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ORIGINAL ARTICLE

Maternal Occupational Exposure to Polycyclic Aromatic Hydrocarbons and Risk of Oral Cleft-Affected Pregnancies

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Objective: **To evaluate whether there is an association between maternal occupational exposure to polycyclic aromatic hydrocarbons and oral clefts in offspring. This is the first human study of polycyclic aromatic hydrocarbons and clefts of which the authors are aware.**

Design: **Case-control study.**

Setting, Participants: **Data for 1997 to 2002 from the National Birth Defects Prevention Study, a large population-based case-control study in the United States, were analyzed. Maternal telephone interviews yielded information on jobs held in the month before through 3 months after conception. Two industrial hygienists independently assessed occupational exposure to polycyclic aromatic hydrocarbons; all jobs rated as exposed or with rating difficulty were reviewed with a third industrial hygienist to reach consensus on all exposure parameters. Logistic regression estimated crude and adjusted odds ratios with 95% confidence intervals for cleft lip with or without cleft palate and cleft palate alone.**

Results: **There were 2989 controls (3.5% exposed), 805 cases of cleft lip with or without cleft palate (5.8% exposed), and 439 cases of cleft palate alone (4.6% exposed). The odds of maternal occupational exposure to polycyclic aromatic hydrocarbons (any versus none) during pregnancy was increased for cleft lip with or without cleft palate cases as compared with controls (odds ratio, 1.69; 95% confidence interval, 1.18 to 2.40); the odds ratio was 1.47 (95% confidence interval 1.02 to 2.12) when adjusted for maternal education. There was a statistically significant adjusted exposure-response relationship for cleft lip with or without cleft palate (P_{trend} = .02). Odd ratios for cleft palate alone were not statistically significant.**

Conclusions: **Maternal occupational exposure to polycyclic aromatic hydrocarbons was associated with increased risk of cleft lip with or without cleft palate in offspring.**

KEY WORDS: *malformations, occupation, oral clefts, PAHs, polycyclic aromatic hydrocarbons*

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 and smoke from other sources of combustion, through ambient air, and through consumption of PAHs particularly in charbroiled foods (Agency for Toxic Substances and Disease Registry [ATSDR], 1995). Although environmental sources contribute to the total exposure burden, some of the highest exposure levels are found in the workplace

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(Brandt and Watson, 2003; Hansen et al., 2008). Occupations where exposure is likely to occur include those involving coke ovens and coal tar use, iron and steel works, carbon electrode and carbon black manufacturing, and asphalt manufacturing and use. Additionally, exposures can occur in more common occupational settings such as restaurants (Sjaastad and Svendsen, 2009).

Maternal exposure to PAHs during gestation has been shown in mice to cause oral clefts (Shum et al., 1979). To the authors' knowledge, no human studies assessing this association have been published. However, a major source of PAH exposure for humans is cigarette smoking (active or secondhand), which has been reported to show a moderate association with clefts in most studies (e.g., Ericson et al., 1979; Khoury et al., 1989; Shaw et al., 1996; Chung et al., 2000; Little et al., 2004b; Zeiger et al., 2005; Honein et al., 2007; Shi et al., 2007; Leite and Koifman, 2009; Shaw et al., 2009, Hackshaw et al., 2011) but not all (e.g., Werler et al., 1990; Grewal et al., 2008). The magnitude of the excess risk may be related to the genotype of the mother and/or fetus (van Rooij et al., 2001; Lammer et al., 2005; Shaw et al., 2005; Shi et al., 2007).

Due to high and potentially common workplace exposures to PAHs and evidence suggesting an association between an important source of PAHs (cigarette smoking) and oral clefts, the objective of this study was to determine whether women's periconceptional occupational exposure to PAHs was associated with risk of oral clefts in offspring.

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). Briefly, case infants were ascertained from birth defects surveillance systems at eight sites (Arkansas, California, Georgia/Centers for Disease Control and Prevention, Iowa, Massachusetts, New Jersey, New York, and Texas). Case records were reviewed by a clinical geneticist at each site prior to inclusion to ensure that case definitions were met (Rasmussen et al., 2003), and syndromic cases (i.e., those having or strongly suspected to have a chromosome abnormality or single-gene condition) were excluded. 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 preconception and pregnancy. The NBDPS and its informed consent procedures were approved by the Office of Management and Budget, and the appropriate institutional review boards at the Centers for Disease Control and Prevention and each participating site.

The current analysis included mothers of cases with cleft lip with or without cleft palate (CL±P), cases with cleft palate alone (CP), 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). Due to the small number of exposed cases that resulted, this study did not break them down into those with only an oral cleft (isolated cases) versus those with other co-occurring major birth defects (multiple defect cases).

Exposure Assessment

For each job reported in the CATI, the mother was asked to provide the employer name, job title, descriptions of the company's product/service, main job activities/duties, chemicals/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 self-reported job was then assigned a set of standard codes corresponding to its occupation and industry using the 2000 Standard Occupational Classification System (SOC) (U.S. Department of Labor, Standard Occupational Classification, 2009) and the 1997 North American Industry Classification System (NAICS) (U.S. Department of Labor, North American Industry Classification System, 2009), respectively. This allowed similar jobs to be grouped together.

Using the CATI data, exposure classification was conducted by industrial hygienists (raters) blinded to case/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 had been previously developed and used for other occupational exposures (solvents, lead, radiation) in the Baltimore-Washington Infant Study (Jackson et al., 2004; Correa et al., 2006). For jobs

considered possibly exposed to PAHs, two industrial hygienists independently assigned the following 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. All jobs rated as exposed by at least one rater and any jobs where raters had difficulty assigning exposure were reviewed at a consensus conference, in which they plus a third industrial hygienist discussed each job and reached an agreement about the appropriate final rating, including all parameters.

