

Maternal Occupational Exposure to Polycyclic Aromatic Hydrocarbons and Risk of Neural Tube Defect-Affected Pregnancies

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BACKGROUND: This study evaluated whether there is an association between maternal occupational exposure to polycyclic aromatic hydrocarbons (PAHs) and neural tube defects (NTDs) in offspring. This is the first such study of which the authors are aware. **METHODS:** Data were analyzed from 1997 to 2002 deliveries in the National Birth Defects Prevention Study, a large population-based case-control study in the United States. Maternal interviews yielded information on jobs held in the month before through 3 months after conception. Three industrial hygienists blinded to case or control status assessed occupational exposure to PAHs. Crude and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were estimated using unconditional logistic regression. **RESULTS:** Of the 520 mothers of children with NTDs, 5.0% were classified as exposed to occupational PAHs, as were 3.5% of the 2989 mothers of controls. The crude OR for PAH exposure was 1.43 (95% CI, 0.92–2.22) for any NTD and 1.71 (95% CI, 1.03–2.83) for spina bifida. Adjusted ORs were smaller in magnitude and not significant. Among women who were normal weight or underweight, the crude OR for spina bifida was 3.13 (95% CI, 1.63–6.03) and adjusted OR was 2.59 (95% CI, 1.32–5.07). Based on estimated cumulative exposure, a statistically significant dose-response trend was observed for spina bifida; however, it was attenuated and no longer significant after adjustment. **CONCLUSION:** Maternal occupational exposure to PAHs may be associated with increased risk of spina bifida in offspring among women who are normal weight or underweight. Other comparisons between PAHs and NTDs were consistent with no association. *Birth Defects Research (Part A) 94:693–700, 2012.* © 2012 Wiley Periodicals, Inc.

Key words: PAHs; polycyclic aromatic hydrocarbons; occupation; maternal exposure; malformations; neural tube defects; spina bifida

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are lipophilic compounds formed during the incomplete burning of coal, tobacco, or other organic substances. Human exposure is common, through inhalation of tobacco smoke, smoke from other sources of combustion, and ambient air, and through consumption of PAHs particularly in charbroiled foods. Although environmental sources are important, some of the highest exposure levels are found in the workplace (Brandt and Watson, 2003; Hansen et al., 2008). Occupations where exposure is likely to occur are those involving coke ovens and coal tar use, iron and steel works, aluminum works, foundries, carbon electrode and carbon black manufacture, and asphalt manufacture and use. In addition, exposures can occur in more common occupational settings such as restaurants (Sjaastad and Svendsen, 2009).

Maternal exposure to PAHs has been shown in laboratory animals to cause neural tube defects (NTDs), birth defects affecting the brain and spinal cord that arise from failure of the embryonic neural tube to close in the first month of pregnancy. This effect can vary depending on the genotype of the fetus and mother, on the specific type of PAH, and on the route of exposure (Faustman-Watts et al., 1984; Barbieri et al., 1986; Stark et al., 1989; Incardona et al., 2004). Four human studies have indicated a positive association between maternal exposure to PAHs and the risk of NTDs using residence location or biomarkers of exposure (Dodds and Seviour, 2001; Rankin et al., 2009; Naufal et al., 2010; Ren et al., 2011). However, no study has assessed maternal occupational exposure to PAHs and risk of NTDs in offspring.

Because of the high and potentially common workplace exposures to PAHs and evidence suggesting an association of PAHs and NTDs, the objective of this study was to determine whether women's periconceptional occupational exposure to PAHs was associated with risk of NTDs in offspring.

MATERIALS AND METHODS

Study Population

This analysis used data from the National Birth Defects Prevention Study (NBDPS), an ongoing, population-based, case-control study of more than 30 major structural birth defects. Detailed study methods have been published elsewhere (Yoon et al., 2001). Case infants were ascertained from birth defects surveillance systems in eight sites: Arkansas, California, Georgia (Centers for Disease Control and Prevention), Iowa, Massachusetts, New Jersey, New York, and Texas. Infants having or strongly suspected to have a chromosome abnormality or single-gene condition were excluded. Cases were reviewed by a clinical geneticist at each site before inclusion to ensure that case definitions were met (Rasmussen et al., 2003). Cases were live births from all sites, fetal deaths from all sites except New Jersey and New York, and pregnancy terminations from all sites except New Jersey, New York, and Massachusetts. Control infants were live born, without birth defects, and were selected at random from birth certificates or birth hospital records from the same populations that provided the cases. All mothers participated in a computer-assisted telephone interview (CATI) in English or Spanish, from 6 weeks

through 24 months after their estimated due dates. Mothers were asked questions on a variety of topics including maternal illnesses and medication use, pregnancy history, diet, vitamin intake, tobacco use, alcohol intake, substance use, and information about jobs held during pre-conception and pregnancy. The NBDPS was approved by the Office of Management and Budget at the Centers for Disease Control and Prevention and the appropriate institutional review boards at each participating site.

