DNA Damage from Polycyclic Aromatic Hydrocarbons Measured by Benzo[*a*]pyrene-DNA Adducts in Mothers and Newborns from Northern Manhattan, The World Trade Center Area, Poland, and China

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Abstract

Polycyclic aromatic hydrocarbons (PAH), of which benzo[*a*]-pyrene is a representative member, are combustion-related environmental pollutants and include known carcinogens. Laboratory animal studies indicate that the dose of PAHs to the fetus is on the order of a 10th that to the mother and that there is heightened susceptibility to PAH-induced carcinogenesis during the fetal and infancy periods. Carcinogen-DNA adducts, a measure of procarcinogenic genetic damage, are considered a biomarker of increased cancer risk. Here we compare the levels of benzo[*a*]pyrene-DNA adducts as a proxy for PAH-DNA damage measured in maternal blood and newborn cord blood obtained at delivery in four different populations of mothers (total of 867) and newborns (total of 822), representing a 30-fold range of exposure to ambient PAHs. The populations include residents in Northern Manhattan, participants in a study of the effects of the World Trade Center disaster, residents in Krakow, Poland, and residents in Tongliang, China. Mean adduct concentrations in both maternal and cord blood and the proportion of samples with detectable adducts, increased across the populations [Northern Manhattan < World Trade Center (WTC) < Krakow < Tongliang], consistent with the trend in estimated ambient exposure to PAHs ($P < 0.001$). For mothers, the means in the respective populations were Northern Manhattan (0.21 adducts per 10^8 nucleotides), WTC (0.23 adducts per 10^8 nucleotides), Krakow (0.28 adducts per 10^8 nucleotides), Tongliang (0.31 adducts per 10^8 nucleotides); the corresponding means in the newborns were Northern Manhattan (0.23), WTC (0.24), Krakow (0.29), Tongliang (0.31). The percentage of mothers with detectable levels of adducts in the respective populations were Northern Manhattan (36.8%), WTC (57.5%), Krakow (72.9%), Tongliang (73.4%); the corresponding percentages among the newborns were Northern Manhattan (42.4%), WTC (60.6%), Krakow (71.1%), Tongliang (79.5%). Despite the estimated 10-fold lower PAH dose to the fetus based on laboratory animal experiments, the adduct levels in the newborns were similar to or higher than in the mothers. This study suggests that the fetus may be 10-fold more susceptible to DNA damage than the mother and that *in utero* exposure to polycyclic aromatic hydrocarbons may disproportionately increase carcinogenic risk. The data support preventive policies to limit PAH exposure to pregnant women and children. (Cancer Epidemiol Biomarkers Prev 2005;14(3):709–14)

Introduction

Polycyclic aromatic hydrocarbons (PAH) are common environmental pollutants present in air, food, and drinking water from incomplete combustion of organic materials. Fossil fuel combustion by motor vehicles, residential heating units, power plants, and industrial activities are major sources of PAHs in urban ambient air (1). PAHs are also present in tobacco smoke and the diet from grilling or broiling of food, and from atmospheric deposition (2, 3).

A number of PAHs, including benzo[*a*]pyrene are known human mutagens, carcinogens, and/or developmental toxicants. Benzo[*a*]pyrene is considered a representative PAH and exerts all three types of toxicity. Some PAHs are transplacental carcinogens in experimental bioassays, producing tumors in the liver, lung, lymphatic tissues, and nervous system of the offspring (4-6). Experimental animal studies have shown greater susceptibility to PAH-induced carcinogenesis when exposure occurs during the fetal and infancy periods than during adulthood (5-9). No comparable human data are available on age-related susceptibility to PAH carcinogenesis (10). The estimated cancer risk for lifetime exposure to 0.1 ng/m^3 of airborne benzo[*a*]pyrene is one excess cancer case in 100,000 exposed individuals (1 per 10^5; ref. 1). PAHs are also developmental toxicants and neurotoxicants (11-13).

PAH-DNA adducts provide a measure of potential cancer risk because they represent a critical step in the carcinogenic pathway and have been associated with cancer risk in both experimental and epidemiologic research (14-18). Benzo[*a*]-]pyrene-DNA as a proxy for PAH-DNA damage in WBC reflect individual variation in exposure, absorption, metabolic activation, and DNA repair, thereby providing an informative individual biological dosimeter and risk marker (15-19). This report presents comparative data on levels of carcinogen-DNA adducts in maternal and newborn blood

Received 6/17/04; revised 10/19/04; accepted 11/19/04.

Grant support: National Institute of Environmental Health Sciences grants P01 009600, R01 ES08977, R01 ES111158, and R01 ES012468; U.S. Environmental Protection Agency grants R827027 and R8260901; Herbert Irving Cancer Center; Core grant 5P30 CA 13696-23; Bauman Family Foundation; Beldon Fund; Educational Foundation of America; Gladys & Roland Harriman Foundation; Hansen Foundation; Horace W. Goldsmith Foundation; New York Community Trust; New York Times Company Foundation; New York Times 9/11 Neediest Fund; September 11th Fund of the United Way and New York Community Trust; and V. Kann Rasmussen Foundation.