The direct and indirect intensity scores were mapped to intensity values of: <0.1, 1, 8, and >10 $\mu g/m^3$. 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 direct and indirect fraction as

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

The calculation formula for I_w is commonly used to combine two exposure intensities of differing levels weighted by the fraction of time spent at each level (Stewart et al., 1998; Checkoway et al., 2004). The intensity values used for this study were based on the exposure data in the PAH database used to rate the job exposures. To combine weighted intensity with frequency and duration, cumulative PAH exposure was calculated as (weighted intensity in μg/h) × [(exposure frequency in h/wk) / (40 h/wk)] × [(hours worked per week) / (7 d/wk)] × (no. of days worked in the periconceptional period). The resulting cumulative exposure value was job-specific rather than woman-specific; a woman's total occupational PAH exposure during the periconceptional period was calculated as the sum of the job-specific cumulative exposures in the periconceptional period. *Occupational exposure* refers here to inhalation exposures inherent in the job or workplace aside from secondhand smoke, and we did not consider exposure through skin or ingestion.

A woman was classified as exposed if her total cumulative occupational PAH exposure in the periconceptional period was more than zero (i.e., if one or more of her jobs held during the periconceptional 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 periconceptional period of zero (i.e., if all her jobs held during the periconceptional period were considered unexposed). Six women whose occupational PAH exposure could not be assigned for one or more of the jobs held during the periconceptional period were excluded; this was due to insufficient information on job title or job duties or insufficient information on job dates to tell whether the job was during the critical window. Other sources of potential PAH exposure included maternal smoking, secondhand smoke at home, and secondhand smoke at work (all obtained from the CATI). However, "total cumulative occupational PAH exposure" does not include smoking or secondhand smoke.

Covariates

Several covariates were considered as potential confounders based on associations either with oral clefts or with PAH exposure as reported in the literature. The CATI yielded data on the following maternal characteristics of interest as potential confounders (categories shown in Table 1): age at delivery; race/ethnicity; education; number of previous live births; prepregnancy body mass index (BMI; categorized according to the National Heart, Lung, and Blood Institute cutoffs as underweight [<18.5 kg/m^2], normal weight [18.5 to 24.9 kg/m^2], overweight [25.0 to 29.9 kg/m^2], and obese [≥ 30.0 kg/m^2]); preexisting diabetes; and plurality of the index pregnancy. Data on the following additional maternal characteristics also obtained during the CATI pertained to exposure in the periconceptional period: use of folate antagonist medications (trimethotrexate, trimetrexate, methotrexate, carbamazepine, valproic acid, dilantin); 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; first-degree family history of clefts; and study site. In the analysis, all covariates were treated as categorical variables.

Statistical Analysis

Crude odds ratios (cORs) and 95% confidence intervals (CIs) were estimated for each potential covariate with CL±P and CP using logistic regression. The associations between each potential covariate and PAH exposure (yes/no) was examined using a chi-square test. Frequency distributions of the 23 SOC major job groups were tabulated for those cases and controls, stratified by occupational PAH exposure status.

Logistic regression was used to estimate crude and adjusted ORs (aORs) and 95% CIs to evaluate the association of maternal occupational exposure to PAHs with risk of CL±P and CP in offspring. All the potential

TABLE 1 Maternal and Infant Factors Among Controls and Oral Cleft Cases, National Birth Defects Prevention Study, 1997–2002*