This analysis included mothers of cases with NTDs (anencephaly, craniorachischisis, spina bifida, and encephalocele) and control infants with estimated dates of delivery from October 1, 1997, through December 31, 2002. Mothers also had to have completed interviews and to have worked at least one job for at least 1 month, from 1 month before conception through 3 months after (defined here as the periconceptional period). There were no inclusion criteria based on minimum number of hours worked per week, but six women who could not be assigned PAH exposure status (described later) were excluded. The resulting study population consisted of 2989 controls and 520 cases of NTDs composed of 136 cases of anencephaly, 321 cases of spina bifida, and 63 cases of encephalocele.

Exposure Assessment

In the CATI, the first occupational question was: "The next section is a series of questions about your work experiences—paid, volunteer, or military service. This includes part-time and full-time jobs, jobs at home, and jobs on a farm or outside your home that lasted 1 month or more. Between (3 months before conception) and (date of infant birth), did you have a job?" For each job, the mother was asked to provide the employer name, job title, descriptions of the company's product or service, main job activities and duties, chemicals or substances handled, and machines used on the job. Mothers also provided job start and end dates and quantitative information on the usual number of days worked per week and hours worked per day. Each job was coded for occupation and industry using the 2000 Standard Occupational Classification (SOC) System (United States Department of Labor, Standard Occupational Classification, 2009) and the 1997 North American Industry Classification System (NAICS; United States Department of Labor, North American Industry Classification System, 2009).

Using the CATI data, exposure classification was conducted independently by two industrial hygienists (raters), blinded to case and control status. The raters' experience in industrial hygiene monitoring ranged from 17 to 27 years; each also had at least 10 years of experience in retrospective exposure assessment and participated in a training session prior to reviewing the job histories (Rocheleau et al., 2011). This expert review strategy was based on an approach that was developed previously and used in the Baltimore-Washington Infant Study (Jackson et al., 2004; Correa et al., 2006). For jobs considered possibly exposed to PAHs, the industrial hygienists assigned these characteristics: (1) whether inhalation exposure was direct, indirect, or both; (2) whether the inhalation exposure was continuous, intermittent, or both; (3) the fraction of total hours worked when exposure was direct (f_{direct}); (4) the fraction of total hours worked when exposure was indirect (f_{indirect}); (5) the intensity of any

direct inhalation exposure (on an ordinal scale from 0 to 4; I_{direct}) during the period of direct exposure; (6) the intensity of any indirect inhalation exposure (same scale, I_{indirect}) during the period of indirect exposure. Two raters independently assigned each of these characteristics; any jobs on which the raters did not have perfect agreement (i.e. discordant jobs) were reviewed at a consensus conference. During the consensus conference, the two raters plus a third industrial hygienist discussed each discordant job and reached an agreement about the appropriate final rating.

The direct and indirect intensity scores were mapped to intensity values of: $<0.1 \mu\text{g}/\text{m}^3$, $1 \mu\text{g}/\text{m}^3$, $8 \mu\text{g}/\text{m}^3$, and $>10 \mu\text{g}/\text{m}^3$. For the purposes of this study, the background intensity of occupational PAH exposure was assumed to be zero. A weighted intensity score (I_w) was computed from the intensity and frequency as:

$$I_w = [I_{\text{direct}} \times f_{\text{direct}}] + [I_{\text{indirect}} \times f_{\text{indirect}}] + [1 - (f_{\text{direct}} + f_{\text{indirect}})]$$

