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Nov. 15, 2013

Millenium Bulk Terminals-Longview EIS
c/o ICF International
701 Second Ave. Suite 550
Seattle, WA 98104

Dear Sirs and Madams:

As a retired pediatrician specializing in the care of children with birth defects during my career, I wish to make the EIS taskforce aware of recent significant epidemiologic research published in peer-reviewed scientific journals regarding the concern of increased risk for serious neural tube defects (spina bifida and anencephaly) in offspring of pregnant women in Shanxi Province, China, where there is widespread use of coal for energy for heating and home cooking. Both epidemiological and placental studies are concerning for indoor exposure to polycyclic aromatic hydrocarbons. Dr. Zhiwen Li is a highly respected epidemiologist in the field of Reproductive Medicine, and Dr. Richard H. Finnell has made major research contributions to our current knowledge of neural tube defects. I should mention that the concern for exposure to these chemicals is coal combustion, not inhaled dust from living nearby to passage of coal trains, so I am not aware of any such risk for pregnant women in Washington State. However, I do feel this research concern does behoove the governments of the United States and China to work toward expediting capability of future exporting of natural gas and use of cleaner energy sources for heating and cooking in the homes of China. I do share the concerns of Dr. Melissa Weakland printed in the "Opinion" section of the Tacoma News Tribune (page A21) on January 25, 2013 regarding the potential adverse impact of coal trains on adults and children with chronic respiratory disorders in communities where coal trains will pass. There is now additional concern from the birth defects scientific community as well regarding indoor pollution exposure for the people of China who use the coal for heat and cooking, especially the offspring of pregnant women. I am enclosing copies of two of several pertinent studies regarding this issue that have been published in the scientific literature for your review.

Thank you for the opportunity for public comment for the EIS process regarding this issue.

Sincerely,

Mark J. Stephan MD

Mark J. Stephan, M.D.

P.O. Box 98450
Lakewood, WA 98496-8450
Nov. 15, 2013

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As a retired pediatric lung specialist in the care of children with birth defects during my career, I wish to make the EIS auditors aware of recent alarming epidemiologic research published in peer-reviewed scientific journals regarding the concern of increased risk for serious neural tube defects (spina bifida and anencephaly) in offspring of pregnant women in Shanxi Province, China, where there is widespread use of coal for energy, for heating and home cooking. Both epidemiological and placental studies are concerning for indoor exposure to polycyclic aromatic hydrocarbons. Dr. Zhiwen Li is a highly respected epidemiologist in the field of Reproductive Medicine and Dr. Richard H. Finnell has made major research contributions to our current knowledge of neural tube defects. I do not pretend that the concern for exposure to these chemicals is usual combustion, and tiny dust from burning results in passage of vital fetal, so I am not aware of any such risk for pregnant women in Washington State. However, I do feel this research concern does behoove the government of the United States and China to work toward expediting capability of future experimenting to natural gas and use of better energy sources for heating and cooking in the homes of China. I do share the concerns of Dr. Melissa Wuellner printed in the "Opinion" section of the Tacoma News Tribune (page A23) on January 23, 2013 regarding the potential adverse impact of coal trains on adults and children with chronic respiratory disorders in communities where coal trains will pass. There is now additional concern from the high details scientific community as well regarding indoor pollution exposure for the people of China who use the coal for heat and cooking, especially the offspring of pregnant women. I am including copies of two of several pertinent studies regarding this issue that have been published in the scientific literature for your review.

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Mark J. Stephens, M.D.

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Original Contribution

Indoor Air Pollution From Coal Combustion and the Risk of Neural Tube Defects in a Rural Population in Shanxi Province, China

Zhiwen Li, Le Zhang, Rongwei Ye, Lijun Pei, Jianmeng Liu, Xiaoying Zheng, and Aiguo Ren*

* Correspondence to Dr. Aiguo Ren, Institute of Reproductive and Child Health, Peking University Health Science Center, 38 Xueyuan Road, Haidian District, Beijing 100191, China (e-mail: renag@bjmu.edu.cn).

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The authors evaluated indoor air pollution from coal combustion (IAPCC) as a potential risk factor for neural tube defects (NTDs) in a rural population in Shanxi Province, China. The studied rural population has both high IAPCC exposure and a high prevalence of NTDs. A population-based case-control study was used to identify 610 NTD cases and 837 normal controls between November 2002 and December 2007. Information was collected within 1 week following delivery or pregnancy termination due to prenatal NTD diagnosis. The authors derived an exposure index by integrating a series of IAPCC-related characteristics concerning dwelling and lifestyle. Compared with women with no IAPCC exposure, women with any exposure at all had a 60% increased risk of having a child with an NTD (adjusted odds ratio (OR) = 1.6, 95% confidence interval (CI): 1.1, 2.1). An increased NTD risk was linked to both residential heating (adjusted OR = 1.7, 95% CI: 1.1, 2.4) and cooking (adjusted OR = 1.5, 95% CI: 1.1, 2.1). The risk increased with increases in the exposure index, showing a dose-response trend ($P < 0.001$). This is the first known study to link IAPCC to NTDs. Additional studies are needed to confirm the link between IAPCC and NTDs.

air pollution, indoor; China; coal; neural tube defects; polycyclic hydrocarbons, aromatic

Abbreviations: CI, confidence interval; IAPCC, indoor air pollution from coal combustion; NTDs, neural tube defects; OR, odds ratio; PAHs, polycyclic aromatic hydrocarbons.

Indoor air pollution caused by the use of solid fuel (coal or biomass) is a major public health challenge in developing countries, where a substantial proportion of the population relies on such fuels for their domestic energy needs (1). In China, more than 70% of households rely on solid fuels for cooking and residential heating (2). While use of biomass fuel has remained relatively constant over the past 20 years in China, coal use in rural homes has tripled (3). Given the limited reserves of petroleum and natural gas and the relative abundance of coal, it is likely that this coal-based energy structure will persist for many years to come in China.

The combustion of coal and biomass indoors emits a substantial amount of toxic pollutants, including particulate matter, polycyclic aromatic hydrocarbons (PAHs), carbon monoxide, nitrogen oxides, and sulfur dioxide (4). People living in households relying on coal-based heating or cooking may experience a high level of exposure to pollutants, particularly women and young children who spend a large proportion of their time indoors. Indoor pollution from coal smoke has been implicated as a causative agent in lung cancer, especially among nonsmoking women (5).

Emerging evidence suggests that ambient air pollution may contribute to birth defects (6–13). These findings linking outdoor air pollution to birth defects may be particularly relevant for rural China, where women are exposed to substantial levels of indoor air pollution from solid fuels. However, little information is available about the role of such indoor exposures in the etiology of birth defects.

Shanxi Province possesses abundant coal reserves and is the leading coal-producing province in China. Almost all local rural Shanxi households use coal for their residential heating and cooking needs. Exposure to substantial levels of indoor air pollution from coal combustion (IAPCC) is highly prevalent in the Shanxi population (14–16). In

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addition, Shanxi Province has the highest prevalence of neural tube defects (NTDs) worldwide, at 10–20 cases per 1,000 births in some counties (17, 18). The Shanxi population provides a unique opportunity to assess the association of IAPCC with the risk of NTDs.

MATERIALS AND METHODS

Location and population

This study was conducted in 4 rural counties (Pingding, Xiyang, Taigu, and Zezhou) in Shanxi Province. The total number of births per year in the study area is approximately 10,000. A population-based birth defect surveillance system, which has been previously described (17), was established in this area in 2002. The surveillance system monitors major external structural birth defects through active case ascertainment. Cases in the study included liveborn infants, stillborn infants (fetal death at ≥20 weeks' gestation), and prenatally diagnosed, electively aborted fetuses of mothers residing in the study areas. County health workers verified the diagnoses by physical examination of the fetal body for all pregnancy outcomes and filled out a reporting form for each case. We requested photographs of every infant born with a suspected birth defect, if available. Pediatricians at Peking University reviewed all case report forms and photographs before assigning final diagnostic codes. Each case was also classified as either isolated, if there was no concurrent major malformation, or nonisolated, if there was at least 1 accompanying major external malformation.