Characteristic	Controls		Cleft Lip With or Without Cleft Palate			Cleft Palate		
	n†	%	n†	%	OR (CI)	n†	%	OR (CI)
Total	2989		805			439		
Age, y								
<20	240	8.0	82	10.2	1.30 (0.97–1.74)	35	8.0	0.99 (0.66–1.48)
20–24	643	21.5	212	26.3	1.25 (1.01–1.56)	93	21.2	0.98 (0.73–1.31)
25–29 (ref)	799	26.7	210	26.1	1.0	118	26.9	1.0
30–34	858	28.7	189	23.5	0.84 (0.67–1.04)	113	25.7	0.89 (0.68–1.17)
35+	449	15.0	112	13.9	0.95 (0.73–1.23)	80	18.2	1.21 (0.89–1.64)
Race/Ethnicity								
White non-Hispanic (ref)	1939	65.0	542	67.4	1.0	324	73.8	1.0
Black non- Hispanic	377	12.6	48	6.0	0.46 (0.33–0.62)	30	6.8	0.48 (0.32–0.70)
Hispanic	527	17.7	165	20.5	1.12 (0.92–1.37)	64	14.6	0.73 (0.55–0.97)
Other	139	4.7	49	6.1	1.26 (0.90–1.77)	21	4.8	0.90 (0.56–1.45)
Education, y								
<12	295	9.9	118	14.7	1.68 (1.33–2.13)	45	10.3	1.05 (0.75–1.46)
12	741	24.8	221	27.5	1.25 (1.04–1.50)	109	24.8	1.01 (0.80–1.27)
12+ (ref)	1951	65.3	465	57.8	1.0	285	64.8	1.0
Parity/No. of previous live births								
0 (ref)	1327	44.4	396	49.2	1.0	208	47.4	1.0
1	1038	34.7	252	31.3	0.81 (0.68–0.97)	144	32.8	0.89 (0.71–1.11)
2+	623	20.8	157	19.5	0.84 (0.69–1.04)	87	19.8	0.89 (0.68–1.16)
Prepregnancy BMI								
Underweight	152	5.2	63	8.0	1.64 (1.20–2.24)	23	5.3	1.06 (0.67–1.68)
Normal weight (ref)	1675	57.2	424	54.1	1.0	238	55.1	1.0
Overweight	663	22.6	162	20.7	0.97 (0.79–1.18)	93	21.5	0.99 (0.76–1.27)
Obese	438	15.0	135	17.2	1.22 (0.98–1.52)	78	18.1	1.25 (0.95–1.65)
Preexisting diabetes								
Yes (type 1 & 2)	15	0.5	12	1.5	3.00 (1.40–6.43)	5	1.1	2.28 (0.83–6.31)
No (ref)	2970	99.5	793	98.5	1.0	434	98.9	1.0
Plurality								
1 (ref)	2877	97.3	765	95.3	1.0	421	96.8	1.0
2+	79	2.7	38	4.7	1.81 (1.22–2.68)	14	3.2	1.21 (0.68–2.16)
Infant sex								
Male	1492	50.0	547	68.2	2.15 (1.82–2.53)	200	45.7	0.84 (0.69–1.03)
Female (ref)	1495	50.0	255	31.8	1.0	238	54.3	1.0
Folate antagonist medication‡§								
Yes	28	0.9	11	1.4	1.47 (0.73–2.96)	3	0.7	0.73 (0.22–2.41)
No (ref)	2957	99.1	791	98.6	1.0	434	99.3	1.0
Folic acid supplements‡								
Yes	2612	87.4	700	87.0	0.96 (0.76–1.21)	386	87.9	1.05 (0.77–1.43)
No (ref)	377	12.6	105	13.0	1.0	53	12.1	1.0
Cigarette smoking‡								
Yes	615	20.6	211	26.2	1.37 (1.14–1.64)	114	26.0	1.35 (1.07–1.71)
No (ref)	2374	79.4	594	73.8	1.0	325	74.0	1.0
Secondhand smoking at home‡								
Yes	534	17.9	158	19.6	1.12 (0.92–1.37)	98	22.3	1.32 (1.04–1.68)
No (ref)	2455	82.1	647	80.4	1.0	341	77.7	1.0
Secondhand smoking at work‡								
Yes	571	19.1	184	22.9	1.25 (1.01–1.51)	82	18.7	0.97 (0.75–1.26)
No (ref)	2418	80.9	621	77.1	1.0	357	81.3	1.0
Alcohol drinking‡								
Yes	1318	44.3	353	44.0	0.99 (0.85–1.16)	197	45.0	1.03 (0.84–1.26)
No (ref)	1660	55.7	449	56.0	1.0	241	55.0	1.0
Household income ($)								
<20,000	683	25.8	230	30.8	1.10 (0.90–1.34)	90	22.3	0.77 (0.58–1.01)
20,000–49,999 (ref)	909	34.3	279	37.3	1.0	156	38.7	1.0
50,000+	1059	40.0	239	32.0	0.74 (0.61–0.89)	157	39.0	0.86 (0.68–1.10)
First generation family history of clefts								
Yes	8	0.3	48	6.0	23.6 (11.1–50.1)	21	4.8	18.7 (8.2–42.5)
No (ref)	2981	99.7	757	94.1	1.0	418	95.2	1.0
Study center								
Arkansas (ref)	373	12.5	83	10.3	1.0	45	10.3	1.0
California	349	11.7	102	12.7	1.31 (0.95–1.82)	41	9.3	0.97 (0.62–1.52)
Iowa	413	13.8	118	14.7	1.28 (0.94–1.75)	50	11.4	1.00 (0.65–1.54)
Massachusetts	429	14.3	120	14.9	1.26 (0.92–1.72)	86	19.6	1.66 (1.13–2.44)
New Jersey	415	13.9	84	10.4	0.91 (0.65–1.27)	49	11.2	0.98 (0.64–1.50)
New York	340	11.4	89	11.1	1.18 (0.84–1.64)	53	12.1	1.29 (0.85–1.97)
Texas	319	10.7	120	14.9	1.69 (1.23–2.32)	51	11.6	1.32 (0.86–2.03)
CDC/Atlanta	351	11.7	89	11.1	1.14 (0.82–1.59)	64	14.6	1.51 (1.01–2.27)

TABLE 2 Standard Occupation Code (SOC) Major Job Groups for Mothers of Cleft Lip With or Without Palate and Cleft Palate Cases and Controls by PAH Occupational Exposure Status, National Birth Defects Prevention Study, 1997–2002

	Controls				*Cleft Lip With or Without Palate*				*Cleft Palate*			
	Exposed		*Unexposed*		*Exposed*		*Unexposed*		*Exposed*		*Unexposed*	
SOC Major Group	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Total*	106	100.0	2883	100.0	47	100.0	758	100.0	20	100.0	419	100.0
Management	3	2.9	289	10.1	0		56	7.4	2	10.0	28	6.7
Business and financial operations	0		129	4.5	0		34	4.5	0		21	5.0
Computer and mathematical	0		50	1.7	0		15	2.0	0		9	2.2
Architecture and engineering	1	1.0	9	0.3	0		0		1	5.0	1	0.2
Life, physical, and social science	0		39	1.4	0		6	0.8	0		3	0.7
Community and social services	0		60	2.1	0		14	1.8	0		11	2.6
Legal	0		32	1.1	0		11	1.5	0		9	2.2
Education, training, and library	0		238	8.3	0		52	6.9	0		32	7.7
Arts, design, entertainment, sports, and media	0		42	1.5	0		19	2.5	0		13	3.1
Health care practitioners and technical	0		228	7.9	0		59	7.8	0		30	7.2
Health care support	1	1.0	130	4.5	1	2.1	26	3.4	0		15	3.6
Protective service	0		20	0.7	0		5	0.7	0		2	0.5
Food preparation and serving related	50	47.6	179	6.2	24	51.1	54	7.1	7	35.0	25	6.0
Building and grounds cleaning and maintenance	1	1.0	61	2.1	1	2.1	28	3.7	0		13	3.1
Personal care and service	7	6.7	124	4.3	5	10.6	32	4.2	0		22	5.3
Sales and related	30	28.6	348	12.1	13	27.7	95	12.5	6	30.0	41	9.8
Office and administrative support	0		636	22.2	1	2.1	177	23.3	0		104	24.9
Farming, fishing, and forestry	2	1.9	44	1.5	0		13	1.7	0		4	1.0
Construction and extraction	0		4	0.1	0		2	0.3	0		1	0.2
Installation, maintenance, and repair	1	1.0	5	0.2	0		3	0.4	0		2	0.5
Production	6	5.7	136	4.7	2	4.3	38	5.0	2	10.0	17	4.1
Transportation and material moving	3	2.9	63	2.2	0		18	2.4	2	10.0	14	3.4
Military specific	0		4	0.1	0		1	0.1	0		0	

* Sum may not add up to total due to missing values.

covariates described in the previous section were included in a full model and submitted to manual backward stepwise logistic regression, removing the covariate with the highest *P* value. Covariates were retained if inclusion resulted in a change of 10% or greater in the effect measure estimate for PAHs and each cleft outcome of interest.