Although the exposure assessment was done for all jobs from 3 months before conception through the end of the pregnancy, for this study the analysis was restricted to jobs held from 1 month before conception through the end of the first trimester, as this corresponds to relevant periods for the formation of neural tube defects (Selevan et al., 2000). To combine weighted intensity with frequency and duration, cumulative PAH exposure during this period was calculated as:

$$\begin{aligned} & \text{(Weighted intensity in mg/hr)} \\ & \times [(\text{Exposure frequency in hr/week}) / (40 \text{ hr/week})] \\ & \times [(\text{Hours worked per week}) / (7 \text{ days/week})] \\ & \times (\text{Number of days worked in the critical period}) \end{aligned}$$

The resulting cumulative exposure value was specific for each job held by each woman; a woman's total occupational PAH exposure during the critical period was calculated as the sum of the job-specific cumulative exposures in the critical period. *Occupational exposure* refers here to inhalation exposures inherent in the job or workplace aside from secondhand smoke, and does not consider exposure through skin or ingestion.

A woman was classified as exposed if her total cumulative occupational PAH exposure in the critical period was more than zero (i.e., if one or more of her jobs held during the critical period was rated as exposed, whether part-time or full-time jobs). She was considered unexposed if her jobs had a cumulative exposure during the critical period of zero (i.e., if all her jobs held during the critical period were considered unexposed). Women whose occupational PAH exposure could not be assigned for one or more of the jobs held during the critical period were excluded. Other sources of potential PAH exposure included maternal smoking, secondhand smoke at home, and secondhand smoke at work (all obtained from the CATI).

Covariates

Several covariates were considered as potential confounders based on associations with NTDs or with PAH exposure as reported in the literature. The CATI yielded

data on the following maternal characteristics (categories shown in Table 1): age at delivery, race or ethnicity, education, number of previous live births, pre-pregnancy body mass index (BMI; categorized according to the National Heart, Lung, and Blood Institute cutoffs as underweight [$<18.5 \text{ kg}/\text{m}^2$], normal weight [$18.5\text{--}24.9 \text{ kg}/\text{m}^2$], overweight [$25.0\text{--}29.9 \text{ kg}/\text{m}^2$], and obese [$\geq 30.0 \text{ kg}/\text{m}^2$]); preexisting diabetes, and plurality of the index pregnancy. Data on the following maternal characteristics pertained to exposure in the periconceptional period: use of folate antagonist medications, consumption of folic acid supplements, cigarette smoking, secondhand smoke exposure at home, secondhand smoke exposure at work, and consumption of alcohol. Also considered as potential covariates were: infant sex, annual household income, family history of NTDs, and study site. In the analysis, all covariates were treated as categorical variables.

Statistical Analysis

Crude odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for each characteristic and NTDs. Frequency distributions of the 23 SOC major job groups were tabulated for cases and controls, stratified by occupational PAH exposure status.

Unconditional logistic regression was used to calculate crude and adjusted ORs and 95% CIs to evaluate the association of maternal occupational exposure to PAHs with risk of NTDs in offspring. Outcomes of interest were individual NTD phenotypes (e.g., anencephaly) and all NTDs combined. Covariates were retained in the final models if inclusion resulted in a change of 10% or greater in the estimate of effect between maternal occupational exposure to PAHs and the risk of NTDs.

Several subanalyses were conducted for combined NTDs and spina bifida, but not anencephaly and encephalocele because case numbers were insufficient. To evaluate the independent effect of occupational PAH exposure, the first subanalysis controlled for exposure to any non-occupational source of PAHs (i.e., smoking and secondhand smoke exposure at home or at work 1 month before conception to 3 months after), whether or not those variables met the 10% change in effect criterion for confounding. Effect measure modification was examined using the Breslow-Day test for homogeneity of ORs in mothers who actively smoked versus those who did not, and in mothers with any smoking exposure (active or secondhand at home or work) versus those who had none. An analysis looked more deeply at the effect of occupational PAH exposure in that last stratum (i.e., women who were unexposed to any nonoccupational PAH). Subjects were stratified by BMI into mothers who were normal or underweight versus obese or overweight, because body fat may influence the storage and transformation of PAHs (Agency for Toxic Substances and Disease Registry, 1995). Lastly, in an analysis for dose response, the cumulative exposure level was categorized into none, low, and high based on the frequency distribution among exposed controls, and the two-sided Cochran-Armitage trend test was used to test for trend.

