Biomarkers of Exposure to Particulate Air Pollution in the Czech Republic

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HUMAN SUBJECT CONSENT

Informed consent was obtained from each of the subjects prior to partcipation in the studies described in this paper.

DISCLAIMER

This paper has been reviewed in accordance with the US Environmental Protection Agency's peer and administrative review policies and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ABSTRACT

The use of biomarkers in the Teplice Program, provided a key tool to relate health outcomes to individual personal exposures and to provide measures of confounding exposures. This research program on the health effects of air pollution studied a population living in the heavily industrialized district of Teplice in Northern Bohemia and compared the exposure and health of this population to that of a non-industrialized district, Prachatice, in Southern Bohemia. The studies included characterization of the environmental and personal air pollution exposure, biomarkers, and studies on reproductive, respiratory, and neurobehavioral effects. Biomarkers were measured in blood, urine, placenta, and sperm. The biomarkers included measures of exposure (e.g., urine metabolites and blood metals), dose (e.g., DNA adducts), DNA damage, genetic and cytogenetic effects, and susceptibility. During winter temperature inversions, unusually high concentrations of a complex mixture of air pollutants were measured, including fine particles, genotoxic organic compounds, and toxic trace elements. This population, however, was also exposed to multiple pollutants via all pathways, and including pollutants resulting from environmental exposures, occupational exposures, and personal habits (e.g., tobacco and alcohol use). Longitudinal and repeated measures used individuals as their own control to examine the influence of environmental exposures as they changed over time and season. Chronic and seasonal exposures to elevated air pollution in the Teplice District were shown to have serious adverse respiratory health consequences for children and reproductive effects in adults. Elevated levels of air pollutants, even for short-term winter inversions resulted in measurable uptake, metabolism, and excretion of polycyclic aromatic hydrocarbons, increased blood concentrations of toxic metals, and resulted in DNA damage. Results of the exposure, biomarker, and health studies indicated that environmental exposure to a complex mixture of air pollutants resulted in significant elevations in personal exposure, uptake, excretion of pollutants and DNA damage.

INTRODUCTION

The Northern Bohemia brown coal basin comprises four mining districts located in the northwestern region of the Czech Republic, including Teplice district. The brown coal (lignite) in this region is very high in sulfur and low in quality. It is surface-mined from open pits. This coal 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 utilized in the former Czechoslovakia. The combustion of this coal combined with heavy industrialization of this region over the past few decades, has resulted in some of the worst environmental pollution in Europe (*1*). This same 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). Prior to 1990, air pollution from these sources caused extensive deforestation. A review of these environmental problems in the former Czechoslovakia was published by Moldan and Schnoor (*1*).

The health consequence of environmental pollution in this region has been a major concern of the public. Although exploratory analysis of data collected prior to 1989 suggested a higher incidence of cancer, reproductive, and behavioral effects in this region(*2*), no comprehensive research had been conducted to address these hypotheses. The Teplice research program was developed in 1989 to evaluate the short-term and long-term health impact of air pollution on the population. 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 in Southern Bohemia, which was originally thought to have some of the cleanest air in the Czech Republic, was selected as a comparison district.

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

to assess environmental health problems in the Northern Bohemian basin area (*3*). Thereafter, a collaborative research program was developed with the U.S. Environmental Protection Agency (US EPA) to include air pollution monitoring, human exposure, biomarker, and health effects studies. This program has succeeded in bringing together many different research organizations and government laboratories in both the Czech Republic and the U.S. to accomplish the multidisciplinary program.

The aim of the Teplice program was 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. The central hypothesis in the Teplice Program is that the polluted air in the Teplice district, adversely affected the health of the population. A summary of the design and inital results of these 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, reproductive, respiratory, and neurobehavioral effects through 1996 has been published(*4*). The personal exposure, biomarker and health effects studies conducted since 1992 and continuing into 2000 is shown in Table 1 by season and year. This paper reviews the role of exposure assessment in these health effects studies through ambient monitoring, personal exposure monitoring and the use of biomarkers of exposure.

CHARACTERIZATION OF MODEL DISTRICTS

Teplice district

The Teplice district 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 Středohoří (Central Bohemian Highlands) in the south. This district is in a basin in which the air pollution emissions are trapped during meteorological temperature

inversions leading to high concentrations of air pollutants, particularly in the winter. Teplice has a population of approximately 132,000, with an average density of about 285 people per km^2. The density is the highest in Northern Bohemia and, with the exception of much larger cities, such as Prague, is one of the highest in the Czech Republic. About 50% of the employed population works in industrial jobs and less than 3% in agriculture.

The district area comprises 469 km^2 including agricultural land (170 km^2) and forests (165 km^2). By 1992, coniferous trees became virtually extinct and the deciduous trees sustained serious damage due to the high air pollution emissions. 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 sulphur dioxide and nitrogen oxides emitted in the Czech Republic originate 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 the brown (lignite) coal as fuel. The Teplice air pollution research program (discussed in more detail below) demonstrated that 30-40% of the human exposure to fine particles in this district was due to home heating (*5*). As a result of this finding, incentives were provided to convert from coal to gas heating in the Teplice district.

Prachatice district

The Prachatice district is situated in the southwestern part of the Southern Bohemia region, near the border with Austria to the south, and the Šumava (Bohemian Forest) Mountains to the southwest which form a natural border with Germany. This area has the lowest number of

inhabitants of Southern Bohemia (50,740) and its population density (39.6 per km^2) 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. The area of the district of Prachatice (1375 km^2) ranks fourth in the region of Southern Bohemia (covering 12% of the region's area). Climatic conditions vary greatly due to the varied elevation and character of the terrain, however, there are also frequent fogs and temperature inversions in this district.

The district has vast, predominantly spruce forests covering the area of 71 thousand hectares (52% of its area). In order to preserve the Šumava Mountain region, its water resources, and tourist attractions, 1630 km^2 of the range were declared 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.

CHARACTERIZATION OF THE AIR POLLUTION

Air pollutant measurements were made at two primary locations during this study(*5*). The main monitoring site was located in the city of Teplice (North Bohemia), and the second was located in the city of Prachatice (South Bohemia). Ambient SO_2 and particulate matter < 10 μm (PM_{10}) was monitored daily in both districts during the health studies. The inorganic and organic composition of fine or respirable particle <2.5 μm ($PM_{2.5}$) and coarse particles (2.5-10 μm) 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 modeling studies 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.