Several subanalyses were conducted. To evaluate the independent effect of occupational PAH exposure, the first subanalysis excluded all women who were considered exposed to any nonoccupational source of PAH exposure (i.e., smoking and secondhand smoke exposure at home or at work). In the second subanalysis, subjects were stratified by prepregnancy BMI (obese mothers versus all others) because body fat may influence the storage and transformation of PAHs (ATSDR, 1995) and stratified ORs were calculated to check for effect measure modification. Mothers were also stratified by age, race/ethnic group, education, folic acid supplement use, active smoking, and any smoking exposure to examine possible effect measure modification. To evaluate the potential for an exposure-response relationship, cumulative exposure level was categorized into none, low, and high based on the frequency distribution among exposed controls, and the two-sided Cochrane-Armitage trend test was used to test for trend. Last, the analyses of occupational PAH exposure categorized as no/yes and none/low/high were repeated, excluding cases with other malformations to check whether the associations changed.

Results

Participation was 69% among control mothers and 76% among case mothers. For the resulting analysis there were 2989 controls, 805 cases of CL±P, and 439 cases of CP.

Risk of CL±P among offspring was statistically significantly higher among mothers who had diabetes before the index pregnancy, a multiple-fetus pregnancy, male children, lower education or household income, or a family history of clefts (Table 1). Risk was also higher among mothers who smoked or were exposed to secondhand smoke at work. Risk of CP was higher among mothers who were non-Hispanic white (compared to non-Hispanic black or Hispanic), who smoked or were exposed to secondhand smoke at home, or who had a family history of clefts. Risk of CP varied across study sites.

Prevalence of occupational PAH exposure in controls was higher ($P < .05$) among mothers who were Hispanic, overweight or obese, had a lower education or household

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* OR = odds ratio; CI = confidence interval; ref = referent; BMI = body mass index; CDC = Centers for Disease Control and Prevention.

† Sum may not add up to total due to missing values.

‡ Exposure 1 month prior to conception to 3 months after.

§ Please see text for a list of medications included.

TABLE 3 Association of Maternal Occupational Exposure to Polycyclic Aromatic Hydrocarbons and the Risk of Cleft Lip With or Without Palate and Cleft Palate in Offspring, National Birth Defects Prevention Study, 1997–2002

		*Cleft Lip With or Without Palate**		*Cleft Palate*†	
Exposure to Occupational PAHs	*Controls (n)*	*n*	*OR (95% CI)*‡	*n*	*OR (95% CI)*
Not exposed (referent)	2883	758	1.00	419	1.00
Exposed (crude)	106	47	1.69 (1.18–2.40)	20	1.30 (0.80–2.12)
Exposed (adjusted)	106	47	1.47 (1.02–2.12)	20	1.24 (0.76–2.03)

* Adjusted for maternal education.
† Adjusted for maternal secondhand smoke exposure at home (B1-P3).
‡ OR = odds ratio; CI = confidence interval.

income, or who smoked or were exposed to secondhand smoke (data not shown). Prevalence of exposure was lower among women who drank alcohol.

Overall, 3.5% of the controls were classified as occupationally exposed to PAHs, as were 5.8% of the CL±P cases and 4.6% of the CP cases. The distribution of SOC major job groups was similar among the 106 control mothers, 47 CL±P mothers, and 20 CP mothers occupationally exposed to PAHs (Table 2). The largest category for each group was "food preparation and serving related" jobs, followed by "sales and related" jobs. Combining those two occupations accounted for 76% of exposed control mothers, 79% of exposed CL±P mothers, and 65% of exposed CP mothers. The occupational settings where 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. Cooking or food preparation accounted for a large portion of jobs with high-intensity exposure to PAHs; whereas, managing, providing personal care, or being a cashier were the most common low–PAH-exposure jobs within the participants' work histories (data not shown).

The cOR (95% CI) for occupational PAH exposure and CL±P was 1.69 (1.18 to 2.40), and 1.47 (1.02 to 2.12) after adjusting for maternal education (Table 3). The ORs for CP were slightly elevated but not statistically significant; the cOR was 1.30 (0.80 to 2.12), and the OR adjusted for maternal secondhand smoke exposure at home was 1.24 (0.76 to 2.03).

In the first subanalysis, the unexposed group and occupationally exposed group were limited to subjects who were not exposed to PAHs from any other source that could be determined using the NBDPS data (smoking or secondhand smoke at home or work) (data not shown). That reduced the number of exposed CL±P cases from 47 to 17. The cOR remained elevated but was no longer statistically significant (1.65; 95% CI, 0.93 to 2.93), as was the aOR (1.70; 95% CI, 0.94 to 3.06). The cOR for the six exposed CP cases was close to unity (1.05; 95% CI, 0.44 to 2.50); the aOR was not estimable.