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samples from four cohorts of mothers and newborns. The four groups of mothers and newborns include (a) residents in Northern Manhattan (448 mothers and 302 babies); (b) participants in a study of the effects of the World Trade Center (WTC) disaster (174 mothers and 208 newborns); (c) residents in Krakow, Poland (181 mothers and 180 newborns); and (d) residents in Tongliang, China (64 mothers and 132 newborns). The small number of Chinese mothers reflects the fact that in the Chinese culture, there is a general reluctance on the part of new mothers to give blood samples of sufficient quantity for adduct analyses. The populations were selected to represent the range of environmental exposure to combustion-related air pollutants worldwide or, in the case of the WTC study, to learn about possible risks from that unprecedented event. Available air-monitoring data from the three cities indicate that the average levels of benzo[*a*]pyrene range from a low of 0.5 ng/m^3 in New York City (NYC) to >15 ng/m^3 in Tongliang. As discussed below, the WTC cohort may have higher exposure to PAHs than the Northern Manhattan cohort due to the WTC fires. Thus, the four populations represent a 30-fold range of ambient PAH exposure (Northern Manhattan < WTC < Krakow < Tongliang).

The purpose of the study was to determine whether adduct levels differ between populations, consistent with the trend in estimated ambient exposure, and to investigate possible differential susceptibility of the fetus to genetic damage, hence potential cancer risk, compared with the mother.

Materials and Methods

All subjects in the four cohort studies are self-reported nonsmokers. Those included in the present analysis had available benzo[*a*]pyrene-DNA adduct data for either mother and/or child. Assays have been done on all samples that were of adequate quantity and quality for analysis. In some cases, a blood sample could not be collected, in others the amount of DNA was inadequate for analysis. All subjects signed consent forms approved by the Columbia Institutional Review Board and the respective collaborating institutions. All subjects were interviewed prenatally using a previously described (11), modified as appropriate, questionnaire to elicit environmental and health histories, including exposure to tobacco smoke at home or work and dietary ingestion of PAH via smoked, broiled, barbecued, and grilled foods. Table 1 presents descriptive data on the demographic and exposure characteristics of the populations. Prenatal personal monitoring of PAH in air was conducted in NYC and Krakow as previously described (11).

The Northern Manhattan Cohort. As previously described, study subjects were nonsmoking Dominican and African American, singleton pregnant women residing in Washington Heights, Central Harlem, and the South Bronx who delivered at New York Presbyterian Medical Center, Harlem Hospital, or their satellite clinics (11). Eligible women were between 18 and 35 years old, had not smoked >1 cigarette/d at any time during pregnancy, and reported no diabetes, hypertension, HIV infection or AIDS, or use of illegal drugs in the last year. Participants were enrolled between 1998 and 2003. Adduct measurements were available from 448 mothers and 302 newborns. The subset in the present analysis did not differ from those subjects without adduct measurements with respect to the demographic and exposure characteristics shown in Table 1.

Air pollution exposures in NYC are representative of those found in many cities in the United States, Western Europe, and other areas of the fully industrialized world. Personal air monitoring of pregnant women in the Northern Manhattan cohort found a mean PAH level of 3.7 + 3.6 ng/m^3, with an average benzo[*a*]pyrene concentration of 0.5 ng/m^3 (11). Current data are not available on annual average ambient concentrations of PAHs or benzo[*a*]pyrene in NYC; however, outdoor 24-hour average benzo[*a*]pyrene concentrations measured in U.S. urban areas have generally been in the range of 0.5.

The WTC Cohort. Singleton pregnant women were enrolled at delivery at three collaborating downtown hospitals located near the WTC: Beth Israel, St. Vincent's, and New York University Downtown. Eligible women were between 18 and 39 years old, had not smoked during pregnancy (>1 cigarette/d at any time), and reported no diabetes, hypertension, HIV infection or AIDS, or use of illegal drugs in the last year. All participants were enrolled between December 13, 2001 and June 26, 2002 (20). The women were Black, White, Hispanic, and Asian. Adduct measurements were available from 174 mothers and 208 newborns. The subset in the present analysis did not differ from those subjects without adduct measurements with respect to the demographic and exposure characteristics shown in Table 1, except that the level of maternal education was higher in the subset with adducts than in those without adduct measurements ($P < 0.05$, χ^2 test).

Monitoring data are not available to estimate population exposure to PAHs following 9-11-01. However, PAHs were released as combustion products from the WTC on 9-11-01 and over a period of several months afterwards (21-26). Thus, the WTC population was potentially exposed to higher concentrations of PAHs than the Northern Manhattan cohort over a portion of the pregnancy.