RESULTS

Participation was 69% among control mothers and 70% among case mothers. Six women who could not be

Table 1
Maternal and Infant Factors Among Neural Tube Defect Cases and Controls, National Birth Defects Prevention Study, 1997–2002

Characteristic	Controls		Cases		OR (95% CI)
	n ^a	%	n ^a	%	
Total	2989		520		
Age (years)					
<20	330	11.0	64	12.5	0.96 (0.70–1.31)
20–24	678	22.7	119	22.8	0.87 (0.67–1.12)
25–29 (referent)	819	27.4	166	31.9	1.00
30–34	795	26.6	102	19.6	0.63 (0.49–0.83)
≥35	367	12.3	69	13.3	0.93 (0.68–1.26)
Race/Ethnicity					
White non-Hispanic (referent)	1939	68.2	309	62.7	1.00
Black non-Hispanic	377	13.3	59	12.0	0.98 (0.73–1.33)
Hispanic	527	18.5	125	25.3	1.49 (1.18–1.87)
Other	139	4.7	27	5.2	1.22 (0.79–1.87)
Education (years)					
<12	295	9.9	75	14.4	1.73 (1.30–2.29)
12	741	24.8	158	30.4	1.45 (1.17–1.79)
>12 (referent)	1951	65.3	287	55.2	1.00
Number of previous live births					
0 (referent)	1327	44.4	195	37.5	1.00
1	1038	34.7	190	36.5	1.25 (1.00–1.54)
≥2	623	20.8	135	26.0	1.47 (1.16–1.87)
Pre-Pregnancy BMI					
Under weight	152	5.2	18	3.6	0.77 (0.47–1.28)
Normal weight (referent)	1675	57.2	256	51.0	1.00
Over weight	663	22.6	106	21.1	1.05 (0.82–1.33)
Obese	438	15.0	122	24.3	1.82 (1.43–2.32)
Preexisting diabetes					
Yes (Type 1 and 2)	15	0.5	5	1.0	1.92 (0.70–5.32)
No (referent)	2974	99.5	515	99.0	1.00
Plurality					
1 (referent)	2892	96.8	492	94.6	1.00
≥2	95	3.2	24	5.4	1.73 (1.12–2.67)
Infant sex					
Male	1492	49.9	237	48.3	1.07 (0.88–1.29)
Female (referent)	1495	50.1	254	51.7	1.00
Folate antagonist medication^{b,c}					
Yes	28	0.9	10	1.9	2.07 (1.00–4.30)
No (referent)	2957	99.1	509	98.1	1.00
Folic acid supplements^b					
Yes	2612	87.4	442	85.0	0.82 (0.63–1.07)
No (referent)	377	12.6	78	15.0	1.00
Cigarette smoking^b					
Yes	615	20.6	98	18.8	0.90 (0.71–1.14)
No (referent)	2374	79.4	423	81.2	1.00
Secondhand smoking at home^b					
Yes	534	17.9	118	22.7	1.35 (1.08–1.69)
No (referent)	2455	82.1	402	77.3	1.00
Secondhand smoking at work^b					
Yes	571	19.1	137	26.4	1.51 (1.22–1.88)
No (referent)	2418	80.9	383	73.6	1.00
Alcohol drinking^b					
Yes	1318	44.3	192	37.1	0.74 (0.61–0.90)
No (referent)	1660	55.7	326	62.9	1.00
Annual household income (\$)					
<20,000	683	25.8	157	32.8	1.71 (1.34–2.19)
20–49,999	909	34.3	179	37.5	1.47 (1.16–1.86)
≥50,000 (referent)	1059	39.9	142	29.7	1.00
1st-generation family history of NTDs					
Yes	5	0.2	3	0.6	3.46 (0.82–14.53)
No (referent)	2984	99.8	517	99.4	1.00
Study center					
Arkansas (referent)	373	12.5	88	16.9	1.00
California	349	11.7	85	16.3	1.03 (0.74–1.44)
Iowa	413	13.8	85	16.3	0.87 (0.63–1.21)
Massachusetts	429	14.3	36	6.9	0.36 (0.24–0.54)
New Jersey	415	13.9	48	9.2	0.49 (0.34–0.72)
New York	340	11.4	44	8.5	0.55 (0.37–0.81)
Texas	319	10.7	73	14.0	0.97 (0.69–1.37)
CDC/Atlanta	351	11.7	61	11.7	0.74 (0.52–1.05)

^aSum may not add up to total because of missing values.

^bExposure 1 month before conception to 3 months after.

^cPlease see text for a list of medications included.