In the second subanalysis, no statistically significant effect measure modification was observed in either CL±P or CP for prepregnancy BMI, maternal age, race/ethnicity, education, folic acid supplement use, active smoking, or any smoking exposure (smoking or secondhand smoke at home or work) (data not shown). This did not seem to be related to statistical power because (1) numbers of exposed cases were acceptable for most strata except some variables among CP, and (2) the ORs for the strata tended to be similar in magnitude, for example, 1.21 to 1.88 for CL±P in each stratum with at least 10 exposed cases.

In the analysis of exposure-response relationships, crude risk of CL±P exhibited a statistically significant monotonic trend by the Cochrane-Armitage test, which remained after adjustment for maternal education ($P = .02$; Table 4). Compared with the no–PAH-exposure group, the aOR for the low PAH exposure level was 1.36 (95% CI, 0.82 to 2.27), and the highest PAH exposure level was 1.66 (95% CI, 1.02 to 2.70). There was no statistically significant exposure-response relationship observed for CP.

TABLE 4 Association Between Cumulative Maternal Occupational Exposure to Polycyclic Aromatic Hydrocarbons (PAHs) and the Risk of Any Oral Clefts in Offspring, National Birth Defects Prevention Study, 1997–2002

	*Cumulative Exposure Level ($\mu g/m^3$ - hours)**	*Controls (n)*	*Cleft Lip With or Without Cleft Palate*		*Cleft Palate*	
			Cases (n)	*OR (95% CI)*†	*Cases (n)*	*OR (95% CI)*
Crude analysis				$P_{trend} = .01$		$P_{trend} = .55$
No PAH exposure	0	2883	758	1.00 (ref)	419	1.00 (ref)
Low PAH exposure	0.32 – 100	55	21	1.45 (0.87–2.42)	11	1.38 (0.71–2.65)
High PAH exposure	108 – 6900	51	26	1.94 (1.20–3.13)	9	1.21 (0.59–2.48)
Adjusted Analysis‡				$P_{trend} = 0.02$		$P_{trend} = 0.47$
No PAH exposure	0	2881	757	1.00 (ref)	419	1.00 (ref)
Low PAH exposure	0.32 – 100	55	21	1.36 (0.82–2.27)	11	1.33 (0.69–2.56)
High PAH exposure	108 – 6900	51	26	1.66 (1.02–2.70)	9	1.15 (0.56–2.36)

* Cumulative exposure calculated as described in Methods.
† OR = odds ratio; CI = confidence interval.
‡ Cleft lip with or without cleft palate adjusted for maternal education. Cleft palate adjusted for maternal secondhand smoke exposure at home.

There were 705 cases of CL±P with no other major malformations; this made up 88% of our total CL±P cases. The associations with occupational PAH exposure observed with these cases were similar to those using the total sample, though sometimes slightly attenuated and often no longer statistically significant. For example, when adjusted for maternal education, the OR for any occupational PAH exposure and CL±P was 1.35 (95% CI, 0.92 to 2.00) (data not shown) versus 1.47 (95% CI, 1.02 to 2.12) in the total sample. Compared with no occupational PAH exposure in the isolated group, the aOR for low exposure was 1.10 (95% CI, 0.62 to 1.97) and for high exposure, 1.61 (95% CI, 0.96 to 2.70); *P* for trend was .08. There were 361 cases or 82% of total CP without other major malformations; among them, the magnitude of associations were similar to total cases, and the test for trend remained not statistically significant.

Discussion

This study found a positive association between maternal occupational exposure to PAHs and risk of CL±P in offspring. This was true of crude and adjusted analyses (the aOR was 1.47; 95% CI, 1.02 to 2.12). Odds ratios increased with increasing total cumulative exposure to occupational PAHs, and the statistical significance of the trend remained after adjustment. Similar patterns were observed among CL±P cases without other major malformations, though there was no longer statistical significance, probably due in part to fewer subjects. When restricted to subjects without exposure to PAHs from active or secondhand smoking, the association with maternal occupational PAHs was no longer statistically significant, but the magnitude of effect was similar. No effect measure modification was observed for CL±P; ORs across strata were similar in magnitude. No statistically significant association was observed with CP.

These observations were consistent with Shum et al. (1979), who found that PAH exposure caused oral clefts in mice. Although no published papers were found regarding PAH exposure in humans and clefts in offspring, the current study was consistent with the frequent reports of cigarette smoking moderately increasing risk of clefts, because smoking is one of the major sources of PAH exposure in human beings. Many of those papers reported an association of smoking with combined oral clefts (Ericson et al., 1979; Van den Eeden et al., 1990; Chung et al., 2000; Van Rooij et al., 2002; Wyszynski et al., 2002; Shi et al., 2007; Shaw et al., 2009, Hackshaw et al., 2011) or found statistically significant associations for both CL±P and CP (Khoury et al., 1987; Khoury et al., 1989; Shaw et al., 1996; Kallen, 1997; Wyszynski et al., 1997; Little et al., 2004a; Little et al., 2004b; Meyer et al., 2004; Zeiger et al., 2005). The current study found statistically significant associations only with CL±P, which is consistent with several studies that examined both phenotypes (Lorente et al., 2000; Honein et al., 2007; Lie et al, 2008; Leite and Koifman, 2009), though one study found effects only with CP (Romitti et al., 1999). Some studies reported no association of smoking with combined clefts or with CL±P or CP (Hemminki et al., 1983; Werler et al., 1990; Christensen et al., 1999; Van Rooij et al, 2001; Grewal et al., 2008).

In the current study, occupational PAH exposure showed statistically significant associations only with CL±P. This might have been due partly to small numbers of exposed cases of CP (20, compared with 47 CL±P) because CP also exhibited a positive, though weaker and not statistically significant, association (aOR = 1.24; 95% CI, 0.76 to 2.03). As such, the current study is consistent with the meta-analysis by Little et al. (2004b) reporting weaker associations with maternal smoking for CP than for CL±P. The difference between phenotype associations might also have been due partly to the fact that the developmental errors that lead to CL±P are different from those that lead to CP (Fogh-Anderson, 1942; Fraser, 1955).