Table 1. Demographic and exposure characteristics of the four groups

	Northern Manhattan (n = 468)	WTC (n = 268)	Krakow (n = 191)	Tongliang (n = 136)
Maternal age (y)	24.8 (4.9)[*,†]	30.3 (5.2)	27.7 (3.8)	25.1 (3.3)
Maternal education (%)				
High school	76.2[‡]			
>High school	23.8	32.5 67.5	40.3 59.7	75.2 24.8
Maternal ETS (% reporting smoker in the home)	37.5[§,‖]	17.0	27.2[‡]	58.1
Maternal alcohol consumption (% drank alcohol during pregnancy)	24.7[§]	8.1	62.3	41.6
PAHs in personal air (ng/m^3)	3.6 (3.8)[§,¶]	NA	52.0 (53.3)	NA

NOTE: The dietary PAH intake group variable differed somewhat due to differences in foods consumed between populations. For each population the variable for dietary PAHs was dichotomized at the median.

*Mean (SD).

† By Bonferroni multiple test, all group pairs are significantly different ($P < 0.001$) except for Northern Manhattan, and Tongliang.

‡ By χ^2 test, all pairs are significantly different ($P < 0.01$) except for Northern Manhattan, Tongliang and Northern Manhattan, Krakow.

§Measurements of personal air PAHs were not available.

‖By χ^2 test, all pairs are significantly different ($P < 0.01$).

¶$P < 0.01$ for Northern Manhattan and Krakow.

The Polish Cohort. Singleton pregnant women who registered at prenatal healthcare clinics in the Srodmiescie/Old Podgorze and the Krowodrza/Nowa Huta/New Podgorze areas were invited to participate in the study (27). The areas of Krakow represent the least and most polluted areas of the city (27). The women were all Caucasian, reflecting the homogeneity of the Krakow population. Subjects were enrolled between 2000 and 2003. The eligibility criteria were essentially the same as in the Northern Manhattan and WTC cohorts listed above. Adduct measurements were available from 181 mothers and 180 newborns. The present subset differed from those subjects without adduct measurements in having higher maternal education and ETS exposure ($P < 0.05$) but were similar in age and alcohol use.

Krakow is representative in terms of air quality of many areas of the developing world. In Krakow in 2002, the monitored average ambient air concentration of benzo[*a*]pyrene was 5.0 ng/m^3 in the less polluted area and 10 ng/m^3 in the most polluted area (Office of Environmental Protection, Krakow). These concentrations are comparable with those reported in the city of Teplice in the Czech Republic (28). Prenatal personal monitoring of air levels of PAHs in the Krakow cohort gave a mean PAH concentration of 39.08 ng/m^3, which was lower than reported by a personal air monitoring study in the high exposure area of Bangkok (74.25 ng/m^3; ref. 29).

The China Cohort. Tongliang is located in Tongliang County in Southwest China. The Tongliang coal-burning power plant is located in the center of the city and is the principal source of local air pollution. Nonsmoking, singleton, pregnant Chinese women residing within a 2-km radius of the power plant and ≥20 years of age were enrolled before delivery between March 1 and June 30, 2002. Adducts were available on 64 mothers and 132 newborns. This subset did not differ from the subjects without adducts with respect to the variables in Table 1.

The estimated mean ambient benzo[*a*]pyrene concentration in Tongliang is 15 ng/m^3.[3] This concentration is at the upper bound of the values monitored in Krakow air. Thus, the four populations span an estimated 30-fold gradient of PAH exposure.

Blood Collection and Adduct Analysis. The method used in this study is an improved version of that previously reported (30, 31). Briefly, a total amount of 100 μg DNA was used for each analysis. Many precautions were taken to avoid the presence of fluorescent contaminants: the absence of any fluorescent material in the purified HCl was checked by high-performance liquid chromatography (HPLC); tubes, HPLC syringes, and other equipment were washed many times with HPLC-grade methanol; and a blank injection was done before each sample was subjected to HPLC analysis. DNA samples were dissolved in 0.1 N HCl and acid hydrolysis carried out at 90°C for 6 hours. The resulting solution was analyzed in a Shimadzu HPLC system with RF-10Axl spectrofluorometric detector. Shimadzu SIL-10A automatic sample injector was used to minimize the batch effect. The tetrol concentrations were calculated by comparing the areas of samples to be analyzed with an external calibration curve, generated from the fluorescence peak of an authentic BPDE tetrol standard, every time a set of samples was analyzed. Calibration was carried out with DNA from calf thymus, alone (background) and added to 2, 4, and 8 pg anti-BPDE tetrol. These standard solutions were then treated in the same way as the tested samples (hydrolyzed in 0.1 N HCl at 90°C for 6 hours). The minimum correlation coefficient was 0.98 and the mean coefficient of variation for analyses repeated on different days was 12%. The detection threshold of anti-BPDE tetrols [r-7,c-10,t-8,t-9-tetrahydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (B[*a*]P tetrol I-1) and r-7,t-9,t-10,t-8-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (B[*a*]P tetrol I-2)] was 0.25 pg (signal-to-noise ratio >3) so that, in the present study, with 100 μg DNA, this assay can measure 0.25 adducts/10^{-8} nucleotides.