Particle mass and elemental composition were measured in Teplice and Prachatice beginning in 1992. The concentrations of all pollutants measured, including particles, sulfur dioxide, anions, toxic metals and polycyclic aromatic hydrocarbons (PAH) were significantly higher in the winter than the spring and summer in both districts. The Teplice district, however, had more severe pollution episodes and higher average winter pollutant concentrations. An example is shown in Table 2 for the winter of 1993 where the average fine particle mass ($PM_{2.5}$) in Teplice was 122 $\mu g/m^3$ compared to 44 $\mu g/m^3$ in Prachatice and the spring and summer average fine particle concentrations for Teplice and Prachatice were 28.7 $\mu g/m^3$ and 17.9 $\mu g/m^3$, respectively. While the fine particle mass, trace elements and organic compounds in Teplice were an average 2-3 fold higher than in Prachatice, the average sulfur dioxide concentrations were generally at least 5-fold higher in Teplice than Prachatice. Seasonal differences in air pollutant concentrations, even within the same district, were often greater than the district differences.

The winter inversions produced the most dramatic pollution changes in Teplice. During an inversion episode, between January 29 and February 6, 1993, the SO_4^{2-} and the organic carbon peak concentrations were 400 and 140 $\mu g/m^3$, respectively. The presence of acidic particles was indicated by the pH level, e.g., one sample collected at the peak of the episode had a pH of 4.1. Fine particles ($PM_{2.5}$) collected during the winter season were dominated by sulfates, organic carbon, and trace metals. The fine particle mass observed in Prachatice was one-third the level found in Teplice during winter episodes. Spring and summer particle concentrations were substantially lower, reflecting the absence of home heating emissions and more favorable meteorological conditions.

Samples were also analyzed for a series of carcinogenic polynuclear aromatic hydrocarbons (PAH). Total PAH concentrations in Teplice in the winter were approximately

twice the average winter concentrations of PAH in Prachatice. The most dramatic differences (10X) were observed between the winter and spring/summer averages in Teplice. Evaluation of the benzo(a)pyrene (BaP) to lead ratios in Teplice over time indicates the presence of at least two sources of PAH (Pinto et al., 1998). During the summer, when mobile sources are the major contributors to BaP, the ratio of BaP 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.

Estimates of the contributions of emissions from power plants, incinerators, automobiles, and home heating to the total fine particle mass measured during the 1993 winter at the main monitoring site in Teplice were made by Pinto et al. (*5*) using chemical mass balance (CMB) receptor modeling. This analysis suggested that most of the fine particle mass was produced by home heating and power plants. Mobile sources accounted 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 30 ± 13% and home heating (and small industrial boilers) contributed 37 ± 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 ± 22% (mainly sulfate) and home heating contributed 29 ± 8% (mainly organic carbon) to the average fine particle mass.

ROLE OF EXPOSURE CHARACTERIZATION IN HEALTH STUDIES

During the 1993 Teplice winter episode, the meteorological conditions and pollution levels were similar to those in London, December 1952 (*6*) where excess mortality was reported to be more than 500 deaths per day. The average particulate matter concentrations (measured by the blackness of the filter, "smoke") and SO_2 concentrations in London were about 1600 $\mu g/m^3$ and 1800 $\mu g/m^3$ (0.7 ppm), respectively. Particulate matter (PM_{10}) and SO_2 concentrations

measured in Teplice were comparable to data for the London episode (*4,5*). The acute effect of particulate air pollution on human mortality has received new attention since studies using time-series analysis in the 90's reported increases in mortality with increasing concentrations of particulate air pollution at concentrations below the U.S.EPA ambient air quality standard for PM_{10} as reviewed by Schwartz (*7*). A recent report by Peters et al. (*8*) on the acute effects of total suspended particle (TSP) concentrations on mortality in Northern Bohemia (including Teplice district) over the 13 years from 1982-1994 found a significant increase in mortality with increasing concentrations of total suspended particulate and an even greater effect for PM_{10} (8.8% increase in mortality by $100\mu g/m^3$), during 1993 and 1994 when PM_{10} measurements were initiated as part of the U.S. EPA's collaborative support.

The initial observations of dramatic seasonal differences in the pollution levels within the Teplice district, at times, resulted in higher differences within district than between the Teplice and Prachatice districts. This finding in the early exposure characterization studies led to an increased emphasis on the longitudinal and time series design that allowed comparisons either within an individual across time or within populations in the same district across seasons as shown in Table 1. These designs also took advantage of the early results from questionnaire data that showed important socioeconomic, behavioral (e.g., smoking differences), and ethnic differences between the populations in these two districts that confounded analysis based solely on comparing Teplice and Prachatice populations (*4,9*).

In addition to characterization of the air pollution using source and ambient monitoring described above, indoor/outdoor and human exposure monitoring studies were planned to provide direct personal exposure measurements. These measurements were critical to the development, validation, and interpretation of biomarkers used in many of the health studies. As shown in Figure 1, these biomarkers range from trace metals in blood which can be directly related to metal

exposures to urinary PAH metabolites and DNA adducts reflecting internal exposure to specific contituents of the complex exposure mixture. Trace metals found in the ambient and personal monitoring were, where possible, measured in blood, hair, and urine in children to facilitate interpretation of the neurobehavioral and respiratory studies. The initial population and air pollution characterization studies in the two districts were critical to the final study designs for the reproductive studies. Personal exposure and biomarker studies were initiated early in the research and several biomarkers were included in the adult male and female reproductive effect studies.

BIOMONITORING EXPOSURE IN THE RESPIRATORY AND NEUROBEHAVIORAL EFFECTS STUDIES

The respiratory and neurobehavioral studies were conducted in school children ranging from 2nd to 8th grade and they included both cross-district and longitudinal design so that evaluations covered the high-pollution winter period and the two lower-pollution periods in Teplice and Prachatice (*9-11*). As shown in Table 1, studies of the respiratory and neurobehavioral effects in 8th grade students was initiated in the winter of 1992 with a cross-sectional respiratory study initiated the following fall. A significantly higher prevalence of adverse respiratory symptoms and decreased lung function were found in the Teplice district than in Prachatice (*9*).

