of pregnancy and evaluated in detail in the final analysis. It should be noted that this preliminary analysis (due to the seasonal variations) cannot really examine the effect of exposure. The preliminary analysis shows that reproductive outcomes may be affected by a number of factors, alone or in combination, such as environment, ethnic composition or lifestyle habits. It is hypothesized that some of the environmental effects found in some earlier studies in these geographic areas could be, in fact, the effect of differences in population structure and social makeup. As the levels of air pollution decrease, the significance of those other determinants of reproduction quality (e.g., smoking and other lifestyle variables) may increase (45).

The preliminary analyses suggest that

a) Prevalence of low birth weight and premature births in the district of Teplice is significantly higher than that in Prachatice. This also holds true for the European majority population alone.

b) This difference is partly due to the higher proportion of Gypsy births and a greater number of people who smoke in the Teplice district. The interdistrict differences in prevalence of low birth weight and premature births remain statistically significant even when these factors are controlled. Future analyses will also examine and control for the effects of other lifestyle and health factors that might affect these outcomes.

c) The increased prevalence of premature births and low birth weight

Table 9. Prevalence of low birth weight and premature births analyzed by conception period and maternal smoking habits.

Smoking habits	Conception period	Birth number	<i>n</i> < 2500 g	% of births < 2500 g	<i>n</i> < 37 weeks	% of births < 37 weeks
Nonsmoker	Summer ^a	810	35	4.3	32	3.9
	Winter ^b	559	31	5.6	28	5.0
Smoker	Summer	516	44	8.5	22	4.3
	Winter	381	59	15.6	48	12.6
				<i>p</i> < 0.005	<i>p</i> < 0.0001	

^aSummer is the low exposure time and for these purposes goes from April through September. ^bWinter is the high exposure time; this analysis uses October through March as the winter period.

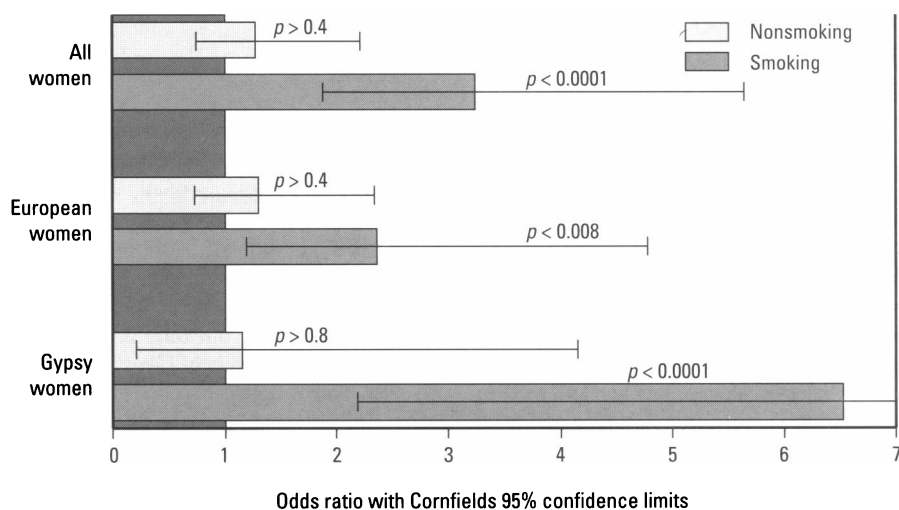


Figure 7. Odds ratios of premature births for infants conceived in winter compared to those conceived in summer in Teplice by smoking habits of mother.

observed in these preliminary analyses for the infants conceived in winter season in the district of Teplice is limited to babies of mothers who smoke. A synergistic effect of air pollution and smoking is only one of many possible explanations of these preliminary results.

Human Semen Study

In response to public perception that people living in the district of Teplice were experiencing problems having children, and a report that the rates of conception and the incidence of congenital malformations might be related to seasonal changes in air pollution (2), reproductive health studies were proposed for inclusion into the Teplice Program. The first of a series of semen studies in young men was initiated in 1992.

The objective of the first study is to compare reproductive health and semen quality outcomes in men living in Teplice with those of men living in Prachatice. Since air pollution is worse during the

winter than the rest of the year, especially in Teplice, and adverse effects on semen quality may be transient in nature, the study was designed to obtain semen samples either during late winter (at the end of the polluted season) or in the early fall (after the cleaner spring and summer months).