PAHs cross the placenta (Gladen 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 (ATSDR, 1995; Choi et al., 2008). Those in turn could affect the directed growth, fusion, and differentiation required for normal palate and lip development. Another possible mechanism is if PAH exposure leads to periods of fetal hypoxia through reduced placental blood flow (Rennie et al., 2011); hypoxic events increase the risk of oral clefts in certain strains of mice (Millicovsky and Johnston, 1981; Bronsky et al., 1986; Bailey et al., 1995).

A major limitation of this study was the potential for exposure misclassification. Although the three industrial hygienists had several years of experience and working knowledge, inaccurate assignment of exposure was nevertheless possible because the occupational information reported during the CATI was limited and did not include potentially important exposure-modifying factors such as use of personal protective equipment and ventilation practices. However, the expert rater approach used in our study was superior to relying exclusively on maternal self-report of PAH exposure, which is likely to be limited and could introduce recall bias (Olsson et al., 2010). In our dichotomized exposure analyses (any/none), nondifferential errors in exposure misclassification would be expected to bias the ORs toward the null (Rocheleau et al., 2011).

There was no information about sources of environmental PAH exposure (e.g., residential proximity to industrial

combustion smoke) other than personal tobacco use and exposure to secondhand smoke at home or at work. However, smoking is an important source of PAHs (ATSDR, 1995), and it is therefore a strength of our study that we were able to account for PAH exposure from smoking. Furthermore, because levels of occupational PAH exposure are generally higher than environmental sources (Brandt and Watson, 2003; Hansen et al., 2008), it is important to consider exposure in the workplace separately from other sources. Biomarkers of exposure to PAHs (e.g., Naufal et al., 2010; Ren et al., 2011) are generally superior to the interview-based data used in this study in terms of accurate measurement of cumulative exposure from all sources, occupational or otherwise. However, biomarkers collected at birth may not accurately reflect PAH exposures experienced during the periconceptional period, which is the critical window of fetal development for the oral cavity and surrounding structures, and thus may introduce undesirable misclassification in the *timing* of exposure.

One of the major strengths of this study was its use of NBDPS data, which provided an extensive occupational exposure database. The occupational exposure assessment improved accuracy compared with the exclusive use of job title or self-reported exposure, and the cumulative exposure estimation allowed examination of exposure-response relationships. Its case-control study design was more practical than occupational cohort studies for very rare outcomes such as birth defects. It yielded data on potentially important confounding factors such as active and passive smoking. The extensive case classification by NBDPS clinical geneticists produced accurate and fairly homogeneous case groups for analysis, reducing outcome heterogeneity and allowing for the examination of specific subgroups of oral clefts.

In summary, this study found a positive association between maternal occupational exposure to PAHs and risk of CL±P in offspring. Risk showed an exposure-response relationship that also persisted after adjustment. No statistically significant association was observed with CP. This may be the first study of PAH exposure and orofacial clefts conducted in humans, and it is generally consistent with many previous studies of cigarette smoking and clefts. Future investigations of PAHs and clefts may benefit from additional measures of exposure such as biomarker data, as well as gathering information on maternal and fetal genotypes related to PAH metabolism (Wassenberg et al., 2005; Shimada, 2006) or genotypes suggested in previous studies of gene-smoking interactions and oral clefts (Shaw et al., 1996; Romitti et al., 1999; Van Rooij et al., 2001; Lammer et al., 2005; Shaw et al., 2005; Zeiger et al, 2005; Shi et al., 2007).

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References

Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs)*. Atlanta: U.S. Department of Health and Human Services, Public Health Service; 1995.

Bailey LJ, Johnston MC, Billet J. Effects of carbon monoxide and hypoxia on cleft lip in A/J mice. *Cleft Palate Craniofac J*. 1995:32:14–19.

Brandt HC, Watson WP. Monitoring human occupational and environmental exposures to polycyclic aromatic compounds. *Ann Occup Hyg*. 2003;47:349–378.

Bronsky PT, Johnston MC, Sulik KK. Morphogenesis of hypoxia-induced cleft lip in CL/Fr mice. *J Craniofac Genet Dev Biol*. 1986;2(suppl):113–128.

Checkoway H, Pearce N, Kriebel D. *Research Methods in Occupational Epidemiology*. 2nd ed. Oxford: Oxford University Press; 2004.

Choi H, Rauh V, Garfinkel R, Tu Y, Perera FP. Prenatal exposure to airborne polycyclic aromatic hydrocarbons and risk of intrauterine growth restriction. *Environ Health Perspect*. 2008;116:658–665.

Christensen K, Olsen J, Norgaard-Pedersen B, Basso O, Stovring H, Milhollin-Johnson L, Murray JC. Oral clefts, transforming growth factor alpha gene variants, and maternal smoking: a population-based case-control study in Denmark, 1991–1994. *Am J Epidemiol*. 1999;149:248–255.

Chung KC, Kowalski CP, Kim HM, Buchman SR. Maternal cigarette smoking during pregnancy and the risk of having a child with cleft lip/palate. *Plast Reconstr Surg*. 2000;105:485–491.

Correa A, Min YI, Stewart PA, Lees PS, Breysse P, Dosemeci M, Jackson LW. Inter-rater agreement of assessed prenatal maternal occupational exposures to lead. *Birth Defects Res A Clin Mol Teratol*. 2006;76:811–824.

Ericson A, Kallen B, Westerholm P. Cigarette smoking as an etiologic factor in cleft lip and palate. *Am J Obstet Gynecol*. 1979;135:348–351.

Fogh-Andersen P. *Inheritance of Harelip and Cleft Palate*. Copenhagen: Munksgaard; 1942.

Fraser FC. Thoughts on the etiology of clefts of the palate and lip. *Acta Genet Stat Med*. 1955;5:358–369.