Biomonitoring children's hair and urine for arsenic (As) and mercury (Hg) was suggested by the relatively high levels of trace metals in $PM_{2.5}$ air pollution derived from the brown coal combustion (Table 2), including arsenic (As), mercury (Hg), cadmium (Cd), and selenium (Se). In 1993, hair and urine samples were also obtained from the 600 2nd-grade children participating in these studies 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 0.27ppm and the average in Prachatice children was 0.89 ppm. Hg levels in urine were similarly low.

Lead (Pb) exposure has been associated with a broad spectrum of neurobehavioral effects (*12*) including the performance of East German children on tests similar to those used in these studies(*13*). Venipuncture sampling, the recommended method to determine Pb exposure, was deemed to be an unacceptable use of invasive biological sampling in this school population. Therefore, blood Pb levels were not measured in the children participating in the neurobehavioral study. Assessment of Pb exposure in children living in the study area utilized blood samples obtained from 200 children that were referred to hospitals in the Teplice and Prachatice districts for blood work not related to these studies. Blood Pb mean levels of 5.0 μg/dl (range 1.0-17.6) were found in children from Teplice and 3.8 μg/dl (range 0.9-14.0) in children from Prachatice as shown in Table 3. While these levels do not suggest that children in either district are at serious risk for lead poisoning, Winneke et al. (*13*) have shown an association of comparable blood Pb levels and performance on similar neurobehavioral tests in 6-year-old East German children.

A majority of the objective neurobehavioral performance measures reported by Otto et al.(*10*) did not differ between the districts, even though significantly higher teacher referrals for neurobehavioral clinical assessments were made in Teplice, than Prachatice. 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 the 4th-grade children (*10*).

Visual contrast sensitivity (VCS) tests have been used successfully in medical diagnosis and subclinical neurotoxicity detection. VCS deficits were seen in exposed 2nd grade children but not with the 4th grade and no consistent relationship as found between visual tests and As or Hg levels in the children's hair. Although hearing impairment was not evaluated in this study, Bencko et al. (*14*) have also reported impaired hearing in children associated with As from the combustion of coal in the nearby Slovak Republic.

PERSONAL EXPOSURE AND BIOMARKERS OF EXPOSURE, DOSE, AND SUSCEPTIBILITY

Personal exposure studies were initially conducted to compare the ambient concentrations and personal air exposures to fine particles, PAH, and organic mutagens. A personal exposure monitor designed for biomarker studies and shown in Figure 2 was used in these studies (*15*). The personal monitor was used to measure the personal air exposure of policemen, coal miners and other workers in the Teplice district(*16*). Stationary medium-volume PM_{10} samplers and high-volume $PM_{2.5}$ and TSP samplers were also used to collect particulate matter for measurement and characterization. The initial studies conducted in the winter of 1991 with a group of Teplice policemen showed that personal BaP exposures averaged 40 ng/m^3. Ambient high-volume air sampling results from 12-h nighttime samples collected in Teplice between February 17 and March 27, 1992 showed particle-associated BaP averaged 12 ng/m^3 and ranged from 2-34 ng/m^3. The sixteen PAH quantified averaged 131 ng/m^3 for the same periods. Approximately 50% of the particle-bound PAH concentrations in Teplice air included compounds that are carcinogenic in animals. The concentration and mutagenic potency of genotoxic substances adsorbed to the particles were determined by the Ames plate incorporation assay. The mutagenic potency of extractable organic matter 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(*16*).

A larger and more comprehensive personal exposure and biomarker study was initiated in 1992 after the pilot studies. The objectives of this project were to evaluate simultaneously personal exposure to air pollution and internal measures of exposure, dose, genetic effects, and susceptibility using a series of biomarkers. The relationship between ambient concentrations of air pollutants and human exposure was also evaluated using simultaneous ambient monitoring for fine particles. PAH were selected as the air pollutant marker for monitoring personal exposure because the major source of air pollution in this region was coal combustion. PAH and other genotoxic polycyclic aromatic compounds adsorbed onto fine soot particles have been estimated to be a significant 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(*17*). Biomarkers of internal exposure and dose to PAH selected for this project are illustrated in Figure 3. These biomarkers included PAH protein adducts, PAH-DNA adducts, PAH urinary metabolites, and urinary mutagenicity. Cytogenetic effects were measured using sister chromatid exchanges and chromosome aberrations. Metabolic susceptibility biomarkers, glutathione-S-transferase(GSTM1) and N-acetyltransferase(NAT2) were measured using the polymerase chain reaction (PCR) method.

Personal exposure monitoring for $PM_{2.5}$ was conducted for the 24 h period prior to collection of blood and urine. Particle extracts were analyzed for carcinogenic PAH (PAH_{car}) and also for their ability to form DNA adducts in-vitro as reported by Binkova, et al.(*18*). A group of 30 women working primarily outdoors in the Teplice district as postal workers and gardeners was compared with a group of 30 women from the Prachatice district who were gardeners and

kindergarten teachers. These 60 women were monitored during the winter season of 1992 (Biopem 1). The mean personal exposures and biomarkers for several of the parameters measured in this study are shown in Table 3. Although most of the personal exposures are higher in the Teplice group, the $PM_{2.5}$ was only three fold higher than Prachatice and the PAH_{car} were only two fold higher. The difference between Teplice and Prachatice biomarkers (e.g., PAH urinary metabolites and blood metals) were less than two-fold different between the two districts.

In 1993, a repeated measures follow-up study (Biopem 2) was started with a group of ten nonsmoking women from Teplice. These ten women had their personal exposure and biomarkers monitored over time during the fall and winter from 1992 through 1994 as shown in Figure 4. 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 (*19,20*). Although both studies showed a significant relationship between DNA adducts and exposure to particle associated carcinogenic PAH, the repeated measures study (Biopem 2) was more statistically powerful than the initial study (Biopem 1)($p<0.001$ compared to $p<0.05$) due to the use of each subject as their own control over time.

The correlation between personal exposure to fine particles, personal exposure to PAH_{car} and several of the other exposure biomarkers are shown in Table 4. Significant correlations were observed between the personal exposures to $PM_{2.5}$ or PAH_{car} and both blood lead and blood selenium concentrations. Urine samples were collected for exposure marker analysis including PAH metabolite analysis, urinary mutagenicity(*21*), and cotinine analysis. Cotinine, a metabolite of nicotine, was used 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}. DNA damage as measured by the comet assay (percentage of DNA in the comet tail) also correlated with exposures to respirable particles (r=0.304, p=0.015). 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 (initial study: r=0.54, p=0.016; follow-up study: r=0.71, p<0.001).