OR, odds ratio; CI, confidence interval; BMI, body mass index; NTD, neural tube defect; CDC, Centers for Disease Control and Prevention.

Table 2
Standard Occupation Code Major Job Groups^a for Jobs of Mothers of Neural Tube Defect Cases and Controls by Occupational Polycyclic Aromatic Hydrocarbon Exposure Status, National Birth Defects Prevention Study, 1997–2002

Major group	Cases				Controls			
	Exposed		Unexposed		Exposed		Unexposed	
	n ^b = 26	%	n = 494	%	n = 106	%	n = 2883	%
Management	0		46	7.3	3	2.8	319	9.3
Business and financial operations	0		26	4.1	0		148	4.3
Computer and mathematical	0		5	0.8	0		52	1.5
Architecture and engineering	0		1	0.2	1	0.9	11	0.3
Life, physical, and social science	0		6	1.0	0		43	1.3
Community and social services	0		10	1.6	0		68	2.0
Legal	0		6	1.0	0		33	1.0
Education, training, and library	0		34	5.4	0		291	8.5
Arts, design, entertainment, sports and media	0		10	1.6	0		50	1.5
Healthcare practitioners and technical	0		48	7.6	0		275	8.0
Healthcare support	0		31	4.9	1	0.9	167	4.9
Protective service	0		4	0.6	0		25	0.7
Food preparation and serving related	13	50.0	50	7.7	52	47.7	230	6.7
Building and grounds cleaning and maintenance	0		29	4.6	1	0.9	73	2.1
Personal care and service	0		31	4.9	7	6.4	157	4.6
Sales and related	8	30.8	92	14.7	32	29.4	443	13.0
Office and administrative support	0		125	19.9	0		745	21.8
Farming, fishing and forestry	1	3.9	20	3.2	2	1.8	49	1.4
Construction and extraction	0		2	0.3	0		5	0.2
Installation, maintenance and repair	0		2	0.3	1	0.9	5	0.2
Production	2	7.7	34	5.4	6	5.5	151	4.4
Transportation and material moving	1	3.9	15	2.4	3	2.8	77	2.3
Military specific	1	3.9	1	0.2	0		4	0.1

^aMultiple jobs may have been held by individual mothers; 109 case mothers and 484 control mothers had more than one job 1 month before conception through 3 months after.

^bTotal number of mothers.

assigned PAH exposure status were excluded. For the resulting analysis, there were 2989 controls, of whom 3.5% were classified as occupationally exposed to PAHs. There were 520 cases of NTDs (5.0% exposed): 136 cases of anencephaly (3.7% exposed), 321 spina bifida (5.9% exposed), and 63 encephalocele (3.2% exposed).

Risk of NTDs among offspring was higher among mothers who were 25 to 29 years old, Hispanic, obese, had a lower education level, more previous live births, or a lower household income (Table 1). Risk was also higher among mothers who were exposed to secondhand smoke at home or work or who used folate antagonist medications (trimethotrexate, trimetrexate, methotrexate, carbamazepine, valproic acid, and phenytoin). Risk of NTDs was lower among mothers with singleton pregnancies and those who reported drinking alcohol. Relative to a single study site (Arkansas), risk varied across sites.

Prevalence of occupational PAH exposure was higher ($p < 0.05$) among mothers who were Hispanic, overweight or obese, had a lower education level or household income, drank alcohol, smoked, or were exposed to secondhand smoke (data not shown).

The distribution of SOC major job groups was similar among the 26 case mothers and 106 control mothers occupationally exposed to PAHs (Table 2). The largest category was "food preparation and serving related" jobs, followed by "sales and related" jobs. Combining those two occupations accounted for 81% of exposed case mothers and 77% of exposed control mothers. The occu-

pational settings in which most exposed case and control mothers worked were restaurants, particularly fast food chains. The most common job duty mentioned by exposed mothers was cooking or food preparation.

The crude OR for occupational PAH exposure and any NTD was 1.43 (95% CI, 0.92–2.22) and the OR was reduced to approximately 1.00 after adjusting for BMI, secondhand smoke at home, and study center (the most parsimonious model, Table 3). The crude OR for spina bifida was 1.71 (95% CI, 1.03–2.83), but decreased to 1.21 (95% CI, 0.69–2.11) upon adjustment for maternal BMI and education. The ORs for anencephaly and encephalocele were consistent with no association or were not estimated because of too few exposed cases (<5).