Eighteen-year-old men were selected for study for several reasons. First, all 18-year-old men in each district receive a physical examination to determine fitness for military service, and it was possible to invite them to participate in a semen study in conjunction with this examination. Second, these young men are likely to have similar environmental exposures since they are still living at home and attending secondary school or undertaking local apprenticeship training. Third, 18-year-old men would be less likely than older men to have been exposed occupationally to reproductive toxicants (solvents, pesticides, metals) or to exhibit personal habits (smoking, drinking alcohol) that may have adverse reproductive effects. Limitations inherent

in sampling young men include a lack of information about their fertility status and the lack of documented normal standards for men of this age.

When each man arrived for his appointment, he was given a physical examination that included the reproductive organs and determination of testes size. He was also interviewed to obtain information about his lifestyle and personal habits, general health, reproductive history, and potential occupational or recreational exposures to chemicals of interest. The objectives of the semen study were explained, and he was asked to donate a semen sample. Of 471 men who were examined and interviewed in the fall of 1992 and 1993 and the late winter of 1993 and 1994, 325 (69%) provided a semen sample: 190 from Teplice and 135 from Prachatice.

To obtain a thorough assessment of semen quality, a variety of semen characteristics were examined. First, a standard semen analysis, as defined by WHO (46), was performed: semen volume, pH, and concentration were determined on site, and smears of sperm were prepared for later assessment of sperm morphology. In addition, aliquots of semen were videotaped and later analyzed to determine the percentage of motile sperm, and to measure various movement and velocity characteristics of individual sperm tracks using computer-assisted sperm analysis (CASA) (47,48). Aliquots of semen were also frozen and later evaluated using the sperm chromatin structure assay (SCSA) (49) to detect changes in the sperm nucleus, which can result from exposure to various chemicals, high fever, and cancer chemotherapy.

Analysis of the questionnaire data showed that the young men from Teplice were quite similar to those from Prachatice with respect to lifestyle, health, and other characteristics (50). The only exception was that men in Teplice were slightly heavier than men in Prachatice (73.7 vs 71.2 kg, $p < 0.05$). There was also no difference in reproductive development as shown by similar means and distributions of self-reported age at first semen appearance (mean = 14.0 and 14.1 years of age) and clinically measured testicular volume (mean = 44.6 and 45.6 cc) in men examined from Teplice and Prachatice, respectively. Nor were there any district differences in the prevalence of developmental defects in the reproductive tract, which might result from perinatal exposure to endocrine disruptors (Table 10). The percentages of men with testicular maldescent (undescended or

retractile testes) are within the range reported in the international literature (51), while our limited sample size precludes meaningful comparisons for hypospadias.

Exposure data from the air monitoring study (see previous section) were used to examine potential relationships between air pollution and the various semen characteristics. Since the database for SO₂ was the most complete on a daily basis, and since air quality standards for SO₂ are established in the United States and Czech Republic, SO₂ was used as a surrogate for air pollution in general. Mean SO₂ values were calculated for the 90-day period immediately preceding each sampling interval (Figure 8). Ninety days was selected to encompass the entire duration of spermatogenesis from stem cell renewal through epididymal maturation. For all sampling times in Prachatice and fall sampling times in Teplice, mean 90-day SO₂ levels were well below the U.S. standard (not to exceed 80 $\mu\text{g}/\text{m}^3$, as an arithmetic annual mean) and these were categorized as low. In contrast, SO₂ levels in Teplice during the winter of 1994 averaged close to 80 $\mu\text{g}/\text{m}^3$ and were categorized as medium, while those in

Teplice during the winter of 1993 were about 2-fold the US standard and were categorized as high. These air pollution categories were used in both linear and logistic regression models developed to examine the influence of air pollution on semen quality. These models always included district, and controlled for appropriate potential modifiers such as abstinence interval, lifestyle (e.g., smoking, drinking), occupational exposures, and health status information obtained from the questionnaire (50,52). For the logistic regression models, cut-off values for normal versus abnormal were derived from WHO standards (46) or from expert opinion.

As expected, sperm counts and concentrations varied widely among men. In general, these outcomes (Table 11) fell within the ranges reported in recent semen studies worldwide. These studies are reviewed by Toppari et al. (51). These measures of sperm production did not differ by district and/or season, nor were they related to air pollution categories in the full linear and logistic regression models. In contrast, significant decrements in sperm motility and morphology were found in samples

Table 10. Prevalence of developmental markers of the reproductive tract in 18-year-old men in Teplice and Prachatice districts.

Abnormality	Teplice (n = 255)	Prachatice (n = 216)	Both districts (n = 471)
Cryptorchidism	3 (1.2%)	3 (1.4%)	6 (1.3%)
Retractile testes	3 (1.2%)	6 (2.8%)	9 (1.9%)
Hypospadias	2 (0.8%)	2 (0.9%)	4 (0.8%)

^aValues are numbers of men affected (% of men examined) and represent cases identified by questionnaire (having been surgically corrected) or by physical examination.