DNA adducts measured in the circulating blood cells appear to be a useful biomarker of internal dose. Futher analysis was conducted of DNA adduct dosimetry over a wider range of exposures from low and moderate environmental exposures to higher occupational exposures(*22*). DNA adduct levels in the WBCs were significantly correlated with environmental exposures to fine particles and carcinogenic PAH adsorbed to particles. However, at the higher occupational levels, such as those found in coke oven workers in Ostrava, CZ, the exposure-DNA adduct relationship became nonlinear(superlinear). Under these extremely high occupational exposure conditions, the relative DNA adduct level per unit of exposure (DNA-binding potency) was significantly lower than measured at environmental exposures (*22*).

Metabolic susceptibility biomarkers were determined by genotyping the DNA isolated from the WBCs. The influence of GSTM1 genotype on the biomarkers of exposure and DNA damage were evaluated by Binkova et al.(*20*) and Costa et al.(*23*). GSTM1 genotype had a significant effect on urinary PAH metabolites (p=0.037), urine mutagenicity (p=0.033) and comet assay (p=0.002) when GSTM1 genotype was considered as a single factor affecting these biomarkers. The influence of GSTM1 and NAT2 genotypes on the dose-response relationship between personal exposure to PAH_{car} and multiple biomarkers in this cohort have been reported by Costa, et al.(*23*). Stratifying the cohort by allele specific genotypes decreased the inter-individual variability and increased the correlation observed between personal exposure and

several biomarkers. This suggests that metabolic susceptibility difference between individuals in the population accounts for some of the interindividual variability in the population.

APPLICATION OF BIOMARKERS TO MALE AND FEMALE REPRODUCTIVE STUDIES

Semen Studies

From 1992 through 1997, a series of collaborative studies to evaluate male reproductive health were conducted as part of the Teplice Program. The first study was designed to evaluate reproductive history and semen quality in young men (18 year old military recruits) living in Teplice or Prachatice. Associations between air pollution levels (averaged over the three months preceding sampling in either late winter or early fall) and semen quality were found for several outcomes indicative of sperm quality (sperm motility and morphology) with no effect on sperm numbers (*24*). This study also included a new measure of sperm chromatin integrity, the sperm chromatin structure assay, that showed propotionally more sperm with abnormal chromatin associated with periods of elevated air pollution (*24*).

Subsets of men from this study were also studied for potential effects of smoking and exposure to air pollution on a molecular marker of sperm nuclear integrity. For this cytogenetic assay, sperm are labeled with chromosome-specific probes such that extra chromosomes (aneuploidy) can be detected using fluorescence in situ hybridization or FISH (*25*). Men who smoked a pack of cigarettes per day had a significantly elevated incidence of sperm with sex chromosome (YY) aneuploidy (*26*), compared with non-smokers, demonstrating the importance of considering smoking as a potential modifier in future studies. These data need to be interpreted with care, since smoking and alcohol consumption were highly correlated in these men; therefore, an effect due to alcohol could not be ruled out. In addition, non-smokers exposed to the highest levels of air pollution exhibited increased sperm aneuploidy when compared with

men from the same district exposed to low levels of air pollution (*27*). If aneuploid sperm fertilize an egg, the resulting conceptus may have serious developmental abnormalities. Thus, inclusion of molecular markers of sperm nuclear integrity (effect biomarkers) provided valuable new information about the potential risk of exposure to air pollution and male-mediated adverse pregnancy outcomes.

Based on these results, a longitudinal semen study was initiated in 1995. This study involves serial sampling of men from Teplice over a period of 2.5 years and is designed to include sampling during periods of both low and high pollution. In addition, internal biomarkers of exposure to metals and cotinine (to control for smoking) were included. This design has several advantages: each man serves as his own baseline; and, changes in semen quality can be related to the timing of spermatogenesis (since the interval between exposure and sampling will be known). Genetic biomarkers of effect are being evaluated in all samples including: sperm aneuploidy (FISH analysis) and measures of DNA damage in sperm, the sperm chromatin structure assay (SCSA), and the deoxynucleotidyl transferase-mediated nick end labeling assay (TUNEL) conducted by flow cytometry, and the single cell gel comet assay for DNA damage (electrophoresis assay). Inclusion of these biomarkers will provide a comprehensive assessment of semen quality, and allow comparisons across methods to help determine the best options in future semen studies.

Pregnancy Outcome

The pregnancy outcome study was initiated in 1994 after extensive planning and peer review. Preliminary analyses on 2,500 pregnancies collected during the first 15 months of the study showed 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%) (*4*). Similarly, the prevalence of premature births was 6.2% in Teplice and 3.4% in the Prachatice district ($P<0.01$).

The populations of the two districts, however, differed 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 (*28*). Differences were observed in their pregnancy outcomes: 13.4% of Gypsy births were premature and about 23.6% of infants weighed less than 2,500g at births. Thus, the difference observed in the two districts fell substantially after exclusion of Gypsy births; however, even after this exclusion the difference remained statistically significant.

A recent publication reports the final results of the impact of maternal exposure to $PM_{2.5}$ and PM_{10} air pollution on fetal growth in the more polluted Teplice district from April 1994 to March 1996 (*29*). This study measured fetal growth in early gestation by determining intrauterine growth retardation (IUGR) which determines birth weight for gestational age. Dejmek et al., (*29*) defined an IUGR birth as one whose weight fell below the 10^{th} percentile, by gender and gestational week, for live births in the Czech Republic from 1991-1993. Only the full term single births of European origin in Teplice district were reported. Nine month exposure windows, from conception to delivery, were examined to determine the relationship between $PM_{2.5}$ and PM_{10} concentrations and IUGR to determine which stages of prenatal development were sensitive to air pollution exposures. Only the first gestational month showed a significant impact of particulate air pollution on IUGR, suggesting that exposure to air pollution early in pregnancy may adversely affect fetal growth. Important parental characteristics such as maternal age, education, alcohol consumption, maternal active and passive smoking, and paternal smoking were evaluated. Season and year were tested for confounding in these studies. The adjusted odds ratios (OR) for risk of IUGR increased with increasing exposure to both $PM_{2.5}$ and PM_{10}, however, only exposure to PM_{10} was statistically significant. The OR and 95% confidence intervals (shown in

parentheses) for IUGR risk in the first month of pregnancy for PM_{10} concentrations between 40 to <50 $\mu g/m^3$ were 1.62 (1.07-2.50) and for high concentrations above 50 $\mu g/m^3$ the OR was 2.64 (1.48-4.71). For $PM_{2.5}$, the trend was similar. The continuous data analysis showed increases in risk of IUGR for the first month of pregnancy for each 20$\mu g/m^3$ increase of PM. The OR for PM_{10} was 1.50 (1.15-1.96); for $PM_{2.5}$ it was 1.34 (0.98-1.82; p= 0.06). These results suggest an increasing dose-response relationship between risk of IUGR and exposure to air pollution as measured by particulate matter.