Using data gathered from the surveillance system, an ongoing population-based case-control study was initiated to explore potential risk factors for external structural birth defects in the study area (19). When an infant was identified as having any major external malformation, a newborn control without any external structural birth defects was matched to the case infant by county, sex, maternal ethnic group, and date of conception (as close as possible). Trained health-care workers conducted face-to-face interviews with the mothers of the cases and controls within 1 week of delivery to collect exposure information using a structured questionnaire. Data were collected on maternal demographic characteristics, lifestyle, illness, medication use, and the use of folic acid supplements. A series of questions related to cooking and heating at home were asked to assess exposure status with respect to IAPCC during the periconceptional period. The participation rate was more than 80% for both case mothers and control mothers. The study protocol was approved by the institutional review board of Peking University. All participants provided written informed consent.

NTD cases identified between November 2002 and December 2007 were included in the study analysis. To increase statistical power, all controls, including controls for NTDs as well as for other major external structural malformations, were included in the analysis. A total of 1,503 participants (637 NTD cases and 866 controls) were available during the study period. We excluded 52 infants (3.5%) with missing data on questions related to IAPCC and 4 infants (0.3%) whose families used biomass as their primary fuel for cooking and heating (biomass exposure may also be a risk factor but was not analyzed because of the small numbers of available biomass-only exposure subjects). Following these exclusions, 610 NTD cases (277 anencephaly, 289 spina bifida, and 44 encephalocele) and 837 controls were available for analysis.

Exposure assessment

Mothers answered a series of 8 questions related to their cooking and heating activities during the periconceptional period, from 1 month before conception to 2 months after conception, to characterize their exposure to IAPCC, including frequency of cooking in the kitchen, primary fuel used for cooking, whether the kitchen was separated from the living room or bedroom, whether their periconceptional period fell within the heating season, whether a coal stove was used for heating, whether the stove was placed in the living room or bedroom, the primary fuel used for heating, and the frequency of house ventilation during the heating season. (The questions and their response options are listed in the Web Appendix, which is posted on the *Journal*'s Web site (http://aje.oxfordjournals.org/).) These questions served as proxy measures for IAPCC in the living environment. We identified 3 potential exposure sources and assigned an exposure value for each source according to the mothers' responses to these questions, as follows.

1. Cooking exposure: When the primary home cooking fuel used was coal, the kitchen exposure value was recorded as 2 if the mother had cooked daily or 1 if the mother had cooked occasionally; otherwise, it was recorded as 0.
2. Attached kitchen exposure: When the primary home cooking fuel was coal and the kitchen was not separated from the living room or bedroom, the attached kitchen exposure value was recorded as 1; otherwise, it was recorded as 0.
3. Heating exposure: When the periconceptional period fell within the heating season, the primary home heating fuel was coal, and a coal stove was used for heating in the living room or bedroom, the heating exposure value was recorded as 2 if the house was almost never ventilated or 1 if the house was occasionally ventilated; otherwise, the value was recorded as 0.

We formed an IAPCC exposure index by summing the exposure values for all individual indoor air pollution sources. The index assumed that the effects of coal smoke were cumulative across the various exposure sources.

Statistical analysis

The original study used a pair-matching design, but for this analysis the matching was broken in order to increase statistical power. Therefore, we used an unmatched analysis to estimate the association between IAPCC and NTDs. We analyzed the IAPCC index as both an ordinal variable and a categorical variable to comprehensively elucidate the exposure effects and any linear trends. Risk was estimated by means of the odds ratio, and the precision of the odds ratio was characterized by its 95% confidence interval. We used χ^2 trend tests and logistic regression with the IAPCC index

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Table 1. Demographic and Obstetric Characteristics of Neural Tube Defect Cases and Controls in Shanxi Province, China, 2002–2007

Characteristic	Cases (n = 610)[a]		Controls (n = 837)[a]		Crude Odds Ratio	95% Confidence Interval
	No.	%	No.	%		
County of residence						
Pingding	171	28.0	248	29.6	1	
Xiyang	90	14.8	113	13.5	1.2	0.8, 1.6
Taigu	97	15.9	161	19.2	0.9	0.6, 1.2
Zezhou	252	41.3	315	37.6	1.2	0.9, 1.5
Season of conception						
Spring	157	26.1	194	23.7	1	
Summer	135	22.5	206	25.2	0.8	0.6, 1.1
Autumn	160	26.7	195	23.9	1.0	0.8, 1.4
Winter	148	24.7	222	27.2	0.8	0.6, 1.1
Maternal age, years						
<20	16	2.7	10	1.2	2.3	1.0, 5.2
20–24	205	34.3	293	35.6	1.0	0.8, 1.3
25–29	201	33.5	290	35.2	1	
30–34	120	20.1	181	22.0	1.0	0.7, 1.3
≥35	56	9.4	49	6.0	1.6	1.1, 2.5
Occupation						
Farmer	406	68.2	555	67.1	1	
Worker	18	3.0	25	3.0	1.0	0.5, 1.8
Professional	17	2.9	29	3.5	0.8	0.4, 1.5
Service/sales	20	3.4	35	4.2	0.8	0.4, 1.4
Other	134	22.5	183	22.1	1.0	0.8, 1.3
Education						
Primary school or lower	100	16.5	50	6.0	1	
Junior high school	435	71.5	641	76.9	0.4	0.3, 0.6
High school or above	73	12.0	143	17.1	0.4	0.2, 0.5

Table continues

Table 1. Continued

Characteristic	Cases (n = 610)[a]		Controls (n = 837)[a]		Crude Odds Ratio	95% Confidence Interval
	No.	%	No.	%		
Ethnicity						
Han	608	99.8	832	99.6	1	
Other	1	0.2	3	0.4	0.3	0.0, 2.7
Parity						
Primiparous	313	55.1	507	62.1	1	
Multiparous	255	44.9	309	37.9	1.2	1.0, 1.4
Multiple births						
No	572	96.3	811	98.8	1	
Yes	22	3.7	10	1.2	3.1	1.5, 6.6
History of pregnancy affected by birth defects						
No	567	93.9	817	97.8	1	
Yes	37	6.1	18	2.2	3.0	1.9, 5.3
Periconceptional use of folic acid						
Yes	64	10.8	114	14.2	1	
No	530	89.2	691	85.8	1.2	0.9, 1.6
Influenza or fever						
No	397	68.4	739	91.3	1	
Yes	183	31.6	70	8.7	4.9	3.6, 6.6
Passive smoking						
No	189	31.1	362	43.4	1	
Yes	418	68.9	473	56.6	1.7	1.4, 2.1
Infant sex						
Male	266	44.4	394	47.5	1	
Female	333	55.6	436	52.5	1.1	0.9, 1.4

[a] Values for some characteristics may not sum to the total number of cases or controls because of missing data.

as an ordinal variable to evaluate the relation between increased exposure to IAPCC and the risk of NTDs. Multivariable logistic regression was used to estimate an adjusted odds ratio controlling for the original matching variables and some potentially confounding variables. The matching variables included county of residence (Zezhou, Pingding, Xiyang, or Taigu), season of conception (spring, summer, autumn, or winter), maternal ethnic group (Han/other), and infant sex (male/female). The potentially confounding variables included maternal age (<20, 20–34, or ≥35 years), education (primary school or less, more than primary school), multiparity (yes, no), multiple births (yes, no), history of pregnancy affected by birth defects (yes, no), maternal influenza or fever (yes, no), and passive smoking (yes, no) during the periconceptional period. These variables were chosen because they were significantly associated with NTD risk in the univariate analysis at the $P < 0.05$ level. All data were analyzed using SPSS 11.5 (SPSS, Inc., Chicago, Illinois).