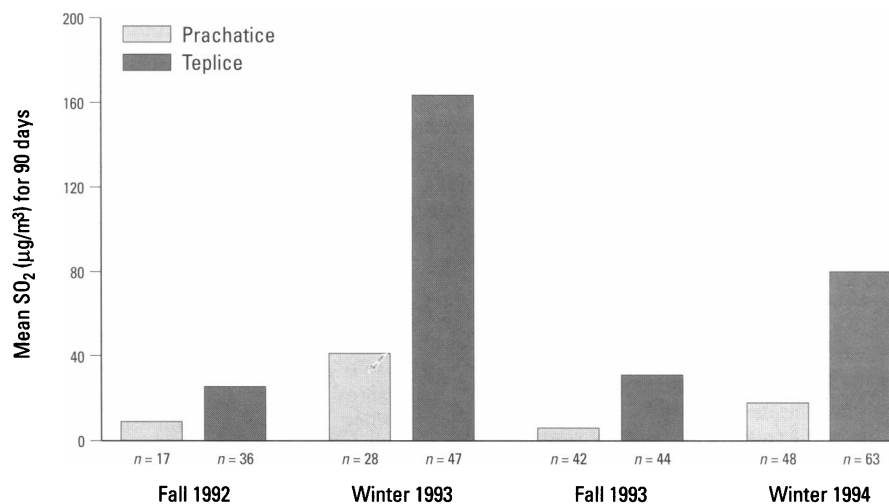


Figure 8. Mean SO₂ levels in Teplice and Prachatice calculated for the 90-day interval preceding each early fall or late winter semen sampling period. The number of men sampled in each survey is shown below the appropriate date of sampling.

Table 11. Semen analysis in 18-year-old men in Teplice and Prachatice districts.

Outcome	Teplice (n = 190)	Prachatice (n = 135)
Semen volume (ml)	1.9 ± 1.0 ^a (0.4–5.5)	2.1 ± 1.1 (0.3–6.0)
Concentration (millions/ml)	60.3 ± 52.9 (0–421)	65.1 ± 74.5 (0–490)
Total sperm count (millions)	111.1 ± 104.0 (0–624)	136.9 ± 191.0 (0–1470)
% (#) with < 20 million sperm/ml	18.9% (36/190)	21.5% (29/135)
% (#) with < 40 million sperm/ml	27.9% (53/190)	25.9% (35/135)

^aNo significant differences were found between districts or seasons, or by exposure category (in regression models) for any of these outcomes (controlled for abstinence intervals < 2 days, and other appropriate modifiers).

^bValues are mean ± SD (range).

collected in Teplice in the winter surveys. For example, preliminary analysis of unadjusted mean data, illustrated in Figure 9, showed that the percentage of progressively motile sperm was significantly lower in samples obtained from Teplice donors in the late winter than in those obtained from Teplice donors in the early fall or Prachatice donors in either season (50). The full linear and logistic regression analyses were consistent with the preliminary analyses and showed that exposures to pollution categorized as medium or high were associated with significant decreases in the percentage of progressively motile sperm, the percentage of morphologically normal sperm, and the percentage of morphologically normal sperm heads (52). Exposure to the high pollution was associated with abnormal sperm chromatin structure. The full study analyses are to be presented elsewhere (unpublished data).

These positive findings prompted us to initiate a second study in which semen quality would be examined in multiple samples from each man, obtained during intervals of both low and high pollution. Accordingly, participants from the first study who were still living in Teplice were invited to participate in a longitudinal study during 1995 to 1996. This study design includes collection of a baseline sample in the early fall (low pollution), three monthly samples during the winter (high pollution), and a final sample in the early fall of 1996. More extensive air monitoring data will be available, including daily PM₁₀ and metals. Urine and blood are also being collected to monitor cotinine, blood metals, and other exposure markers. This approach has the advantage that each man serves as his own baseline, and it allows better definition of the interval between exposure and effect.

A preliminary study was also conducted to examine the potential effect of smoking on sperm aneuploidy. Simultaneous assessment of three fluorescent probes specific for the sex chromosomes and chromosome 8 and fluorescence *in situ* hybridization (53) was used to evaluate sperm aneuploidy in a subset of smokers and nonsmokers selected from one survey period. Smoking status was found to be related to an increased incidence of sperm aneuploidy (54). This information is vital to the interpretation of the ongoing analyses designed to evaluate the potential impact of air pollution on this outcome.