DNA adducts in placentas of mothers from a subset of the women in the more polluted Teplice district were significantly higher than in the placentas from Prachatice women (*30*). Further analysis of the DNA adducts showed a highly significant relationship with IUGR, NAT2 genotype, Vitamin C level and smoking (*31*). Chromosomal aberrations were significantly higher in the Teplice mothers during pregnancy than the Prachatice mothers (*32*) but no differences were found at delivery in the blood of the mothers or the cord blood. No differences were found in the venous and cord blood DNA damage when measured as single strand breaks with the comet assay (*32*). In vitro studies of the genotoxicity and embryotoxicity of the particulate during the summers and winters of 1993 and 1994 showed that the organic fraction containing PAH is the most active in forming DNA adducts and inducing embryotoxicity (*33*).

CONCLUSION

The use of biomarkers in these studies provided a key tool to relate ambient exposures and personal exposures to health outcomes and to provide measures of confounding exposures such as tobacco smoke. Biomarkers of exposure, dose, susceptibility, and molecular effects were directly related to health outcomes. The biomarker studies also used individuals as their own control to examine the influence of environmental exposures as they changed over time and season.

Chronic and seasonal exposures to elevated air pollution in the Teplice district were shown to have serious adverse respiratory health consequences for children and reproductive effects in adults. Elevated levels of air pollutants, even for short winter inversions resulted in measurable uptake, metabolism, and excretion of genotoxic organic compounds (polycyclic aromatic hydrocarbons), increased blood concentrations of toxic metals, and resulted in DNA damage. Pregnancy outcomes were affected by a number of factors including the environmental air pollution, ethnicity, and lifestyle (e.g., smoking). Results of this study thus indicate that exposure to a complex mixture of air pollutants can affect multiple functional endpoints and produce DNA damage.

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Exposure to Complex Mixtures in Various Human Tissues and Experimental Systems, *Environmental Health Perspectives,* 99: 89-97; 1993.

FIGURE LEGENDS

Fig. 1 Biomarkers in air pollution studies, including pollutants measured in blood, urine metabolites, and DNA or protein (e.g., hemoglobin) adducts are related to ambient and personal exposures to specific constituents of the complex air pollution mixture.

Fig. 2 EPA's personal exposure monitor used in biomarker studies and described in detail by Williams et al., 1999.

Fig. 3 Polycyclic aromatic hydrocarbon (PAH) biomarkers of exposure and dose.

Fig. 4 Repeated measures study of DNA adducts, personal exposure to respirable particles(RSP), and carcinogenic PAH in ten nonsmoking women in Teplice plotted over time from 1992 to 1994.

Table 1. Summary of Studies by Season and Year

Year	Winter: High Pollution Period	Low Pollution Period[a]
1992	Exposure & Biomarker Studies Initiated Respiratory Study of 8th Grade Children Neurobehavioral Study of 8th Grade	Respiratory Cross-Sectional Study Neurobehavioral Study of 8th Grade Semen Study (18 yr old men)
1993	Exposure & Biomarker Repeated Measures Respiratory Cross-Sectional Study Semen Study	Respiratory Cross-Sectional Study Neurobehavioral Study of 2nd Grade Semen Study
1994	Pregnancy Outcome Study Initiated Semen Study	Pregnancy Outcome Study Neurobehavioral Study of 4th Grade
1995	Exposure & Biomarker Repeated Measures Pregnancy Outcome Study Semen Study (18 yr old men)	Longitudinal Respiratory Study Pregnancy Outcome Study Longitudinal Semen Study Baseline
1996	Pregnancy Outcome Study Longitudinal Semen Study	Pregnancy Outcome Study Longitudinal Semen Study
1997	Pregnancy Outcome Study Longitudinal Semen Study	Pregnancy Outcome Study Longitudinal Semen Study
1998	Pregnancy Outcome Study	Pregnancy Outcome Study

[a] Includes spring, summer and fall

Table 2. Fine Particle Composition in Teplice and in Prachatice, Winter and Summer 1993[a]

	Teplice		Prachatice	
	Winter	**Summer**	**Winter**	**Summer**
SO_2 ($\mu g/m^3$)	153 (60)	na	29 (11)	4.4(1.6)
$PM_{2.5}$($\mu g/m^3$)				
Total Mass	122 (3.0)	28.7 (1.2)	44.0 (0.8)	17.9 (0.4)
Metal Oxides	6.5 (0.5)	1.9 (0.1)	1.8 (0.1)	1.1 (0.07)
Sulfate[b]	41.9 (5.7)	10.2 (1.3)	9.5 (1.2)	6.7 (0.9)
PAH (ng/m^3)				
Sum of PAHs	278 (12)	27 (1)	163 (8)	24 (1)
Benzo[*a*]pyrene	8 (0.4)	0.5 (0.4)	4.7 (0.24)	0.14(0.01)
Trace Elements (ng/m^3)				
As	44.5 (4.5)	nd	30. (2.6)	1.9 (0.6)
Se	8.1 (1.0)	nd	2.1 (0.4)	0.5 (0.2)
Br	18.5 (2.1)	5.1 (0.9)	11. (1.1)	4.2 (0.5)
Pb	108 (11)	39.8 (4.4)	54. (4.6)	23. (2.3)

[a] Fine particles (<2.5μ) or $PM_{2.5}$ data are shown with estimated uncertainty in parentheses; na = measurements not available; nd = detected at the 3 standard deviation level in fewer than half the samples; Winter included January through March for both Teplice and Prachatice and Summer included May through August for both Districts.