RESULTS

The demographic characteristics of the study population are presented in Table 1. Nearly all mothers were of Han ethnicity. Most of the mothers were aged 20–34 years and had not taken folic acid supplements during their periconceptional period. Compared with control mothers, case mothers were more likely to be aged <20 years or ≥35 years, to have a primary school or lower education, to be multiparous, to have had multiple births, to have a history of pregnancy affected by birth defects, to have influenza or fever, and

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Table 2. Distribution of IAPCC Exposure Variables in Neural Tube Defect Cases and Controls, Shanxi Province, China, 2002–2007

IAPCC Exposure Variable	Cases (n = 610)[a]		Controls (n = 837)[a]		Crude Odds Ratio	95% Confidence Interval
	No.	%	No.	%		
Primary fuel used for cooking						
Natural gas/other[b]	60	9.9	145	17.4	1	
Coal	544	90.1	688	82.6	1.9	1.4, 2.6
Frequency of cooking in the kitchen						
Almost none	91	14.9	154	18.4	1	
Sometimes	183	30.0	319	38.1	1.0	0.7, 1.3
Daily	336	55.1	364	43.5	1.6	1.2, 2.1
Separate kitchen from living room/bedroom						
Yes	523	85.7	719	85.9	1	
No	87	14.3	118	14.1	1.0	0.8, 1.4
Conceiving during the heating season						
No	288	47.2	398	47.6		
Yes	322	52.8	438	52.4	1.0	0.8, 1.3
Using a stove for heating						
No	90	28.2	169	38.8	1	
Yes	229	71.8	267	61.2	1.6	1.2, 2.2
Primary fuel used for heating						
Natural gas/other[b]	1	0.4	2	0.7	1	
Coal	228	99.6	265	99.3	1.7	0.2, 19.1
Stove in the living room/bedroom						
No	60	26.2	90	33.8	1	
Yes	169	73.8	176	66.2	1.4	1.0, 2.1
Frequency of ventilation in the living room/bedroom						
Daily	85	37.1	124	46.4	1	
Sometimes	120	52.4	134	50.2	1.3	0.9, 1.9
Almost none	24	10.5	9	3.4	3.9	1.7, 8.8

Abbreviation: IAPCC, indoor air pollution from coal combustion.

[a] Values for some characteristics may not sum to the total number of cases or controls because of jump terms or missing data.

[b] Does not include biomass fuel.

to have been passively exposed to smoking during the periconceptional period. No significant differences were found between the case and control groups for any other characteristics.

Table 2 shows the distribution of variables related to cooking and heating in the case and control groups. More than 80% of households utilized coal as their primary domestic fuel for cooking, and nearly all families (>99%) used coal for residential heating. Compared with control mothers, NTD case mothers were more likely to use coal as their primary fuel (relative to nonbiomass solid fuels), to have a higher frequency of cooking in the kitchen, to use a coal stove for heating, to have a coal stove in the living room/bedroom, and to ventilate the living room/bedroom less frequently. All of these items indicate a higher IAPCC exposure in the NTD group.

Table 3 shows the association between the IAPCC exposure index and the risk of NTDs. Overall, 80.2% of case mothers and 72.9% of control mothers had IAPCC exposure during the periconceptional period. IAPCC exposure was associated with a 60% increase in NTD risk relative to no exposure (adjusted odds ratio (OR) = 1.6, 95% confidence interval (CI): 1.1, 2.1). Both crude and adjusted odds ratios tended to increase with increasing score on the exposure index, demonstrating a clear linear trend ($P < 0.001$). Similar results were observed for anencephaly and spina bifida when these defects were analyzed separately (data not shown).

We further analyzed the association of NTDs with each of the 3 different exposure sources separately to understand their respective effects (Table 4). An increased risk of NTDs was associated with IAPCC exposure from heating (adjusted OR = 1.7, 95% CI: 1.1, 2.4) and from cooking (adjusted OR = 1.5, 95% CI: 1.1, 2.1) but not with exposure from having a kitchen attached to the living room/bedroom (adjusted OR = 1.1, 95% CI: 0.7, 1.6). Approximately 70% of women in the study were exposed to IAPCC from a cooking source, while less than 20% of women were exposed to IAPCC from a heating source.

We further examined the IAPCC index as an ordinal variable to analyze its association with each type of NTD. As Table 5 shows, a 1-unit increase in the exposure index was significantly associated with a 30% increased overall risk of NTDs (adjusted OR = 1.3, 95% CI: 1.1, 1.5) and a 20%–40% increase in the risk of the various subtypes of NTDs.

DISCUSSION

In this population-based case-control study, we found that among a rural population in Shanxi Province, maternal exposure to indoor coal smoke was significantly associated with an increased risk of anencephaly, spina bifida, and NTDs overall. The NTD risk increased with increases in the exposure index. IAPCC exposures from heating and cooking were both linked to increased NTD risk.

Maternal active or passive cigarette smoking during pregnancy has long been associated with several birth defects, including NTDs (19–22). In recent years, outdoor air pollution has also been linked to some birth defects (6–13). Most of the deleterious pollutants in urban air pollution, tobacco smoke, or biomass smoke, such as PAHs, carbon monoxide, and heavy metals, are also prominent in coal smoke. Because of decreased dispersal or photo-degradation, some pollutants exist at even higher levels indoors in rural households of

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Table 3. IAPCC Exposure Index Scores for Neural Tube Defect Cases and Controls and Association With Risk of Neural Tube Defects in Shanxi Province, China, 2002–2007

IAPCC Exposure Index Score	Cases		Controls		Crude OR*	95% CI	Adjusted OR*[a]	95% CI
	No.	%	No.	%				
0[b]	121	19.8	227	27.1	1		1	
1	140	22.9	223	26.6	1.2	0.9, 1.6	1.2	0.9, 1.8
2	239	39.2	289	34.5	1.6	1.2, 2.1	1.7	1.2, 2.5
3	81	13.3	90	10.8	1.7	1.2, 2.5	1.8	1.1, 2.9
≥4	29	4.8	8	1.0	6.8	3.0, 15.3	5.4	2.0, 14.1
Any[c]	489	80.2	610	72.9	1.5	1.2, 1.9	1.6	1.1, 2.1

Abbreviations: CI, confidence interval; IAPCC, indoor air pollution from coal combustion; OR, odds ratio.

* $P_{trend} < 0.001$.

[a] Adjusted for county of residence (Zezhou, Pingding, Xiyang, Taigu); season of conception (spring, summer, autumn, winter); maternal ethnic group (Han, other); infant sex (male, female); maternal age (<20 or ≥35 years, 20–34 years); maternal education (primary school or less, more than primary school); multiparity (yes, no); multiple births (yes, no); history of pregnancy affected by birth defects (yes, no); maternal influenza or fever (yes, no); and passive smoking during the periconceptional period (yes, no).

[b] Reference category.

[c] Exposure index scores 1–5 combined.

China. Thus, there is good reason to expect that this pollution exposure would affect pregnancy outcomes in these settings.