Taken together, the findings to date suggest that exposure to high levels of air pollution does not appear to impair sperm production, but may be associated with transient decrements in sperm quality (motility, morphology, nuclear integrity), although the specific component(s) responsible for such effects are unknown. Ongoing studies should better characterize these effects and provide information that may identify suspect components.

Conclusions

Intensive mining and combustion of brown coal in the Northern Bohemian basin has resulted in unusually high concentrations of air pollutants. Fine particles collected during the winter season were dominated by acidic sulfates, genotoxic organic carbon compound (e.g., PAH), and toxic trace elements. The major source of airborne fine particles resulted from the burning of coal for residential heating and in power plants. The concentrations found in this study in the winter episode of 1993 were similar to those observed in London in 1952. Human exposure and biomarker studies demonstrated that variations in air pollution were greater within the Teplice district than between Teplice and Prachatice. The exceptionally high air pollution exposures during inversion episodes, however, are much greater in Teplice than in Prachatice. Seasonal differences are consistently significant, with winter inversions resulting in much higher concentrations of air pollutants in both districts than and spring/summer periods. These findings led to the investigation of within district effects across season for several health outcomes. Personal exposures to respirable fine particles and organic carcinogens (e.g., PAH) were correlated with excretion of PAH metabolites in urine, several trace metals in blood, and DNA adducts in blood cells. Studies are in progress that apply these biomarkers of exposure, dose, and metabolic susceptibility to samples from the male and female reproductive cohorts to determine the role of exposure and metabolic susceptibility in genetic damage and reproductive outcomes.

Respiratory and neurobehavioral studies of school-age children (2nd–8th grade) were conducted using both questionnaires and clinical measures. The respiratory studies demonstrated a significantly higher prevalence of spastic bronchitis and allergies in the Teplice district compared to Prachatice, with lower air pollutant concentrations. Symptoms of cough, phlegm, and wheezing were also higher in Teplice children. Respiratory spirometry tests of lung function also showed significant adverse outcomes in Teplice children as compared to those in Prachatice as measured by several established tests of lung function. The neurobehavioral studies of children indicated significant district differences in some, but not the majority of, objective measures of motor and cognitive performance. Based on questionnaires administered to the teachers, the children

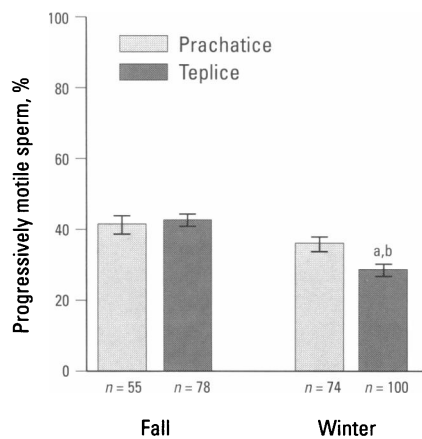


Figure 9. Effect of district and season on sperm motility. Bars indicate the mean (±SEM) percentage of sperm with progressive motility for samples obtained in Prachatice (P) or Teplice (T) in the early fall (after exposure to low air pollution) or the late winter (after exposure to higher pollution). n = number of samples in each group; a = different by SEASON within district; b = different by DISTRICT within season by Wilcoxon rank sum tests or ANOVA.

in Teplice were more than twice as likely to be referred for clinical assessment of learning or behavioral problems. This finding is important because referral to the Pedagogical Clinics is the method used in schools throughout the Czech Republic to identify children with learning disabilities. These clinics are responsible for diagnosing learning and behavioral problems and for prescribing appropriate remediation. Further analysis is needed to determine if the incidence of learning disability is associated with pollutant exposure or other factors.

Reproductive studies were conducted in both male and female populations.

Preliminary analysis of the pregnancy outcome study in progress, indicated the prevalence of low birth weight and premature births were significantly greater in Teplice than in Prachatice. In addition, these adverse outcomes in Teplice were higher in infants conceived in the winter season for smoking mothers. Pregnancy outcomes were affected by a number of factors including the environmental air pollution, ethnicity, and lifestyle (e.g., smoking). The reproductive health of males was evaluated in a reproductive development and semen study of young men. These studies indicated no differences between the districts in reproductive

development; however, measures of semen quality between districts and between seasons suggest that exposure to high levels of air pollution may be associated with transient decrements in semen quality.

These studies consistently suggest that elevated levels of airborne fine particle pollution can result in measurable uptake, metabolism, and cellular DNA damage in populations exposed to high concentrations, even for short-term winter inversion periods. Chronic and seasonal exposures to elevated air pollution in the Teplice district may have serious adverse respiratory health consequences for children and adverse reproductive risk in adult populations.

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