Results

The demographic and exposure characteristics of the subjects included in the present analysis are provided in Table 1. Tables 2 and 3 provide the means, SDs, range, and percent of samples with detectable adducts for each of the four populations (cord and maternal blood samples, respectively).

Among unpaired samples, the mean adduct levels in cord blood increased across the four populations: Northern Manhattan < WTC < Poland < Tongliang (*t* test for trend $P < 0.01$). By regression analysis, the trend was also significant ($P < 0.001$). Among the newborns, by the Wilcoxon rank sum test, the Northern Manhattan population did not differ significantly from the WTC population, but both the Northern Manhattan and WTC newborns had significantly lower adduct values than either the Polish or Chinese newborns ($P < 0.003$). Among the mothers, there also was a significant trend across the four populations: Northern Manhattan < WTC < Poland < Tongliang (*t* test for trend and regression analysis both, $P < 0.001$). The Northern Manhattan mothers had significantly lower levels of adducts than the WTC mothers. The adduct concentrations in the Northern Manhattan and WTC mothers were significantly lower than in Polish and Chinese mothers ($P < 0.001$). However, the maternal adduct means did not differ significantly between Krakow and Tongliang probably due to small numbers of samples in the Tongliang group (see Table 3). The percent of samples with detectable adducts increased from Northern Manhattan < WTC < Krakow < Tongliang in both mothers and newborns (χ^2 test in proportion, $P < 0.001$). Detectable adduct levels were found in 37% of mothers and 42% of newborns in NYC and 73% and 80% of Tongliang mothers and newborns, respectively. These differences were significant ($P < 0.001$, χ^2 test for both mothers and newborns).

Among the paired samples, the results were similar to those for unpaired samples, except that maternal adduct means did not differ significantly between Northern Manhattan and WTC (Table 4).

In all four populations, the levels of adducts in the newborns were either similar to or higher than those in the paired mothers, despite the estimated 10-fold lower dose to the fetus, based on laboratory animal experiments. In Northern Manhattan, adducts were significantly higher in the newborns compared with their mothers ($P = 0.03$, by paired Wilcoxon test); but the other three mother/newborn comparisons were not significant.

Table 2. PAH-DNA adducts in maternal blood: all subjects

	Northern Manhattan	WTC	Krakow	Tongliang
n	448	174	181	64
Mean (adducts/10^8 nucleotides)	0.21*	0.23	0.28	0.31
Median	0.125	0.26	0.29	0.31
SD	0.13	0.10	0.11	0.17
Detectable rate (%)	36.8*	57.5	72.9	73.4

*Trends for means and detectable proportions across populations: both $P < 0.001$.

[3] J. Chow, Report on air monitoring in Tongliang, a collaborative study with Columbia University, Desert Research Institute, in preparation.

Table 3. PAH-DNA adducts in umbilical cord blood: all subjects

	Northern Manhattan	WTC	Krakow	Tongliang
n	302	208	180	132
Mean	0.23*	0.24	0.29	0.33
Median	0.125	0.27	0.29	0.32
Std deviation	0.14	0.10	0.16	0.14
Detectable rate (%)	42.4*	60.6	71.1	79.5

*Trend for means and detectable proportions across populations: both $P < 0.001$.

By Spearman's rank test, among both mothers and newborns in all four populations, the correlations between adducts and ETS (presence/absence of smoker(s) in the home) or dietary PAH (high/low frequency of consumption of fried, broiled or barbecued food) were nonsignificant. Levels of PAHs in prenatal personal air sample (available for Northern Manhattan and Polish cohorts only) were not significantly correlated with maternal or cord adducts.

Discussion

The ~30-fold gradient of PAH concentrations represented by the four studies provides an opportunity to analyze the dose-response relationship between prenatal PAH exposure and genetic damage in the fetus measured by benzo[*a*]pyrene-DNA adducts over the range of PAH exposure currently experienced worldwide. The analysis is possible because of the availability of a large number of measurements in nonsmoking mothers and newborns (822 in cord blood, 867 in maternal blood including 606 mother/newborn pairs) and the comparability of data obtained by the same laboratory method from subjects who met similar eligibility criteria and provided similar questionnaire data.

There are two major findings from this work. The first is that, in the exposure range studied, there is no apparent threshold for adduct formation and that adducts generally increase across the gradient of exposure. In both maternal and newborn samples, there was a significant increasing trend in levels of adducts across the four populations from Northern Manhattan to the WTC study to Krakow to Tongliang. Thus, even low levels of prenatal exposure to PAHs may increase the child's risk of cancer in a dose-related fashion. The observed continuum of adduct formation over a wide range of PAH exposure (0.5-15 ng/m^3 benzo[*a*]pyrene) is of concern in light of the associations seen previously in molecular epidemiologic research between PAH-DNA adduct levels and cancer risk (14-17, 32).