Chronic exposure to PAHs may be the primary mechanism by which coal smoke increases the risk of NTDs. PAHs are the most common complex mixtures in coal smoke. Some PAHs, such as benzo(*a*)pyrene, are well-known human carcinogens and also demonstrate teratogenic effects in animal studies (23–25). Our previous studies indicated very high levels of benzo(*a*)pyrene pollution in local households,

Table 4. Association of Various IAPCC Exposure Sources With Risk of Neural Tube Defects in Shanxi Province, China, 2002–2007

IAPCC Exposure Source and Exposure Index Score[a]	Cases		Controls		Crude OR	95% CI	Adjusted OR[b]	95% CI
	No.	%	No.	%				
Heating								
0	501	82.1	748	89.4	1		1	
1	90	14.8	82	9.8	1.6	1.2, 2.3	1.7	1.1, 2.5
2	19	3.1	7	0.8	4.1	1.7, 9.7	1.6	0.6, 4.3
Any[c]	109	17.9	89	10.6	1.8	1.4, 2.5	1.7	1.1, 2.4
Cooking								
0	139	22.8	256	30.6	1		1	
1	164	26.9	254	30.3	1.2	0.9, 1.6	1.2	0.9, 1.7
2	307	50.3	327	39.1	1.7	1.3, 2.2	1.9	1.3, 2.7
Any[c]	471	77.2	581	69.4	1.5	1.2, 1.9	1.5	1.1, 2.1
Attached kitchen								
0	535	87.7	737	88.1	1		1	
1	75	12.3	100	11.9	1.0	0.7, 1.3	1.1	0.7, 1.6

Abbreviations: CI, confidence interval; IAPCC, indoor air pollution from coal combustion; OR, odds ratio.

[a] An exposure index score of 0 was the reference category.

[b] Adjusted for county of residence (Zezhou, Pingding, Xiyang, Taigu); season of conception (spring, summer, autumn, winter); maternal ethnic group (Han, other); infant sex (male, female); maternal age (<20 or ≥35 years, 20–34 years); maternal education (primary school or less, more than primary school); multiparity (yes, no); multiple births (yes, no); history of pregnancy affected by birth defects (yes, no); maternal influenza or fever (yes, no); passive smoking during the periconceptional period (yes, no); and the 3 exposure sources (heating, cooking, and attached kitchen; yes, no).

[c] Exposure index scores 1 and 2 combined.

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Table 5. Association of a 1-Unit Increase in IAPCC Exposure Index Score (Ordinal) With the Risk of Neural Tube Defects, Overall and by Subtype, in Shanxi Province, China, 2002–2007

	No. of Cases	No. of Controls	Crude OR[a]	95% CI	Adjusted OR[a,b]	95% CI
All NTDs	610	837	1.3	1.2, 1.4	1.3	1.1, 1.5
NTD subtype						
Anencephaly	277	837	1.4	1.2, 1.6	1.4	1.2, 1.7
Spina bifida	289	837	1.2	1.1, 1.4	1.2	1.0, 1.4
Encephalocele	44	837	1.2	0.9, 1.7	1.4	0.9, 2.1
Isolated or nonisolated[c] defect						
Isolated NTDs	577	837	1.3	1.2, 1.4	1.3	1.1, 1.5
Nonisolated NTDs	33	837	1.2	0.9, 1.7	1.4	0.9, 2.3

Abbreviations: CI, confidence interval; IAPCC, indoor air pollution from coal combustion; NTD, neural tube defect; OR, odds ratio.

[a] Odds ratio for a 1-unit change in IAPCC exposure index score.

[b] Adjusted for county of residence (Zezhou, Pingding, Xiyang, Taigu); season of conception (spring, summer, autumn, winter); maternal ethnic group (Han, other); infant sex (male, female); maternal age (<20 or ≥35 years, 20–34 years); maternal education (primary school or less, more than primary school); multiparity (yes, no); multiple births (yes, no); history of pregnancy affected by birth defects (yes, no); use of folic acid supplements (yes/no); maternal influenza or fever (yes, no); and passive smoking during the periconceptional period (yes, no).

[c] Associated with other major external structural birth defects.

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especially in the winter. Of the 48 pairs of samples collected from kitchens and bedrooms in winter, 97.9% and 93.8% of samples exceeded recommended national standards for indoor air benzo(*a*)pyrene levels (1 ng/m^3), and the median concentration reached 15.0 ng/m^3 in kitchen samples and 11.84 ng/m^3 in bedroom samples. Even in summer, 63.3% of kitchens had a benzo(*a*)pyrene concentration that was over national standards (14). A comparison study indicated that the median PAH content of high-molecular-weight (4 ring and larger) compounds in the settled house floor dust in the rural households of Shanxi Province (162 μg/m^2) was 40-fold higher than what has been recorded in Azerbaijan (2.9 μg/m^2) and more than 200-fold higher than reported levels in Texas (0.11 μg/m^2). The surface loadings of carcinogenic PAHs followed the same trend, with median loading being 50-fold higher in China than in Azerbaijan and levels of carcinogenic PAHs being below detection limits in most of the Texas samples (15). High levels of genotoxic mixtures of PAHs were detected in the settled dust of the average rural households sampled in Shanxi Province (16). A recent case-control study in this population showed that the mothers of newborn children with an NTD exhibited significantly elevated levels of PAHs in their blood as compared with the levels observed in normal controls. However, the study did not indicate a correlation with PAH-DNA adducts, which may suggest a possible nongenotoxic mechanism (26).

Other suspected pollutants from domestic coal combustion that may be involved in the increased risk of NTDs include carbon monoxide and some toxic heavy metals such as arsenic and lead. Carbon monoxide reduces the capacity of the blood to carry oxygen to the body tissues by binding to hemoglobin and forming carboxyhemoglobin. Experimental studies have demonstrated that chronic low-level maternal carbon monoxide exposure may decrease the metabolism of xenobiotics such as benzo(*a*)pyrene, interfere with the metabolic and transport functions of the placenta, and have a toxic effect on the developing nervous system of rats (6, 27). Arsenic and lead are neurotoxins. While the risks of human maternal exposure to arsenic or lead during pregnancy are not well known, studies involving pregnant animals exposed to the metals have linked them to a variety of congenital malformations, including NTDs (13).

Our study showed that exposure to indoor air pollution from both heating and cooking sources contributed significantly to an increased risk of NTDs. In the study population, more than 85% of kitchens are separated from living areas with poor ventilation. Although the time spent in the kitchen is relatively short, the kitchen is significantly more polluted than the living area (14). In addition, the percentage of women exposed to cooking source pollution (approximately 70%) was far higher than that of women exposed to heating source pollution (<20%) because heating was only required in the winter and most households had relatively good ventilation in the living area. Domestic cooking is an important duty of most local housewives, and most women usually persist in this cooking role throughout pregnancy. Our results showed that more than 80% of the women cooked frequently, and approximately 50% of them had cooked daily during the periconceptional period. Chen et al. (28) found that cooking frequency was associated with higher levels of urinary 1-hydroxypyrene in Chinese women, indicating that a higher PAH exposure was also associated with frequent cooking. Therefore, cooking exposure may play an important role in the development of NTDs in the rural population of Shanxi Province.

Tobacco smoking is another source of indoor air pollution. In China, approximately 2 of every 3 adult men are regular smokers, whereas very few women smoke actively. Passive smoking, however, is widespread in China. In the

study population, less than 2% of women were active smokers, whereas some 60% of women had been passively exposed to tobacco smoke during the periconceptional period. Our previous study demonstrated that maternal passive smoking was associated with an increased risk of NTDs (19). When this potential confounder was included in the multivariate model used here, the risk of NTDs attributed to IAPCC was not substantially changed.

It is noteworthy that the effect estimate for heating source index level 2 decreased from 4.1 to 1.6 with adjustment (Table 4). The dramatic change may be related to the close correlation between heating exposure and the variable "influenza or fever." The 2 factors are both related to heating season and poor ventilation. If this "influenza or fever" factor was removed from the final adjustment model, the effect estimate for the index level 2 heating source variable was 3.0 (95% CI: 1.2, 7.7) (data not shown).

Our study had several strengths. The study was population-based, with a relatively large sample size, a high participation rate, and adjustment for several potential confounders. The very high prevalence of NTDs in the study population increased the likelihood of identifying important risk factors. The study population was relatively homogeneous. All birth defect cases were reviewed by pediatricians at Peking University using clinical descriptions and photographs. This additional review decreased the likelihood of misclassification. Our study considered all types of pregnancies affected by NTDs, including elective terminations due to prenatal diagnosis. Participants were interviewed within the first week after delivery or pregnancy termination, and the interviewers helped them to confirm the period of exposure according to the dates of their last menstrual period. Therefore, misclassification due to exposure timing was minimized. To our knowledge, this study was the first to assess the effects of IAPCC and the risk of NTDs in a Chinese population.