The first subanalysis controlled only for smoking or secondhand smoke at home or work. The resulting ORs for occupational PAH exposure were between the crude and parsimonious model results for both NTDs and spina bifida, and were not statistically significant (Table 3). Controlling for all smoking exposures together with other covariates in the original parsimonious models resulted in nonsignificant ORs similar to the parsimonious models.

There was no evidence of interaction with smoking status. For example, comparing the NTD OR for smokers (1.51; 95% CI, 0.90–2.53) with nonsmokers (1.31; 95% CI, 0.57–3.05) yielded a p value for homogeneity of 0.78; for spina bifida, the corresponding ORs (1.86; 95% CI, 1.03–3.34) and 1.40 (95% CI, 0.53–3.71) had a p value for homogeneity of 0.63 (data not shown). Similar results were

Table 3
Association of Maternal Occupational Exposure to Polycyclic Aromatic Hydrocarbons and the Risk of Any Neural Tube Defect or Specific Birth Defect in Offspring, National Birth Defects Prevention Study, 1997–2002

Exposure to Occupational PAHs	Controls		Any Neural Tube Defect		Anencephaly		Spina Bifida		Encephalocele	
	n		n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR
Not exposed (referent)	2883	494	131	1.00	131	1.00	302	1.00	61	1.00
Exposed (crude)	106	26	5	1.43 (0.92–2.22)	5	1.04 (0.42–2.59)	19	1.71 (1.03–2.83)	2	n/a ^a
Exposed (adjusted, most parsimonious model)	103	22	4	1.01 (0.61–1.66) ^b	4	n/a	17	1.21 (0.69–2.11) ^c	1	n/a
Exposed (adjusted for all smoking exposure)	106	25	4	1.26 (0.80–1.97) ^d	4	n/a	19	1.55 (0.93–2.58) ^d	1	n/a
Exposed (adjusted for all smoking exposure and other covariates in original parsimonious model)	103	22	4	1.00 (0.62–1.62) ^e	4	n/a	17	1.20 (0.69–2.06) ^f	1	n/a

^aNot available; the number of exposed cases was too low for meaningful analysis.

^bAdjusted for BMI, secondhand smoking at home, study center.

^cAdjusted for BMI and education.

^dAdjusted for smoking, secondhand smoking at home, and secondhand smoking at work.

^eAdjusted for smoking, secondhand smoking at home, secondhand smoking at work, BMI, and study center.

^fAdjusted for smoking, secondhand smoking at home, secondhand smoking at work, BMI, and education. PAH, polycyclic aromatic hydrocarbon; OR, odds ratio; CI, confidence interval; BMI, body mass index.

found when stratifying by any smoking exposure (smoking, secondhand smoke at home or work) versus no smoking exposure. In these analyses for interaction, the smallest number of cases in a cell was 5 for smokers who had occupational PAH exposure and were mothers of children with spina bifida.

When limiting the analysis to subjects who were not exposed to smoking or secondhand smoke at home or work, the crude OR (cOR) for NTDs was 1.28 (95% CI, 0.59–2.75) and the adjusted OR (aOR) was 0.69 (95% CI, 0.26–1.80). The cOR for spina bifida was 1.87 (95% CI, 0.83–4.24) and the aOR was 0.98 (0.37–2.60). In other words, these ORs were not substantially different than the original results with the entire subject pool.

When stratified by BMI, the cOR was significantly higher for normal and underweight mothers than for overweight or obese mothers, for NTDs (p for homogeneity < 0.03) and spina bifida (p < 0.01; Table 4). In particular, the cOR for spina bifida was 3.13 (95% CI, 1.63–6.03). This OR was slightly attenuated but still statistically significant after adjustment for education (aOR, 2.59; 95% CI, 1.32–5.07).

In the analysis of exposure-response relationships, crude risk of combined NTDs did not show a statistically significant monotonic trend by the Cochrane-Armitage test. However, mothers in the highest exposure category had a cOR of 1.95 (1.11–3.40) compared to no PAH exposure (Table 5). After adjustment for covariates, the trend was attenuated and the OR for the highest exposure category was 1.30 (95% CI, 0.69–2.44). Spina bifida showed a significant crude dose-response trend, where the highest exposure category had an OR of 2.43 (95% CI, 1.31–4.53), but risk was attenuated to 1.73 (95% CI, 0.87–3.42) after adjustment and the trend lost significance.