The second finding is that the level of PAH-induced genetic damage measured by PAH-DNA adducts in the fetus is consistently higher on an estimated unit of exposure basis than in the mother. This finding holds true for matched pairs across the four populations as well. Although there are no data in humans on maternal versus fetal dose of PAHs, experimental studies in laboratory animals using radiolabeled PAH indicate that the dose to the fetus is generally an order of magnitude lower than the dose to paired maternal tissues (33-35). In a number of rodent bioassays, fetal levels of PAH-DNA adducts have also been higher than expected given the lower estimated transplacental dose of PAH (36-38). Previous studies by ourselves and others, involving smaller numbers of subjects and using different methods to analyze adducts, have also reported either comparable or higher levels of PAH-DNA adducts in the fetus compared with the mother (39, 40). We recently reported this result in the Northern Manhattan and Krakow cohorts (41), and here we extend the finding to the WTC and Tongliang populations. Although there may be interspecies differences in metabolism of PAHs, in light of the available experimental data on transplacental dose and adduct formation, our findings suggest that the amount of DNA damage per unit dose of PAH may be on the order of 10-fold higher in the fetus relative to the mother.

Increased susceptibility to DNA damage may contribute to the greater carcinogenic effect of PAHs when given to experimental animals prenatally or neonatally compared with later in life (5-9). Experimental and human evidence indicates that the developing fetus and neonate have heightened susceptibility to a number of chemical carcinogens, including PAHs, nitrosamines, pesticides, tobacco smoke, air pollution, and radiation, compared with the adult (reviewed in refs. 10, 39, 42). Compared with exposures occurring in adult life, exposures *in utero* and in the early years can disproportionately increase the risks of many types of cancer later in life (19, 43, 44). The mechanisms underlying fetal susceptibility to genetic damage and carcinogenesis could include greater absorption or retention of toxicants, reduced detoxification and DNA repair, and the higher rate of cell proliferation during early stages of development. With respect to carcinogenesis, other factors include lower immunologic competence in the fetus and the fact that cancers initiated in the womb and in the early years have the opportunity to develop over many decades (10, 42).

Our observation that within all four populations the maternal and fetal adduct levels are not correlated with each other is consistent with our prior study in Poland using ELISA (39). The absence of a correlation is possibly due to the effect of enzyme activity of cytochrome *P*4501A1 and glutathione *S*-transferases in placental tissue which influence the formation of adducts in WBC (45).

The consistent lack of a significant correlation between adducts and individual measures of exposure including PAHs in personal air (available in Northern Manhattan and Krakow only), ETS, and dietary PAH consumption probably reflects individual variation in adduct formation due to coexposures, nutritional, and genetic factors. Individual variation in biological response may explain why, although group level differences are generally found, few studies have found a direct correlation between PAH-DNA adducts and estimated PAH exposure concentrations at the individual level.

The trends for adduct means and rates of detectable adducts are both significant across the populations over an estimated 30-fold range of ambient PAH exposure. However, whereas the exposure increased by 30-fold from lowest to highest exposure (Northern Manhattan to Tongliang), the adduct means increased by a much smaller percentage: 48% in mothers and 43% in newborns from Northern Manhattan to Tongliang. The proportion of subjects with detectable adducts increased by 99% in mothers and 88% in newborns across the same two populations. This is consistent with a possible plateau effect due to saturation of activating enzyme systems and/or to cell death triggered by higher levels of adducts.

Table 4. Pairwise comparison between maternal and cord blood PAH-DNA in four populations: all subjects

	Maternal adducts*	Cord adducts*
Northern Manhattan		
WTC	<0.001	0.22
Krakow	<0.001	<0.001
Tongliang	<0.001	<0.001
WTC		
Krakow	<0.001	0.001
Tongliang	<0.001	<0.001
Krakow		
Tongliang	0.24	0.001

NOTE: The median and the mean levels are provided in Tables 2 and 3.
*P of Wilcoxon rank sum test.

The means seen here in association with air pollution exposure are lower than those seen in active smokers by the same HPLC fluorescence method. We previously found a benzo[*a*]pyrene-DNA adduct mean of 0.80 ± 0.06 per 10^8 nucleotides in 284 active cigarette smokers who averaged ~1 pack per day (46) which is consistent with the higher inhaled dose of benzo[*a*]pyrene from smoking. The mean levels of adducts reported here are also lower than that we have seen in a prior study assessing fetal susceptibility to DNA damage (39). First, the HPLC/fluorescence method used in the present study is specific to one member of the class of PAHs (benzo[*a*]pyrene), whereas the assays which were used in the prior study in Poland measure a far broader range of compounds. The ELISA detects benzo[a]pyrene and a number of structurally related PAHs; the ^{32}P-postlabeling assay detects multiple PAHs as well as other aromatic compounds. Second, when the prior study was conducted in Poland, uncontrolled coal burning was a far greater source of pollution and the ambient levels of air pollution (represented by benzo[*a*]pyrene), were 2- to 5-fold higher than at the present time.