Our study had several limitations. As in any other case-control study, recall bias is a major concern. Case mothers may tend to overreport factors they believe to have influenced their NTD-affected pregnancies in comparison with control mothers. However, because there have been no published scientific reports regarding the possible association between indoor air pollution and the risk of NTDs so far, and because the interval from birth or pregnancy termination to interview was relatively short, any recall bias should have been minimal. In our questionnaire, potential data indicating IAPCC exposure were distributed throughout several separate and relatively objective questions about dwelling or lifestyle around the time of conception. Therefore, it is unlikely that recall bias substantially affected our results. In our study, one of the matching criteria for control selection was the date of conception. This criterion was originally designed to control for the effect of seasonal dietary variation. However, because heating is highly correlated with season, matching by date of conception may lead to overmatching, resulting in underestimation of the effect of IAPCC. Questions about dwelling and lifestyle characteristics are indirect measures of coal smoke exposure and may not accurately reflect individual exposure. Ventilation during cooking was not investigated; thus, its effect on the exposure index could not be estimated. These limitations could have resulted in nondifferential exposure misclassification and therefore weakened the true association. In addition, the IAPCC exposure index was newly created for use in this study and has not been validated in other studies. Therefore, it may need to be validated with other data sets and in other settings in the future. In this study, information on dietary folate intake was not collected, preventing us from adjusting for its potentially confounding effect. However, the diet of the local residents is not very diverse. Variation in folate intake from diet cannot explain the observed association between IAPCC exposure and NTD risk.

China has the largest coal production and consumption in the world. IAPCC is a major public health issue in rural China because of the abundant use of coal and low awareness of potential health risks among the population. Women may experience high IAPCC exposure because of the large amount of time they spend both indoors and cooking. Our results, together with evidence of the association of other negative pregnancy outcomes with IAPCC, may draw more attention to the potential damage caused by IAPCC in rural China. The intriguing findings of our study warrant further research. More powerful study designs are necessary to confirm the association between IAPCC and NTDs and to understand which contaminants, if any, in this process might render exposed women more susceptible to NTD-affected pregnancies.

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Author affiliations: Institute of Reproductive and Child Health, Peking University Health Science Center, and Reference Laboratory on Reproductive Health, Ministry of Health, Beijing, China (Zhiwen Li, Le Zhang, Rongwei Ye, Jianmeng Liu, Aiguo Ren); and Institute of Population Research/WHO Collaborating Center on Reproductive Health and Population Science, Peking University, Beijing, China (Lijun Pei, Xiaoying Zheng).

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Association of selected persistent organic pollutants in the placenta with the risk of neural tube defects

Aiguo Ren[a,1,2], Xinghua Qiu[b,1], Lei Jin[a], Jin Ma[b], Zhiwen Li[a], Le Zhang[a], Huiping Zhu[c], Richard H. Finnell[c,d], and Tong Zhu[b,2]

[a]Institute of Reproductive and Child Health and Ministry of Health Key Laboratory of Reproductive Health, Peking University Health Science Center, Beijing 100191, China; [b]State Key Joint Laboratory for Environmental Simulation and Pollution Control, College of Environmental Sciences and Engineering and Center for Environment and Health, Peking University, Beijing 100871, China; and [c]Dell Pediatric Research Institute, Department of Nutritional Sciences and [d]Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX 78723

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Persistent organic pollutants (POPs) have been associated with a wide range of adverse health effects. Our case–control study was performed to explore the association between placental levels of selected POPs and risks for neural tube defects (NTDs) in a Chinese population with a high prevalence of NTDs. Cases included 80 fetuses or newborns with NTDs, whereas the controls were 50 healthy, nonmalformed newborn infants. Placental concentrations of polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides, polychlorinated biphenyls, and polybrominated diphenyl ethers were analyzed by gas chromatography–mass spectrometry. The medians of PAHs, o,p′-isomers of dichlorodiphenyltrichloroethane (DDT) and metabolites, α- and γ-hexachlorocyclohexane (HCH), and α-endosulfan were significantly higher in case placentas than in controls. PAH concentrations above the median were associated with a 4.52-fold [95% confidence interval (CI), 2.10–9.74) increased risk for any NTDs, and 5.84- (95% CI, 2.28–14.96) and 3.71-fold (95% CI, 1.57–8.79) increased risks for anencephaly and spina bifida, respectively. A dose–response relationship was observed between PAH levels and the risk of NTDs, with odds ratios for the second, third, and fourth quartiles, compared with the first, of 1.77- (95% CI, 0.66–4.76), 3.83- (95% CI, 1.37–10.75), and 11.67-fold (95% CI, 3.28–41.49), respectively. A dose–response relationship was observed for anencephaly and spina bifida subtypes. Similar results were observed for o,p′-DDT and metabolites, α-HCH, γ-HCH, and α-endosulfan, whereas no dose–response relationship was observed for the last two pollutants. Elevated placental concentrations of PAHs, o,p′-DDT and metabolites, and α-HCH were associated with increased risks of NTDs in this population.

congenital abnormalities | indoor air pollution

Neural tube defects (NTDs) are serious birth defects that result from the failure of the neural tube to close by the 28th day of gestation. NTDs that are restricted to the cranial region of the neural tube are referred to as anencephaly (1). This condition is characterized by the absence of the cranial vault and absent or markedly diminished cerebral hemispheres. In addition, the cerebellum is usually absent, and the brainstem may be hypoplastic. Most fetuses with anencephaly are aborted or stillborn. However, a small proportion of anencephalic infants are live-born, and they can survive for short periods without significant medical support. Closure defects that are restricted to the caudal portion of the neural tube are referred to as meningomyeloceles or spina bifida (1). This condition is associated with bony defects in the overlying neural arches, through which the meninges and spinal cord tissue are exposed to the body surface. Clinically, spina bifida may be further characterized by anatomic level and extent of the lesion. The majority of fetuses with spina bifida are live-born and, with proper treatment, survival into adulthood is common. Infants with spina bifida often have paralysis, urinary and bowel problems, learning difficulties, or hydrocephalus. It has been estimated that annually, more than 320,000 infants worldwide are affected by NTDs (2).

NTDs have a multifactorial etiology, with both genetic and environmental contributions to the observed phenotypes. Folate deficiency during the periconceptional period, maternal epilepsy with concomitant anticonvulsant drug exposure, maternal obesity, diabetes mellitus, and maternal hyperthermia are all known risk factors for NTDs (3). Environmental pollutants have also been suggested to be involved in the etiology of these malformations, although limited corroborating evidence has been reported to date (3, 4).

Persistent organic pollutants (POPs) are ubiquitous chemicals that can accumulate in the human body because of their lipid solubility and resistance to metabolism. Common POPs include: organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and polycyclic aromatic hydrocarbons (PAHs), among others. Exposure to POPs can be associated with a wide range of adverse health effects, including carcinogenicity and teratogenicity (5, 6). These compounds not only behave as endocrine disruptors, but they also have the ability to induce cellular oxidative stress (7, 8). Both of these mechanisms have been reported to disrupt normal embryonic development.

There have been few reported studies of the association between these pollutants and the risk of NTDs in humans. The only publication exploring the association between PAH biomarkers and NTD risk showed that the total PAH concentration in the blood of women whose pregnancies were affected by NTDs was higher than that of women who gave birth to healthy infants (9). In a case–control study conducted in the United States, pregnant women who reported that an insect control professional applied pesticides to their homes had a 60% increased risk of having an NTD-affected pregnancy compared with those women who had no such pesticide exposure during their pregnancies (10). Mexican American women who reported using pesticide in their homes or yards were twice as likely to have pregnancies affected by NTDs than those who reported no pesticide use (11). In California, the NTD risk was found to be weakly associated with periconceptional maternal residential proximity to National Priority List sites containing PCBs (12). However, a major limitation of most of these epidemiological studies is that they did not use biomarkers to quantify maternal or fetal exposure, relying solely on more circumstantial evidence.