[b]Sulfur expressed as ammonium sulfate.

Table 3. Comparison of Personal Exposure of Nonsmokers in Teplice and Prachatice during November and December of 1992[a]

Exposure Measurement	Teplice	Prachatice
Personal Exposure Monitor		
$PM_{2.5}$ ($\mu g/m^3$)	46 ± 25 (n=21)	16 ± 17 (n=30)
PAH_{car} (ng/m^3)	13 ± 7 (n=21)	7 ± 4 (n=30)
Benzo(a)pyrene (BaP) (ng/m^3)	2.5 ±1 (n=21)	1.1 ± 1 (n=30)
Urinary Metabolites		
(ng/mg creatinine)		
Total PAH Metabolites	231 ± 72 (n=21)	126 ± 39 (n=30)
BaP Metabolites	5 ± 6 (n=21)	3 ± 2 (n=30)
Trace Metals in Blood		
($\mu g/dl$)		
Mercury (Hg)	0.1 ± 0.05 (n=21)	0.09 ± 0.05 (n=30)
Selenium (Se)	10.0 ± 2.1 (n=21)	6.9 ± 1.2 (n=30)
Lead (Pb)		
Biomarker Study	6.9 ± 3.3 (n=21)	4.7 ± 2.0 (n=30)
Children's Study (n = 200)	5.0 (1.0-17.6)[b]	3.8 (0.9-14.0)[b]

[a] mean ± standard deviation (n=number of subjects)
[b] range

Table 4. Correlation between Personal Exposure and Biomarker Measurements

Exposure Measure	$PM_{2.5}$	PAH_{car}
Personal Exposure Monitor		
Carcinogenic PAH (PAH_{car})	0.79[a] $p < 0.0001$[b] (60)[c]	0.79[a] $p < 0.0001$[b] (60)[c]
Benzo(a)pyrene (BaP)	0.85 $p < 0.0001$ (60)	ND
Trace Metals in Blood		
Blood Pb	0.39 $p = 0.003$ (58)	0.35 $p = 0.009$ (58)
Blood Se	0.55 $p < 0.0001$ (60)	0.49 $p = 0.0002$ (58)
Urinary PAH Metabolites	0.48 $p = 0.0002$ (58)	0.40[a] $p = 0.002$[b] (60)[c]
NAT2-slow Genotype	ND	0.58[a] $p = 0.001$[b] (29)[c]
Urinary Mutagenicity (MS YG1041 -S9)[d]	ND	0.67 $p = 0.016$ (21)[e]
DNA adducts		0.16 $p = 0.32$ (51)[e]
GSTM1-positive Genotype Subjects from both Districts		0.59 $p = 0.005$ (21)
Teplice Subjects		0.54 $p = 0.01$ (21)[e]
Repeated Analysis of 10 Teplice Subjects		0.71 $p < 0.001$ (10)[e]

[a] Spearman rank correlation; [b] significance; [c] number of observations
[d] Microsuspension assay
[e] Nonsmokers only

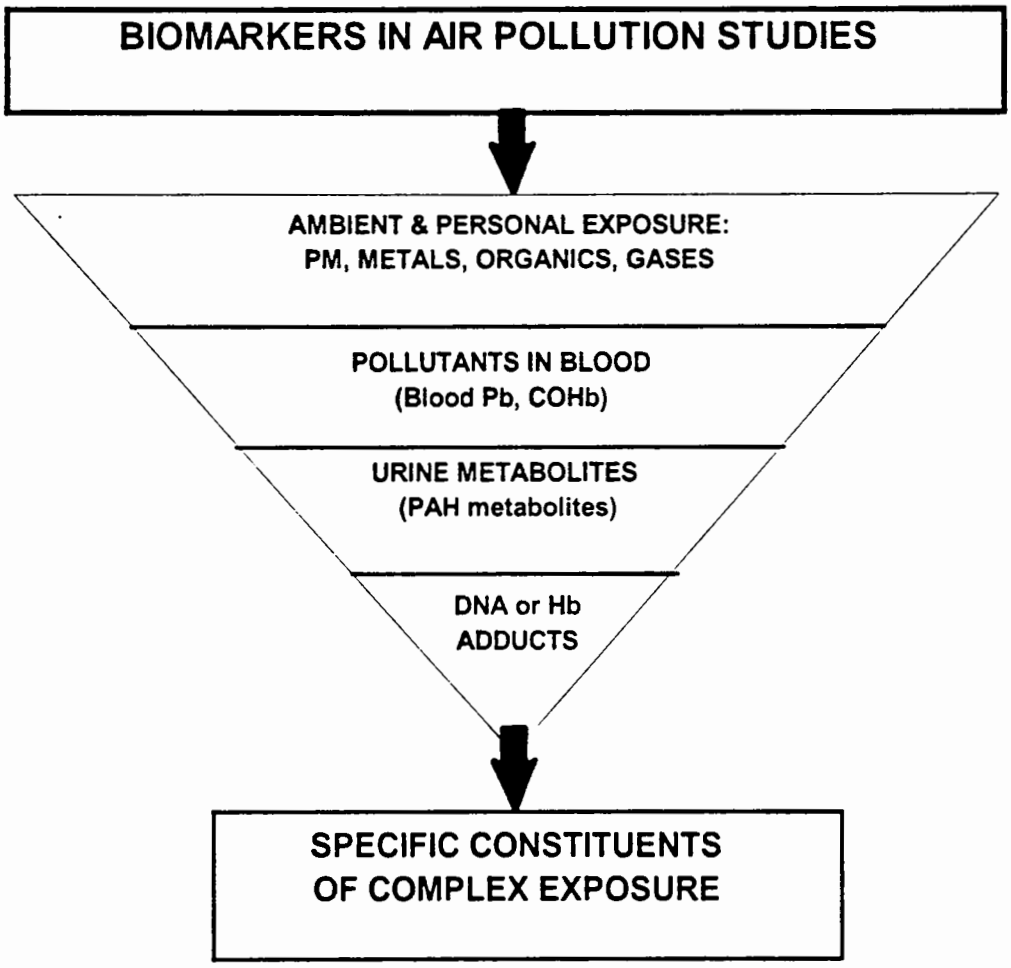
BIOMARKERS IN AIR POLLUTION STUDIES
AMBIENT & PERSONAL EXPOSURE:
PM, METALS, ORGANICS, GASES
POLLUTANTS IN BLOOD
(Blood Pb, COHb)
URINE METABOLITES
(PAH metabolites)
DNA or Hb
ADDUCTS
SPECIFIC CONSTITUENTS
OF COMPLEX EXPOSURE