A limitation of the analysis is that the four populations are of different ethnicity and we are not able to explore possible genetic/ethnic contributions to the observed differences between them. There are limited data on the effect of race/ethnicity on molecular pathways in adduct formation and human cancer, but genetic polymorphisms involved in metabolism and DNA repair have been shown to influence risk of both outcomes (reviewed in refs. 47, 48).

Adding to the significance of the results, PAH/aromatic DNA adducts have been associated with somatic mutation in newborns (49) and increased cancer risk in a number of molecular epidemiologic studies (15-18, 32, 49). It has also been shown experimentally that benzo[*a*]pyrene induces a pattern of mutations in the *p53* tumor suppressor gene (mutated in an estimated 40-50% of lung and other epithelial cancers) that is consistent with the types of benzo[*a*]pyrene-DNA adducts formed (50, 51). There is not a one-to-one relationship between adducts and risk because additional molecular events that can be determined by both inherited and acquired factors are required for tumorigenesis. In addition, the carcinogenicity of PAHs is not only due to their ability to form adducts with DNA, thereby inducing mutations and cancer, but also to their ability to interfere with transcription, DNA replication and protein synthesis, and to bind to the cytosolic aryl hydrocarbon, with subsequent up-regulation of genes involved in growth and differentiation (see ref. 1, for review). Whereas it is not possible to estimate individual cancer risk based on adduct measurements, inferences can be made at the group level.

This study adds to a growing body of evidence that there are substantial benefits in terms of health and economic savings to reducing combustion-related ambient pollution (52, 53). For example, in the United States, the benefits of reductions in air pollution predicted to occur by 2010 as a result of the Clean Air Act regulations are in the range of US $110 billion (53).

In conclusion, the present findings highlight the need for international pollution prevention programs to protect women of childbearing age and their children from PAHs. Individuals are generally exposed throughout most of their life to low levels of carcinogens. Given the present evidence of fetal susceptibility to procarcinogenic damage, if they also experience prenatal exposure to carcinogens, the possibility that they will develop cancer over their lifetime may be disproportionately increased (4). Moreover, there is a higher probability that low level-long latency effects will be manifested as cancer during the individual's life span if these effects are initiated *in utero* rather than later in life. Therefore, the results presented here have implications for risk assessment and environmental health policy and highlight the need to protect pregnant women and especially their children as a sensitive subset of the population.

Acknowledgments

We thank the assistance of Lirong Qu and Jingzi Zhou for DNA adduct analysis; Susan Illman for program coordination; Drs. Wei Yann Tsai and Howard Andrews, Yi Hsuan Tu, and Lori Heopner for statistical support; Drs. Jeffrey King, Janet L. Stein, and Giuseppe DelPriore for clinical support; the Children's Environmental Health Center research staff: Andria Reyes, Diurkia Diaz, Mejico Borjas, Yesenia Cosme, Linda Ali Cruz, and Darrell Holmes; the WTC research staff: Kristin Lester and Lisa Weiss; the Polish research staff: Ivona Bendkowska, Hyunok Choi, and Agnieszka Penar; and the China project research team including Drs. Ting Yu Li and Judy Chow.