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The authors declare no conflict of interest.

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[1]A.R. and X.Q. contributed equally to this work.

[2]To whom correspondence may be addressed. E-mail: tzhu@pku.edu.cn or renag@bjmu.edu.cn.

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Understanding the environmental causes of NTDs can facilitate research into the underlying mechanisms responsible for the development of NTDs and help formulate population prevention strategies that target these modifiable environmental factors. In the present study, we tested the hypothesis that elevated concentrations of POPs in the placenta may be associated with increased risks of NTDs by measuring PAHs, OCPs, PCBs, and PBDEs in the placentas from 130 women in a rural area of China with a reported NTD prevalence rate as high as 13.9 per 1,000 births (13).

Results

Characteristics of the case and control mothers are summarized in Table 1. There was no significant difference between the two groups with respect to maternal age, educational level, occupation, parity, previous history of birth defect-affected pregnancies, folic acid supplementation, or passive smoking. One-third of the case mothers reported fever and/or influenza during early pregnancy, compared with 8% of the controls. None of the case mothers and only one control mother reported smoking during the periconceptional period.

Table 2 and detail the concentrations of selected POPs in the placental samples of cases and controls. In total, 10 PAH congeners were detected in 82–100% of the samples. Phenanthrene was the PAH compound with the highest concentration, followed by fluorene, fluoranthene, pyrene, and anthracene. Median concentrations of all individual PAHs were higher in case placentas than in those of controls, although the differences for benzo[a]anthracene and benzo[g,h,i]perylene were not statistically significant. When all 10 PAHs were considered together, the median concentration of the sum (Σ_{10}PAHs) for PAH burden was significantly higher in case placentas than in controls.

Table 1. Characteristics of women who had pregnancies affected by NTDs (cases) and women who delivered healthy infants (controls)

Characteristic	Cases (n = 80)	Controls (n = 50)
Maternal age (y)		
<25	31 (39)	15 (32)
25–29	23 (29)	11 (23)
≥30	25 (32)	21 (45)
Maternal education		
Primary or lower	15 (19)	5 (10)
Junior high	54 (68)	41 (82)
High school or above	10 (13)	4 (8)
Maternal occupation		
Farmer	64 (83)	45 (92)
Nonfarmer	13 (17)	4 (8)
Parity		
1	45 (60)	23 (46)
≥2	30 (40)	27 (54)
Previous birth defects history		
Yes	6 (8)	0 (0)
No	73 (92)	50 (100)
Periconceptional folate supplementation		
Yes	7 (9)	6 (13)
No	71 (91)	41 (87)
Fever or flu during early pregnancy		
Yes	25 (33)	4 (8)
No	50 (67)	46 (92)
Maternal smoking		
Smoking	0 (0)	1 (2)
Nonsmoking	79 (100)	49 (98)
Maternal passive smoking		
Yes	50 (63)	24 (48)
No	29 (37)	26 (52)

Data are number (percentage). Total number may not be equal to the number of cases or controls due to missing or unknown data.

Target pesticides were successfully quantified in 78–100% of the placental samples. p,p′- dichlorodiphenyldichloroethylene (p,p′-DDE) was the dominant pesticide measured. Concentrations of p,p′-dichlorodiphenyltrichloroethane (p,p′-DDT) and its metabolites [p,p′-DDE and p,p′-dichlorodiphenyldichloroethane (p,p′-DDD)] or their sum (Σ_3p,p′-DDTs) showed no significant difference between case and control placentas. In contrast, concentrations of o,p′-DDT and its metabolites (o,p′-DDE and o,p′-DDD) or their sum (Σ_3o,p′-DDTs) were significantly higher in the case placentas compared with the control placentas. In addition, the ratio of metabolites (p,p′-DDE+p,p′-DDD) to parent (p,p′-DDT) was significantly lower in placental samples of cases than that of controls (41.5 vs. 56.0, P = 0.004). Hexachlorocyclohexanes (HCHs) also showed isomer-specific differences between the two groups. Concentrations of α-HCH and γ-HCH were significantly higher in case than in control placentas, but this was not the case for β-HCH, the most abundant HCH isomer found in the placentas. Additionally, case placentas had a higher concentration of α-endosulfan than did control placentas, although the concentrations were low in both groups compared with the other pesticides. For all other halogenated aromatic hydrocarbons, including hexachlorobenzene (HCB), PCBs, and PBDEs, there was no significant difference between the two groups. Pollutant concentrations in the case group were compared in placentas collected before and after 28 wk of gestation; no differences were observed ( ).

The risk of NTDs in association with a higher level of a specific pollutant or a group of pollutants was also analyzed (Table 3). A concentration higher than the median of Σ_{10}PAHs was associated with a 4.52-fold [95% confidence interval (CI), 2.10–9.74)] increased risk of NTDs, and an odds ratio of 2.91 (95% CI, 1.39–6.08) was observed for both Σ_3o,p′-DDTs and α-HCH. In addition, higher levels of γ-HCH and α-endosulfan were associated with 3.36- (95% CI, 1.10–6.44) and 2.53-fold (95% CI, 1.03–5.99) increased risks for any NTDs, respectively. When potential confounding factors were adjusted for with an unconditional multivariate logistic model, the associations between a concentration above the median of Σ_{10}PAHs, Σ_3o,p′-DDTs, α-HCH, γ-HCH, and α-endosulfan and the risk of any NTDs remained (Table 3).

The association between selected pollutants and the risk of subtypes of NTDs was further analyzed. Median concentrations of Σ_{10}PAHs, Σ_3o,p′-DDTs, α-HCH, γ-HCH, and α-endosulfan were all higher in the placental samples of cases with anencephaly and spina bifida than those of controls, although differences in the concentration of γ-HCH and α-endosulfan for the two subtypes of NTDs were not statistically significant (Table 3). Having a concentration above the median of Σ_{10}PAHs was associated with 5.84- (95% CI, 2.28–14.96) and 3.71-fold (95% CI, 1.57–8.79) increased risk for anencephaly and spina bifida, respectively. Higher concentrations of Σ_3o,p′-DDTs, α-HCH, γ-HCH, and α-endosulfan were also associated with elevated risk of anencephaly and spina bifida. After adjustment for the potential confounding factors, most of the significant associations between these pollutants and the risk of subtypes of NTDs remained (Table 3).

The association between higher levels of Σ_{10}PAHs, Σ_3o,p′-DDTs, and α-HCH with the risk of NTDs showed a clear dose–response relationship (Fig. 1 and). When the lowest quartile was used as the referent, increases in the risk of NTDs of 1.77- (95% CI, 0.66–4.76), 3.83- (95% CI, 1.37–10.75), and 11.67-fold (95% CI, 3.28–41.49) were observed for women whose placental concentration of Σ_{10}PAHs was in the second, third, and fourth quartiles, respectively. For Σ_3o,p′-DDTs, the risks of any NTDs in association with the second, third, and fourth quartiles were 1.20 (95% CI, 0.45–3.19), 2.16 (95% CI, 0.79–5.92), and 5.10 (95% CI, 1.66–15.70), respectively. The results for α-HCH

were similar to those of Σ_3o,p′-DDTs. The dose–response relationship was present for both anencephaly and spina bifida. In contrast, no apparent dose–response relationships between levels of γ-HCH or α-endosulfan and the risk of any subtype of NTDs were observed.