Figure 1

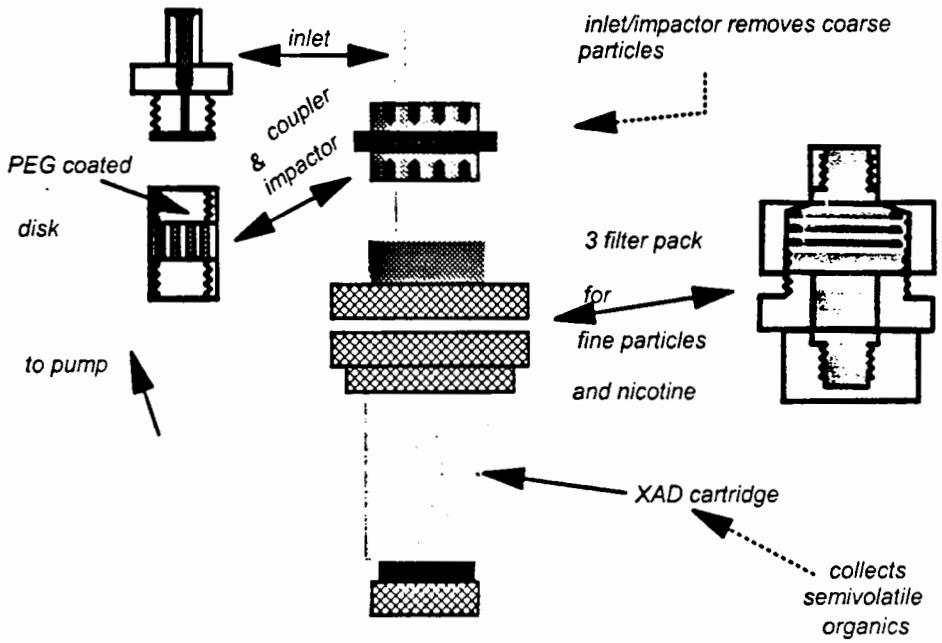
inlet
inlet/impactor removes coarse particles
coupler & impactor
PEG coated
disk
3 filter pack
for
fine particles
and nicotine
to pump
XAD cartridge
collects semivolatile organics

Figure 2.

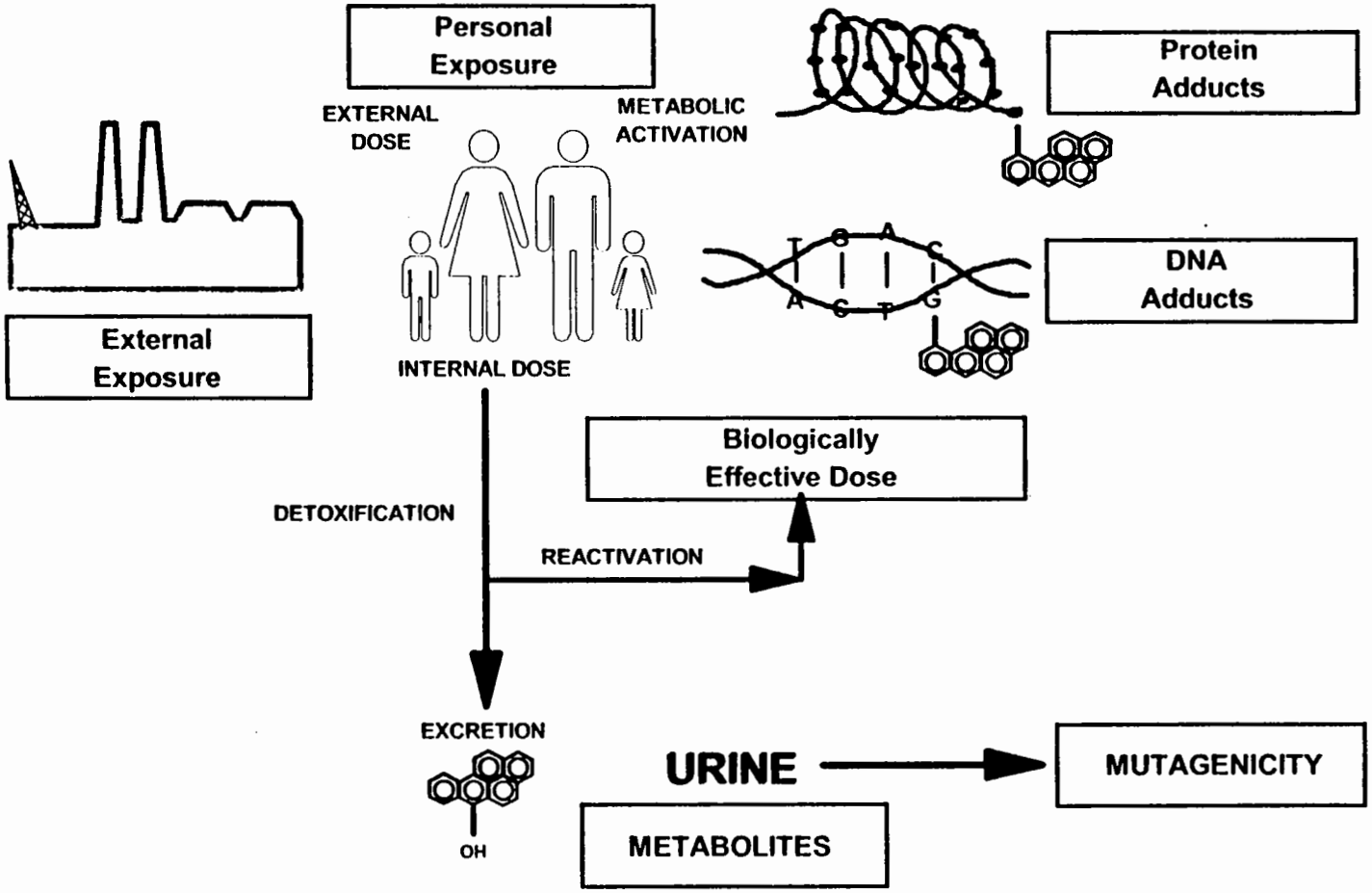
Personal Exposure
EXTERNAL DOSE
METABOLIC ACTIVATION
Protein Adducts
External Exposure
INTERNAL DOSE
DNA Adducts
Biologically Effective Dose
DETOXIFICATION
REACTIVATION
EXCRETION
OH
URINE
METABOLITES
MUTAGENICITY