References

1. Bostrom CE, Gerde P, Hanberg A, et al. Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environ Health Perspect 2002;110:451–88.
2. IARC. Polynuclear aromatic compounds. Part l. Chemical, environmental, and experimental data. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Lyon (France): IARC; 1983; 32:l–453.
3. U.S. Environmental Protection Agency. Aerometric information retrieval system (AIRS), data for 1985-1990. Research Triangle Park (NC): U.S. Environmental Protection Agency; 1990.
4. Bulay OM, Wittenberg LW. Carcinogenic effects of polycyclic hydrocarbon carcinogens administrated to mice during pregnancy on the progeny. J Natl Cancer Inst 1971;46:397–402.
5. Rice JM, Ward JM. Age dependence of susceptibility to carcinogenesis in the nervous system. Ann N Y Acad Sci 1982;381:274–89.
6. Vesselinovitch SC, Kandala DR, Mihailovich N. Neoplastic response of mouse tissues during perinatal age periods and its significance in chemical carcinogenesis. J Natl Cancer Inst Monogr 1975;51:230–50.
7. Soyka LF. Hepatic drug metabolizing enzyme activity and tumorigenesis in mice following perinatal exposure to benzo(*a*)pyrene. Pediatr Pharmacol 1980;1:85–96.
8. Toth B, Rappaport H, Shubik P. Influence of dose and age on the induction of malignant lymphomas and other tumors by 7,12-dimethylbenx[*a*] anthracene in Swiss mice. J Natl Cancer Inst 1963;30:723–41.
9. Walters MA. The induction of lung tumours by the injection of 9,10-dimethyl-1,2-benzanthracene (DMBA) into newborn suckling and young adult mice. A dose response study. Br J Cancer 1966;20:148–60.
10. Anderson LM, Diwan BA, Fear NT, Roman E. Critical windows of exposure for children's health: cancer in human epidemiological studies and neoplasms in experimental animal models. Environ Health Perspect 2000;108:573–94.
11. Perera F, Rauh V, Tsai WY, et al. Effects of transplacental exposure to environmental pollutants on birth outcomes in a multi-ethnic population. Environ Health Perspect 2003;111:201–5.
12. Wu J, Ramesh A, Nayyar T, Hood DB. Assessment of metabolites and AhR and CYP1A1 mRNA expression subsequent to prenatal exposure to inhaled benzo(*a*)pyrene. Int J Dev Neurosci 2003;21:333–46.
13. Dejmek J, Solansky I, Benes I, Lenicek J, Sram RJ. The impact of polycyclic aromatic hydrocarbons and fine particles on pregnancy outcome. Environ Health Perspect 2000;108:1159–64.
14. Perera FP, Weinstein IB. Molecular epidemiology and carcinogen-DNA adduct detection: new approaches to studies of human cancer causation. J Chron Dis 1982;35:58l–600.
15. Bartsch H, Hietanen E. The role of individual susceptibility in cancer burden related to environmental exposure. Environ Health Perspect 1996;104:569–77.
16. Poirier MC, Beland FA. DNA adduct measurements and tumor incidence during chronic carcinogen exposure in animal models: implications for DNA adduct-based human cancer risk assessment. Chem Res Toxicol 1992;5:749–55.
17. Stowers SJ, Anderson MW. Formation and persistence of benzo[*a*]pyrene metabolite-DNA adducts. Environ Health Perspect 1985;62:31–9.
18. Veglia F, Matullo G, Vineis P. Bulky DNA adducts and risk of cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2003;12:157–60.
19. Perera FP. Molecular epidemiology: on the path to prevention? J Natl Cancer Inst 2000;92:602–12.
20. Lederman SA, Rauh V, Weiss L, et al. The effects of the World Trade Center event on birth outcomes among term deliveries at three lower Manhattan hospitals. Environ Health Perspect 2004;doi:10.1289/ehp.7348. [Online 2004 Sept 8].
21. Chen LC, Thurston G. Word Trade Center cough. Lancet 2002;360:S37–8.
22. Jeffrey NL, D'Andrea C, Leighton J, et al. Potential exposures to airborne and settled surface dust in residential areas of lower Manhattan following