DISCUSSION

The crude results of this study suggested a positive association between maternal occupational exposure to PAHs and risk of NTDs, particularly spina bifida, in offspring. However, after adjustment, the ORs for NTDs and spina bifida were attenuated and no longer significant; therefore, the association might have been largely due to confounding. Similarly, risk of spina bifida in offspring increased with increasing cumulative exposure in the crude estimates, but not the adjusted estimates. The only statistically significant associations after adjustment for confounding were observed among women who were underweight or normal weight.

These observations are generally inconclusive and as such neither support nor refute the literature. Experiments report that PAHs cause NTDs in laboratory animals (Faustman-Watts et al., 1984; Barbieri et al., 1986; Stark et al., 1989; Incardona et al., 2004). Human populations with likely environmental exposures had elevated risk of NTDs in Canadian areas near large-scale coke oven operations, (adjusted relative risk, 1.25; 95% CI, 1.04–1.51; Dodds and Seviour, 2001) and nervous system anomalies were higher in regions in the United Kingdom exposed to total black smoke (OR, 1.10 per increase of 1000 $\mu\text{g}/\text{m}^3$, 95% CI, 1.03–1.18; Rankin et al., 2009). Most importantly, one human study of biomarkers of exposure (cumulative from all sources) reported that high maternal blood PAH conferred an 8.70-fold risk of NTDs (95% CI, 2.02–37.04; Naufal et al., 2010) and another that high placental concentrations were associated with a 4.52-fold

Table 4
Association Between Maternal Occupational Exposure to Polycyclic Aromatic Hydrocarbons and the Risk of Any Neural Tube Defect or of Spina Bifida in Offspring, National Birth Defects Prevention Study, 1997–2002

BMI Category	Controls		Any neural tube defect			Spina bifida			
	n	n	Crude OR (95% CI)	n	Adjusted OR ^a (95% CI)	n	Crude OR (95% CI)	n	Adjusted OR ^b (95% CI)
Normal or Underweight									
Not exposed (referent)	1779	260	1.00	260	1.00	142	1.00	142	1.00
Exposed	48	14	2.00 (1.08–3.67)	14	1.71 (0.91–3.19)	12	3.13 (1.63–6.03)	12	2.59 (1.32–5.07)
Overweight or Obese									
Not exposed (referent)	1046	220	1.00	220	1.00	152	1.00	152	1.00
Exposed	55	8	0.69 (0.32–1.47)	8	0.62 (0.29–1.34)	5	0.63 (0.23–1.59)	5	0.60 (0.23–1.54)

^aAdjusted for secondhand smoking at home and study center.

^bAdjusted for education.

BMI, body mass index; PAH, polycyclic aromatic hydrocarbon; OR, odds ratio; CI, confidence interval.

increased risk (95% CI, 2.20–9.74; Ren et al., 2011). The lower estimates of risk in the present analysis could have been due to (1) the less accurate measurement of exposure based on a retrospective exposure assessment of interview data instead of on biologic markers, (2) the focus on occupational exposures alone instead of cumulative exposure from all sources, and (3) the higher PAH exposures experienced by some rural Chinese populations such as the one used in both the Naufal and Ren studies (Naufal et al., 2010; Ren et al., 2011), who use coal for home heating and cooking (Naufal et al., 2010) or are exposed to high ambient PAH emissions from coal production (Zhang et al., 2009).

PAHs are known to cross the placenta (Gladden et al., 2000) and have been found in cord blood (Madhavan and Naidu, 1995). They have been shown to form bulky DNA adducts in mothers and offspring (Topinka et al., 2009). Workers exposed to PAHs have higher levels of PAH-DNA adducts compared with the general population (Perera et al., 1994; Brandt and Watson, 2003). If not repaired, these adducts can disrupt the cell's microenvironment, leading to inhibition of important enzymes, cell death, and alteration of other cells (Agency for Toxic Substances and Disease Registry, 1995; Choi et al., 2008). Because PAHs are highly lipophilic and tend to be stored mostly in kidneys, liver, and fat (Agency for Toxic Substances and Disease Registry, 1995), they could become sequestered in fat stores in overweight or obese women,

resulting in lower exposure to the fetus. That observation was consistent with the results from the current study.