Figure 3

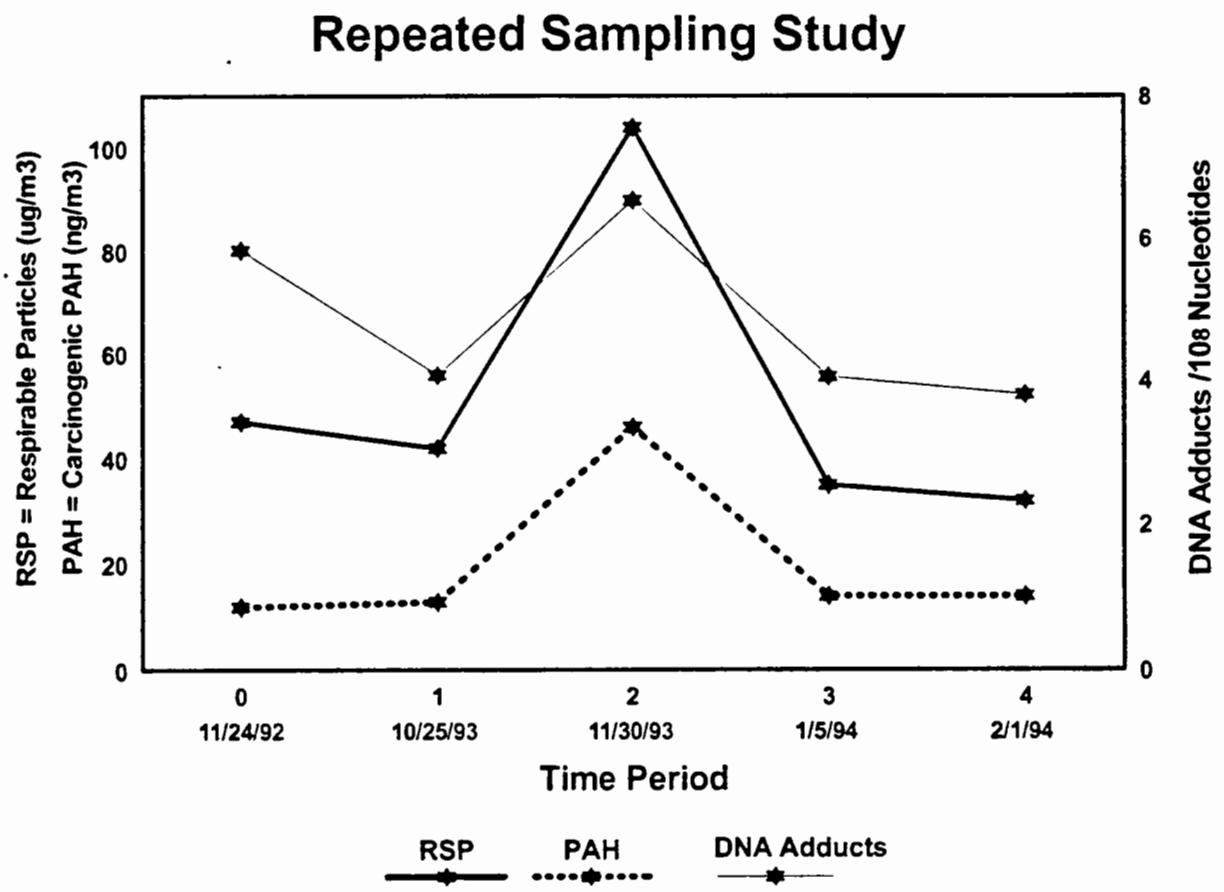
Repeated Sampling Study
RSP = Respirable Particles (ug/m3)
PAH = Carcinogenic PAH (ng/m3)
DNA Adducts /10⁸ Nucleotides
0
20
40
60
80
100
0
2
4
6
8
0
11/24/92
1
10/25/93
2
11/30/93
3
1/5/94
4
2/1/94
Time Period
RSP
PAH
DNA Adducts

Figure 4

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16. ABSTRACT

The use of biomarkers in the Teplice Program, provided a key tool to relate health outcomes to individual personal exposures and to provide measures of confounding exposures. This research program on the health effects of air pollution studied a population living in the heavily industrialized district of Teplice in Northern Bohemia and compared the exposure and health of this population to that of a non-industrialized district, Prachatice, in Southern Bohemia. The studies included characterization of the environmental and personal air pollution exposure, biomarkers, and studies on reproductive, respiratory, and neurobehavioral effects. Biomarkers were measured in blood, urine, placenta, and sperm. The biomarkers included measures of exposure (e.g., urine metabolites and blood metals), dose (e.g., DNA adducts), DNA damage, genetic and cytogenetic effects, and susceptibility. During winter temperature inversions, unusually high concentrations of a complex mixture of air pollutants were measured, including fine particles, genotoxic organic compounds, and toxic trace elements. This population, however, was also exposed to multiple pollutants via all pathways, and including pollutants resulting from environmental exposures, occupational exposures, and personal habits (e.g., tobacco and alcohol use). Longitudinal and repeated measures used individuals as their own control to examine the influence of environmental exposures as they changed over time and season. Chronic and seasonal exposures to elevated air pollution in the Teplice District were shown to have serious adverse respiratory health consequences for children and reproductive effects in adults. Elevated levels of air pollutants, even for short-term winter inversions resulted in measurable uptake, metabolism, and excretion of polycyclic aromatic hydrocarbons, increased blood concentrations of toxic metals, and resulted in DNA damage. Results of the exposure, biomarker, and health studies indicated that environmental exposure to a complex mixture of air pollutants resulted in significant elevations in personal exposure, uptake, excretion of pollutants and DNA damage.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
exposure, biomarkers, air pollution	particulate matter, polycyclic aromatic hydrocarbons, trace metals, urinary metabolites, DNA adducts, fine particles,	environmental health and exposure

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