the collapse of the World Trade Center-New York City, November 4-December 11, 2001. JAMA 2003;289:1498–500.
23. Lioy PJ, Weisel CP, Millette JR, et al. Characterization of the dust/smoke aerosol that settled east of the World Trade Center (WTC) in lower Manhattan after the collapse of the WTC 11 September 2001. Environ Health Perspect 2002;110:703–14.
24. McKinney K, Benson S, Lempert A, Singal M, Wallingford K, Snyder E. Occupational exposures to air contaminants at the World Trade Center Disaster site- New York, September-October, 2001. MMWR Morb Mortal Wkly Rep 2002;51:453–6.
25. Offenburg JH, Eisenreich SJ, Chen LC, et al. Persistent organic pollutants in the dusts that settled across lower Manhattan after September 11, 2001. Environ Sci Technol 2003;37:502–8.
26. Service RF. Chemical studies of 9/11 disaster tell complex tale of ''bad stuff''. Science 2003;301:1649.
27. Jedrychowski W, Whyatt RM, Camann DE, et al. Effect of prenatal PAH exposure on birth outcomes and neurocognitive development in a cohort of newborns in Poland. Study design and preliminary ambient data. Int J Occup Med Environ Health 2003;16:21–9.
28. Binkova B, Lewtas J, Miskova I, Lenicek J, Sram R. DNA adducts and personal air monitoring of carcinogenic polycyclic aromatic hydrocarbons in an environmentally exposed population. Carcinogenesis 1995;16:1037–46.
29. Ruchirawa M, Mahidol C, Tangjarukij C, et al. Exposure to genotoxins present in ambient air in Bangkok, Thailand: particle associated polycyclic aromatic hydrocarbons and biomarkers. Sci Total Environ 2002;287:121–32.
30. Alexandrov K, Rojas M, Geneste O, et al. An improved fluorometric assay for dosimetry of benzo(*a*)pyrene diol-epoxide-DNA adducts in smokers' lung: comparisons with total bulky adducts and aryl hydrocarbon hydroxylase activity. Cancer Res 1992;52:6248–53.
31. Rojas M, Alexandrov K, van Schooten FJ, Hillebrand M, Kriek E, Bartsch H. Validation of a new fluorometric assay for benzo[*a*]pyrene diolepoxide-DNA adducts in human white blood cells: comparisons with 32P-postlabeling and ELISA. Carcinogenesis 1994;15:557–60.
32. Tang D, Phillips DH, Stampfer M, et al. Association between carcinogen-DNA adducts in white blood cells and lung cancer risk in the physicians health study. Cancer Res 2001;61:6708–12.
33. Neubert D, Tapken S. Transfer of benzo(*a*)pyrene into mouse embryos and fetuses. Arch Toxicol 1988;62:236–9.
34. Withey JR, Shedden J, Law FC, Abedini S. Distribution of benzo[*a*]pyrene in pregnant rats following inhalation exposure and a comparison with similar data obtained with pyrene. J Appl Toxicol 1993;13:193–202.
35. Srivastava VK, Chauhan SS, Srivastava PK, Kumar V, Misra UK. Fetal translocation and metabolism of PAH obtained from coal fly ash given intratracheally to pregnant rats. J Toxicol Environ Health 1986;18:459–69.
36. Lu LJ, Wang MY. Modulation of benzo(*a*)pyrene-induced covalent DNA modification in adult and fetal mouse tissues by gestation stage. Carcinogenesis 1990;11:1367–72.
37. Lu LJ, Disher RM, Reddy MV, Randerath K. 32P-postlabeling assay in mice of transplacental DNA damage induced by the environmental carcinogens safrole, 4-aminobiphenyl and benzo(*a*)pyrene. Cancer Res 1986;46:3046–54.
38. Wang MY, Lu LJ. Differential effect of gestation stage on benzo(*a*)pyrene-induced micronucleus formation and/or covalent DNA modifications in mice. Cancer Res 1990;50:2146–51.
39. Whyatt RM, Jedrychowski W, Hemminki K, et al. Biomarkers of polycyclic aromatic hydrocarbon-DNA damage and cigarette smoke exposures in paired maternal and newborn blood samples as a measure of differential susceptibility. Cancer Epidemiol Biomarkers Prev 2001;10:581–8.
40. Mumford JL, Lee X, Lewtas J, Young TL, Santella RM. DNA adducts as biomarkers for assessing exposure to polycyclic aromatic hydrocarbons in tissues from Xuan Wei women with high exposure to coal combustion emissions and high lung cancer mortality. Environ Health Perspect 1993;99:83–7.
41. Perera FP, Rauh V, Whyatt RM, et al. Molecular evidence of an interaction between prenatal environmental exposures on birth outcomes in a multi-ethnic population. Environ Health Perspect 2004;112:662–30.
42. National Academy of Sciences. Pesticides in the diets of infants and children. Washington (DC): National Academy Press; 1993.
43. Goldman LR. Children: unique and vulnerable. Environmental risks facing children and recommendations for response. Environ Health Perspect 1995;103:13–8.
44. National Academy of Sciences. Science and judgment of risk assessment. Washington (DC): National Academy Press; 1994.
45. Whyatt RM, Perera FP, Jedrychowski W, Santella RM, Garte S, Bell DA. Association between polycyclic aromatic hydrocarbon-DNA adduct levels in maternal and newborn white blood cells and *glutatione S-transferase P1* and *CYP1A1* polymorphisms. Cancer Epidemiol Biomarkers Prev 2000;9:207–12.
46. Mooney LA, Madsen AN, Tang D, et al. Antioxidant vitamin supplementation reduces benzo[*a*]pyrene-DNA adducts and potential cancer risk in female smokers. Cancer Epidemiol Biomarkers Prev 2005;14:237–42.
47. Nebert DW. Drug-metabolizing enzymes, polymorphisms and interindividual response to environmental toxicants. Clin Chem Lab Med 2000;38:857–61.
48. Wiencke JK. Impact of race/ethnicity on molecular pathways in human cancer. Nat Rev Cancer 2004;4:79–84.
49. Perera F, Hemminki K, Jedrychowski W, et al. *In utero* DNA damage from environmental pollution is associated with somatic gene mutation in newborns. Cancer Epidemiol Biomarkers Prev 2002;11:1134–7.
50. Denissenko M, Pao A, Tang MS, Pfiefer G. Preferential formation of benzo[*a*]pyrene adducts at lung cancer mutational hotspots in *p53*. Science 1996;274:430–2.
51. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 1994;55:4855–78.
52. Hutchinson EJ, Pearson PJ. An evaluation of the environmental and health effects of vehicle exhaust catalysts in the UK. Environ Health Perspect 2004;112:132–41.
53. Wong EY, Gohlke J, Griffith WC, Farrow S, Faustman EM. Assessing the health benefits of air pollution reduction for children. Environ Health Perspect 2004;112:226–32.

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Cancer Epidemiol Biomarkers Prev 2005;14:709-714.