Gladen BC, Zadorozhnaja TD, Chislovska N, Hryhorczuk DO, Kennicutt MC 2nd, Little RE. Polycyclic aromatic hydrocarbons in placenta. *Hum Exp Toxicol*. 2000;19:597–603.

Grewal J, Carmichael SL, Ma C, Lammer EJ, Shaw GM. Maternal periconceptional smoking and alcohol consumption and risk for select congenital anomalies. *Birth Defects Res A Clin Mol Teratol*. 2008;82:519–526.

Hackshaw A, Rodeck C, Boniface S. Maternal smoking in pregnancy and birth defects: a systematic review based on 173,687 malformed cases and 11.7 million controls. *Human Reprod Update*. 2011;17:589–604.

Hansen AM, Mathiesen L, Pedersen M, Knudsen LE. Urinary 1-hydroxypyrene (1-HP) in environmental and occupational studies—a review. *Int J Hyg Environ Health*. 2008;211:471–503.

Hemminki K, Mutanen P, Saloniemi I. Smoking and the occurrence of congenital malformation and spontaneous abortions: multivariate analysis. *Am J Obstet Gynecol*. 1983;145:61–66.

Honein MA, Rasmussen SA, Reefhuis J, Romitti PA, Lammer EJ, Sun L, Correa A. Maternal smoking and environmental tobacco smoke exposure and the risk of orofacial clefts. *Epidemiology*. 2007;18:226–233.

Jackson LW, Correa-Villasenor A, Lees PS, Dominici F, Stewart PA, Breysse PN, Matanoski G. Parental lead exposure and total anomalous pulmonary venous return. *Birth Defects Res A Clin Mol Teratol*. 2004;70:185–193.

Kallen K. Maternal smoking and orofacial clefts. *Cleft Palate Craniofac J*. 1997;34:11–16.

Khoury MJ, Gomez-Farias M, Mulinar J. Does maternal cigarette smoking during pregnancy cause cleft lip and palate in offspring? *Am J Dis Child*. 1989;143:333–337.

Khoury MJ, Weinstein A, Panny S, Holtzman NA, Lindsay PK, Farrel K, Eisenberg M. Maternal cigarette smoking and oral clefts: a population-based study. *Am J Public Health*. 1987;77:623–625.

Lammer EJ, Shaw GM, Iovannisci DM, Finnell RH. Maternal smoking, genetic variation of glutathione s-transferases, and risk for orofacial clefts. *Epidemiology*. 2005;16:698–701.

Leite IC, Koifman S. Oral clefts, consanguinity, parental tobacco and alcohol use: a case-control study in Rio de Janeiro, Brazil. *Brazil Oral Res*. 2009;23:31–37.

Lie RT, Wilcox AJ, Taylor J, Gjessing HK, Saugstad OD, Aabyholm F, Vindenes H. Maternal smoking and oral clefts: the role of detoxification pathway genes. *Epidemiology*. 2008;19:606–615.

Little J, Cardy A, Arslan MT, Gilmour M, Mossey PA. Smoking and orofacial clefts: a United Kingdom-based case-control study. *Cleft Palate Craniofac J*. 2004a;41:381–386.

Little J, Cardy A, Munger RG. Tobacco smoking and oral clefts: a meta-analysis. *Bull World Health Org*. 2004b;82:213–218.

Lorente C, Cordier S, Goujard J, Ayme S, Bianchi F, Calzolari E, De Wall HE, Knill-Jones R. Tobacco and alcohol use during pregnancy and risk of oral clefts. *Am J Public Health*. 2000;90:415–419.

Madhavan ND, Naidu KA. Polycyclic aromatic hydrocarbons in placenta, maternal blood, umbilical cord blood and milk of Indian women. *Hum Exp Toxicol*. 1995;14:503–506.

Meyer KA, Williams P, Hernandez-Diaz S, Cnattingius S. Smoking and the risk of oral clefts: exploring the impact of study designs. *Epidemiology*. 2004;15:671–678.

Millicovsky G, Johnston MC. Hyperoxia and hypoxia in pregnancy: simple experimental manipulation alters the incidence of cleft lip and palate in CL/Fr mice. *Proc Natl Acad Sci U S A*. 1981;9:4723.

Naufal Z, Zhiwen L, Zhu L, Zhou GOD, McDonald T, He LY, Mitchell L, Ren A, Zhu H, Finnell R, et al. Biomarkers of exposure to combustion by-products in a human population in Shanxi, China. *J Expo Sci Environ Epidemiol*. 2010;20:310–319.

Olsson AC, Fevotte J, Fletcher T, Cassidy A, 't Mannetje A, Zaridze D, Szeszenia-Dabrowska N, Rudnai P, Lissowska J, Fabianova E, et al. Occupational exposure to polycyclic aromatic hydrocarbons and lung cancer risk: a multicenter study in Europe. *Occup Environ Med*. 2010;67:98–103.

Perera FP, Dickey C, Santella R, O'Neill AP, Albertini RJ, Ottman R, Tsai WY, Mooney LA, Savela K, Hemminki K. Carcinogen-DNA adducts and gene mutation in foundry workers with low-level exposure to polycyclic aromatic hydrocarbons. *Carcinogenesis*. 1994;15:2905–2910.

Rasmussen SA, Olney RS, Holmes LB, Lin AE, Keppler-Noreuil KM, Moore CA. Guidelines for case classification for the National Birth Defects Prevention Study. *Birth Defects Res A Clin Mol Teratol*. 2003;67:193–201.

Ren A, Qiu X, Jin L, Ma J, Li Z, Zhang L, Zhu H, Finnell RH, Zhu T. Association of selected persistent organic pollutants in the placenta with the risk of neural tube defects. *Proc Natl Acad Sci U S A*. 2011;108:12770–12775.