Factors that might be associated with higher placental concentrations of PAHs or pesticides were compared between the case and the control groups. Five case mothers (6%) reported exposure to pesticides, solvents or heavy metals during the periconceptional period; no control mothers reported such exposures ( ). No difference was found between the two groups with regard to domestic coal combustion exposure. When the analysis was performed separately in the case group and in the control group, case mothers who had a kitchen attached to her bedroom or living room, and who used hard coal for cooking and residential heating tended to have higher placental levels of PAHs, but the differences were not statistically significant. No similar tendency was observed in the control group. Maternal passive smoking was not associated with an elevated level of PAHs ( ).

Discussion

We found that higher levels of PAHs, o,p′-DDT and metabolites, and α-HCH in the placental tissues were associated with elevated risks of NTDs, and these risks increased with the concentrations of these pollutants. Increased placental levels of γ-HCH and α-endosulfan were also associated with elevated NTD risks, but no dose–response relationships were observed. No association was found between placental levels of p,p′-DDT and metabolites, β-HCH, HCB, PCBs, and PBDEs and risks of NTDs.

From the time of its conception and implantation in the uterus, the embryo requires a means of nutrient exchange from the mother as well as some means of protecting it from a maternal immune response (14). Within the first weeks of development, the embryonic cells are separated from the maternal tissues by a layer of trophoblastic cells (15). This arrangement remains throughout gestation. With time, the trophoblasts have not only penetrated the endometrial blood supply but have initiated chorionic villi formation. Those villi closest to the maternal blood supply will develop and expand into a mass of chorionic tissue that is ultimately considered to be the placenta. In addition to nutrients and oxygen, xenobiotics, especially lipophillic pollutants, can readily cross the preplacental structures and potentially impact embryonic development (16). Although much of this development has occurred after the embryo's neural tube has closed, the continuum of development from the trophoblast to the placenta proper makes this a reasonable surrogate in which environmental exposures that occurred earlier in gestation can be

Table 2. Concentrations (ng/g lipid) of typical pollutants in the placentas of women who had pregnancies affected by NTDs (cases) and women who delivered healthy infants (controls)

	Cases (n = 80)		Controls (n = 50)		
Pollutant	%*	Median (IQR)	%*	Median (IQR)	$P^{\dagger}$
PAH					
Fluorene	100	108 (81–151)	100	80 (57–97)	<0.001
Phenanthrene	100	308 (250–486)	100	210 (142–322)	<0.001
Anthracene	100	20 (11–39)	100	12 (7.6–19)	0.002
Fluoranthene	100	46 (29–68)	100	30 (22–47)	<0.001
Pyrene	100	45 (27–85)	100	24 (12–30)	<0.001
Benzo[a]anthracene	85	3.5 (2.0–5.4)	86	2.9 (1.5–4.1)	0.10
Chrysene	100	12 (7.0–23)	100	8.1 (4.2–11)	<0.001
Benzo[b]fluoranthene	96	2.7 (1.5–4.0)	96	2.1 (1.1–3.2)	0.03
Benzo[k]fluoranthene	98	1.7 (1.1–2.6)	96	1.2 (0.70–1.7)	0.003
Benzo[g,h,i]perylene	89	1.3 (0.51–3.1)	82	0.87 (0.24–1.8)	0.09
Σ_{10}PAHs		597 (460–835)		392 (268–547)	<0.001
DDT and metabolites					
o,p′-DDT	88	1.1 (0.47–2.2)	90	0.63 (0.25–1.0)	0.011
o,p′-DDE	100	0.95 (0.70–1.8)	100	0.72 (0.53–1.0)	0.001
o,p′-DDD	96	1.6 (0.84–3.0)	98	1.3 (0.76–1.9)	0.05
Σ_3o,p′-DDTs		4.3 (2.5–7.6)		2.7 (2.0–3.8)	0.001
p,p′-DDT	78	1.1 (0.40–2.0)	82	0.73 (0.30–1.1)	0.06
p,p′-DDE	100	52 (26–79)	100	51 (37–76)	0.5
p,p′-DDD	100	3.7 (2.2–7.6)	100	3.9 (2.4–6.2)	0.86
Σ_3p,p′-DDTs		55 (31–85)		59 (39–83)	0.60
Σ_6DDTs		60 (35–98)		61 (41–88)	0.77
HCH					
α-HCH	100	1.5 (0.99–2.2)	100	0.96 (0.57–1.4)	0.001
β-HCH	100	39 (13–65)	100	33 (22–52)	0.60
γ-HCH	98	1.6 (0.70–2.7)	98	0.99 (0.55–1.7)	0.02
Σ_3HCHs		44 (18–71)		36 (24–56)	0.47
α-Endosulfan	94	0.079 (0.038–0.15)	98	0.054 (0.035–0.089)	0.03
HCB	100	15 (10–22)	100	17 (13–21)	0.37
Σ_8PCBs[‡]		0.90 (0.62–1.7)		0.87 (0.64–1.1)	0.43
Σ_6PBDEs[§]		0.54 (0.37–0.94)		0.54 (0.34–0.79)	0.74

IQR, Interquartile range.

*Detection rate of a specific pollutant in placental samples.

†In comparison with the median of controls, Mann–Whitney U test.

‡Includes PCB congeners of -105, -118, -156, -157, -167, -189, -206, and -209.

§Includes PBDE congeners of -47, -66, -99, -100, -153, and -154.

Table 3. Median concentrations (ng/g lipid) of $\sum_{10}$PAHs, $\sum_{3}$o,p′-DDTs, α-HCH, γ-HCH, and α-endosulfan in the placentas of women who had pregnancies affected by NTDs (anencephaly or spina bifida) and women who delivered healthy infants (controls), and the risk in association with a concentration above the median of the pollutant(s)

Pollutant	Median (IQR)	Unadjusted odds ratio (95% CI)[†]	Adjusted odds ratio (95% CI)[†,‡]
$\sum_{10}$PAHs[§]			
Any NTDs	597 (460–835)***	4.52 (2.10–9.74)	6.00 (1.95–18.50)
Anencephaly	633 (462–844)***	5.84 (2.28–14.96)	8.71 (1.63–46.45)
Spina bifida	571 (452–825)***	3.71 (1.57–8.79)	9.09 (2.07–39.86)
Controls	392 (268–547)	1.00	1.00
$\sum_{3}$o,p′-DDTs[¶]			
Any NTDs	4.3 (2.5–7.6)***	2.91 (1.39–6.08)	5.19 (1.70–15.82)
Anencephaly	4.4 (2.4–6.8)*	2.43 (1.01–5.85)	9.52 (1.45–62.65)
Spina bifida	4.2 (2.8–8.5)***	3.40 (1.45–7.93)	8.68 (1.94–38.76)
Controls	2.7 (2.0–3.8)	1.00	1.00
α-HCH			
Any NTDs	1.5 (0.99–2.2)***	2.91 (1.39–6.08)	3.89 (1.26–11.97)
Anencephaly	1.4 (1.0–2.4)***	3.43 (1.40–8.42)	5.26 (0.96–28.79)
Spina bifida	1.5 (0.78–1.9)*	2.55 (1.11–5.89)	2.78 (0.76–10.24)
Controls	0.96 (0.57–1.4)	1.00	1.00
γ-HCH			
Any NTDs	1.6 (0.70–2.7)	3.36 (1.10–6.44)	11.75 (2.95–46.88)
Anencephaly	1.6 (0.63–2.5)	2.66 (1.59–7.08)	9.91 (1.35–72.76)
Spina bifida	1.7 (0.91–3.0)	4.11 (1.74–9.72)	20.40 (3.27–127.39)
Controls	0.99 (0.55–1.7)	1.00	1.00
α-Endosulfan			
Any NTDs	0.079 (0.038–0.15)	2.53 (1.03–5.99)	3.26 (1.10–9.71)
Anencephaly	0.082 (0.035–0.17)	2.49 (1.22–5.25)	3.00 (0.61–14.81)
Spina bifida	0.078 (0.039–0.15)	2.57 (1.12–5.91)	4.43 (1.14–17.19)
Controls	0.054 (0.035–0.089)	1.00	1.00

IQR, interquartile range.
[†]In comparison with controls; exposure was defined as above the median concentration.
[‡]Adjusted for maternal occupation, age, educational level, parity, folic acid supplementation, passive smoking, and fever or flu during early pregnancy, season of conception, mother's residence, and infant sex.
[§]Includes fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[g,h,i]perylene.
[¶]Includes o,p′-DDT, o,p′-DDE, and o,p′-DDD.
***$P \leq 0.001$, **$P \leq 0.01$, *$P \leq 0.05$ in comparison with the median of controls, Mann–Whitney *U* test.

evaluated. Thus, placental levels of pollutants of interest can be used as biomarkers of early in utero exposures even before the embryo proper is established (17).