The major limitation of this study was potential exposure misclassification. Although industrial hygienists had the knowledge and experience to assign PAH exposure based on maternal job history, there is a possibility for inaccurate assessment. However, this approach was superior to a strategy that relies completely on maternal self-report, in which knowledge of PAH exposure is likely to be limited (Olsson et al., 2010). Similarly, the NBDPS did not collect information about the use of local exhaust ventilation and personal protective equipment; those were estimated by the industrial hygienists when evaluating each job. Many of the case and control mothers reported working in restaurants, especially fast food establishments; the exposure assessment focused on inhalation exposures and did not assess PAH exposure owing to consumption of food prepared at high temperatures. Information was lacking on other environmental sources of PAHs, although occupational exposures are generally higher than those found in the environment (Brandt and Watson, 2003; Hansen et al., 2008). Secondhand smoke exposure at home and especially at work may have been inaccurately reported in the CATI, though both showed increased risk of NTDs in our data. Exposure biomarkers such as those used in studies of rural Chinese populations (Naufal et al., 2010; Ren et al., 2011) are generally superior to the interview data used in this study, in accuracy and in their mea-

Table 5
Association Between Cumulative Maternal Occupational Exposure^a to Polycyclic Aromatic Hydrocarbons and the Risk of Any Neural Tube Defect or of Spina Bifida in Offspring, National Birth Defects Prevention Study, 1997–2002

PAH Exposure	Controls		Any Neural Tube Defect			Spina Bifida			
	n	n	Crude OR (95% CI)	n	Adjusted OR ^b (95% CI)	n	Crude OR (95% CI)	n	Adjusted OR ^c (95% CI)
None	2883	494	1.00 (referent)	480	1.00 (referent)	302	1.00 (referent)	294	1.00 (referent)
Low (0.32–100 µg/m ³ -hr) ^d	55	9	0.96 (0.47–1.95)	9	0.82 (0.40–1.68)	6	1.20 (0.37–3.89)	6	0.85 (0.36–2.01)
High (108–3800 µg/m ³ -hr)	51	17	1.95 (1.11–3.40)	13	1.30 (0.69–2.44)	13	2.43 (1.31–4.53)	11	1.73 (0.87–3.42)

^aCumulative PAH exposure during this period was calculated as: (Weighted intensity in mg/hr) × [(Exposure frequency in hr/week) / (40 hr/week)] × [(Hours worked per week) / (7 days per week)] × (Number of days worked in the critical period). Please see Materials and Methods.

^bAdjusted for BMI, secondhand smoking at home, and study center.

^cAdjusted for BMI and education.

^dLow versus high based on median value of cumulative PAH exposure among the controls.

PAH, polycyclic aromatic hydrocarbon; OR, odds ratio; CI, confidence interval; BMI, body mass index.

surement of cumulative exposure from all sources, occupational or otherwise. One potential drawback is that biomarkers collected at birth might not accurately reflect the mother's exposure during the critical period of fetal development. In addition, by focusing on PAHs from occupational exposures instead of from all sources, the current study examined consequences of some of the highest exposure levels humans are likely to encounter (Brandt and Watson, 2003; Hansen et al., 2008), and was potentially more relevant to any focused public health intervention. Despite the fact that the NBDPS is one of the largest population-based case-control studies of the causes of birth defects to date, the statistical power of the present study was hindered by the small number of exposed subjects, leading to a lack of precision. For example, the OR for spina bifida remained elevated (1.73) but with a wide 95% CI (0.87–3.42) for the highest cumulative exposure category after adjustment for covariates; this result might have been more conclusive with more subjects. Although adjustment was made for many important maternal factors, there is the potential for confounding by unidentified or unmeasured factors.

The use of NBDPS data is one of the strengths of this study. It has an extensive occupational exposure database. The case-control study design is more practical than occupational cohort studies for rare outcomes such as birth defects. It yielded data on potentially important confounding factors, such as BMI and smoking. The extensive case classification by NBDPS clinical geneticists produced accurate and fairly homogeneous case groups for analysis.

Although generally inconclusive, this study suggests that underweight or normal weight women exposed to occupational PAHs have a greater risk of spina bifida in offspring than similar women who are not exposed. Future investigations of PAHs and NTDs may benefit from stratification by maternal obesity, gathering detailed data on other factors affecting risk of NTDs, additional measures of exposure such as biomarker data, and gathering information on maternal and fetal genotypes related to PAH metabolism (Wassenberg et al., 2005; Shimada, 2006).

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