Rennie MY, Detmar J, Whiteley KJ, Yang J, Jurisicova A, Adamson SL, Sled JG. Vessel tortuousity and reduced vascularization in the fetoplacental arterial tree after maternal exposure to polycyclic aromatic hydrocarbons. *Am J Physiol Heart Circ Physiol*. 2011;300:H675–H684.

Rocheleau CM, Lawson CC, Waters MA, Hein MJ, Stewart PA, Correa A, Echeverria D, Reefhuis J. Inter-rater reliability of assessed prenatal maternal occupational exposures to solvents, polycyclic aromatic hydrocarbons, and heavy metals. *J Occup Environ Hyg*. 2011;8:718–728.

Romitti PA, Lidral AC, Munger RG, Daack-Hirsch S, Burns TL, Murray JC. Candidate genes for nonsyndromic cleft lip and palate and maternal cigarette smoking and alcohol consumption: evaluation of genotype-environment interactions from a population-based case-controls study of orofacial clefts. *Teratology*. 1999;59:39–50.

Shaw GM, Carmichael SL, Vollset SE, Yang W, Finnell RH, Blom H, Midttun O, Ueland PM. Mid-pregnancy cotinine and risks of orofacial clefts and neural tube defects. *J Pediatr*. 2009;154:17–19.

Shaw GM, Iovannisci DM, Yang W, Finnell RH, Carmichael SL, Cheng S, Lammer EJ. Endothelial nitric oxide synthase (NOS3) genetic variants, maternal smoking, vitamin use, and risk of human orofacial clefts. *Am J Epidemiol*. 2005;162(12):1207–1214.

Shaw GM, Wasserman CR, Lammer EJ, O'Malley CD, Murray JC, Basart AM, Tolarova MM. Orofacial clefts, parental cigarette smoking, and transforming growth factor-alpha gene variants. *Am J Hum Genet*. 1996;58:551–561.

Shi M, Christensen K, Weinberg CR, Romitti P, Bathum L, Lozada A, Morris RW, Lovett M, Murray JC. Orofacial cleft risk is increased with maternal smoking and specific detoxification-gene variants. *Am J Hum Genet*. 2007;80:76–90.

Shimada T. Xenobiotic-metabolizing enzymes involved in activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons. *Drug Metab Pharmacokinet*. 2006;21:257–276.

Shum S, Jensen NM, Nebert DW. The murine Ah locus: *in utero* toxicity and teratogenesis associated with genetic differences in benzo(a)pyrene metabolism. *Teratology*. 1979;20:365–376.

Sjaastad AK, Svendsen K. Exposure to polycyclic aromatic hydrocarbons (PAHs), mutagenic aldehydes, and particulate matter in Norwegian a la carte restaurants. *Ann Occup Hyg*. 2009;53:723–729.

Stewart PA, Stewart WF, Siemiatycki J, Heineman EF, Dosemeci M. Questionnaires for collecting detailed occupational information for community-based case control studies. *Am Ind Hyg Assoc J*. 1998;59:39–44.

Topinka J, Milcova A, Libalova H, Novakova Z, Rossner P Jr, Balascak I, Sram RJ. Biomarkers of exposure to tobacco smoke and environmental pollutants in mothers and their transplacental transfer to the foetus. Part I: bulky DNA adducts. *Mutat Res*. 2009;669:13–19.

U, S. Department of Labor, Bureau of Labor Statistics. North American industry classification system. Available at http://www.bls.gov/bls/naics.htm. Accessed August 29, 2009.

U.S. Department of Labor, Bureau of Labor Statistics. Standard occupational classification. Available at http://www.bls.gov/soc/soc_majo.htm. Accessed August 29, 2009.

van den Eeden SK. Karagas MR, Daling JR, Vaughan TL. A case-control study of maternal smoking and congenital malformations. *Paediatr Perinat Epidemiol*. 1990;4:147–155.

van Rooij IA, Groenen PM, van Drongelen M, Te Morsche RH, Peters WH, Steegers-Theunissen RP. Orofacial clefts and spina bifida: N-acetyltransferase phenotype, maternal smoking, and medication use. *Teratology*. 2002;66:260–266.

van Rooij IA, Wegerif MH, Roelofs HM, Peters WH, Kuijpers-Jagtman AM, Zielhuis GA, Merkus HM, Steegers-Theunissen RP.

Smoking, genetic polymorphisms in biotransformation enzymes, and nonsyndromic oral clefting: a gene-environment interaction. *Epidemiology*. 2001;12:502–507.

Wassenberg DM, Nerlinger AL, Battle LP, Di Giulio RT. Effects of the polycyclic aromatic hydrocarbon heterocycles, carbazole and dibenzothiophene, on *in vivo* and *in vitro* CYP1A activity and polycyclic aromatic hydrocarbon-derived embryonic deformities. *Environ Toxicol Chem*. 2005;24:2526–2532.

Werler MM, Lammer EJ, Rosenberg L, Mitchell AA. Maternal cigarette smoking during pregnancy in relation to oral clefts. *Am J Epidemiol*. 1990;132:926–932.

Wyszynski DF, Duffy DL, Beaty TH. Maternal cigarette smoking and oral clefts: a meta-analysis. *Cleft Palate Craniofac J*. 1997;34:206–210.

Wyszynski DF, Wu T. Use of US birth certificate data to estimate the risk of maternal cigarette smoking for oral clefting. *Cleft Palate Craniofac J*. 2002;39:188–192.

Yoon PW, Rasmussen SA, Lynberg MC, Moore CA, Anderka M, Carmichael SL, Costa P, Druschel C, Hobbs CA, Romitti PA, et al. The National Birth Defects Prevention Study. *Public Health Rep*. 2001;116(suppl 1):32–40.

Zeiger JS, Beaty TH, Liang KY. Oral clefts, maternal smoking, and TGFA: a meta-analysis of gene-environment interaction. *Cleft Palate Craniofac J*. 2005;42:58–63.