Possible associations between PAH exposure and the risk of NTDs have been reported in several previously published epidemiological studies. Elevated risk of NTDs (relative risk, 1.83; 95% CI, 1.08–3.09) was observed in Sydney, Nova Scotia, Canada, where PAHs were reported as some of the most common pollutants emitted from large-scale coke oven operations, compared with a neighboring community (18). A 10% excess risk of congenital anomalies of the nervous system was observed in a region of the United Kingdom, where an increased exposure to total black smoke was recorded (19). In animal experiments, PAHs have been consistently shown to be teratogenic. The embryonic development of fish embryos was disturbed (20), and congenital malformations of various organ systems were induced by benzo(*a*)pyrene exposure to pregnant mice (21).

Environmental PAHs have multiple sources (22). In northern China, PAH exposure comes primarily from coal combustion. Shanxi Province produced 300 million tons of coal in 2003 and ranked first in PAH emission among all Chinese provinces (23). Coincident with PAH emission, the prevalence of NTDs in Shanxi Province was the highest in the country (24), with a prevalence rate of as high as 13.9 per 1,000 in some rural communities (13). In addition to industrial emission, exposure to indoor coal combustion is another major source of exposure to PAHs. In the rural area of the province, local residents use coal for cooking and residential heating in the winter. Our previous study conducted in the same population found that the concentration of PAHs was higher in the venous blood of mothers who had NTD-affected pregnancies than in those who delivered healthy infants (9). Exposure to indoor coal combustion, based on an index constructed from questionnaire data, has been shown to be associated with an elevated NTD risk (25). These findings support the hypothesis that NTD risk may be associated with environmental PAH exposure. In the present study, however, an association between indoor coal use and placental PAH concentration was not observed, likely owing to the small sample size.

Because each of the 10 individual PAHs assessed in the present study had higher concentration in case placentas than in controls, total PAHs concentration was used as a summary exposure indicator in subsequent analyses. The similarities in their molecular structure and metabolism of these PAHs justify the use of the total PAHs as such an exposure indicator. Although fluorene and phenanthrene had the most weight, the difference in total PAHs remained significant even if fluorene and phenanthrene were excluded in calculating the total (median, 162 ng/g lipid for cases, and median 91 ng/g lipid for controls).

A possible association between pesticide exposure and the risk of NTDs has been suggested in several epidemiological studies (10, 11). However, all of these studies relied on self-reported pesticide exposure or used occupation or job title as surrogates for actual pesticide exposure data. This may compromise the studies by increasing the chance of recall bias and exposure mis-

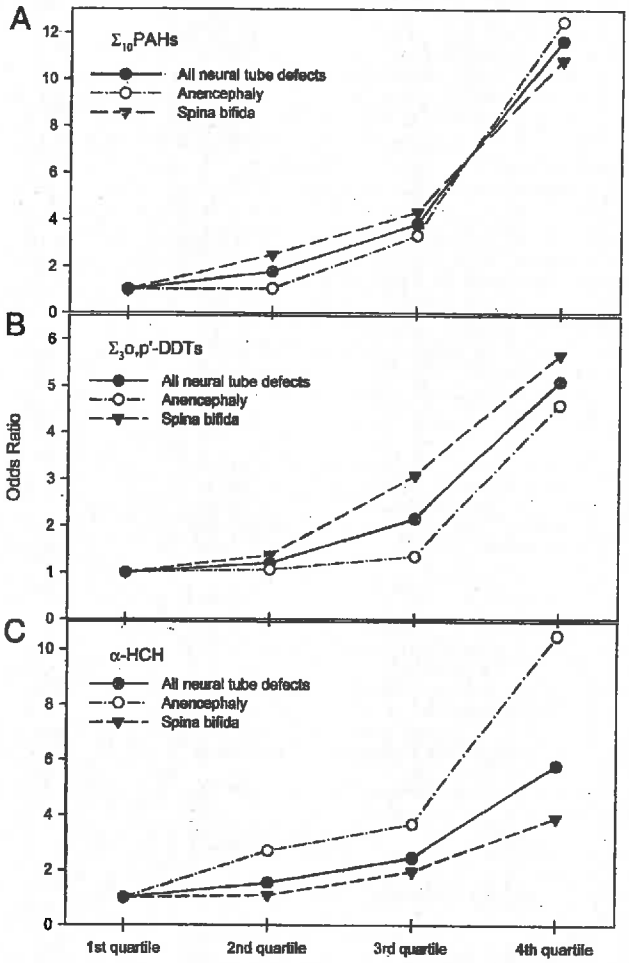

Fig. 1. Quartiles of Σ_{10}PAHs (*A*), Σ_3o,p′-DDTs (*B*), and α-HCH (*C*) in the placenta and the risks of NTDs.

classifications (26). In the present study, we used placental concentrations of several pesticides and their metabolites as biomarkers of in utero exposure. We found that elevated levels of o,p′-DDTs and α-HCH in the placenta were associated with increased risks of NTDs. Because DDT and HCH were banned in 1983 in China, the presence of these pesticides in the placenta must reflect their residues persisting in the environment.

Placental levels of pollutants are determined by both maternal exposure and the metabolic rate of the mother. Higher levels of pollutants in case placentas could be an indication of excess exposure to these pollutants by case mothers compared with controls. Although no difference in exposures to residential coal combustion was observed between the case and the control groups, the possibility of increased exposure to coal combustion for the case group cannot be ruled out. On the other hand, variations in maternal metabolism may also contribute to the differences in placental levels of these pollutants (i.e., case mothers may have a lower metabolic rate than the control mothers, presumably due to genetic factors). This was supported by the lower metabolites to parent ratio ([p,p′-DDE+p,p′-DDD]/p,p′-DDT) in the case group than in the control group. Lower metabolic rates in case mothers may result in higher placental levels of relatively less persistent pollutants, such as PAHs, o,p′-DDTs, and α-HCH, but not for more persistent pollutants, such as HCB, p,p′-DDE, and β-HCH. Lower metabolic activities mean slower elimination of xenobiotics from the maternal body, and thus may increase exposure of the embryo/fetus. Such extended exposure to these teratogenic compounds may increase the risk of birth defects (27).

Although PAHs and organochlorine pesticides are structurally different types of chemical pollutants, they need to be activated by cytochrome P450 enzymes before they can exert their biological impact (28). Studies have shown that PAHs and organochlorine pesticides could induce oxidative stress (29–33). Oxidative stress has long been considered to be a mechanism of teratogenic action for many compounds, resulting in the misregulation of redox-sensitive signal transduction pathways. These pathways are critical for developmental processes, including cellular proliferation, differentiation, and apoptosis (34).

Maternal hyperthermia has been shown to induce NTDs in experimental animal systems (35, 36). In humans, fever and/or influenza during the periconceptional period has been found to be a risk factor for fetal NTDs (37–39). In the present study, maternal fever and/or influenza was associated with an elevated risk of NTDs in a univariate analysis. In a multivariate logistic regression analysis that adjusted for maternal fever and/or influenza, along with other factors that are potentially associated with increased risks of NTDs, the association of higher levels of PAHs and several organochlorine pesticides in the placenta with the risk of NTDs remained significant, suggesting that these pollutants may be independent risk factors for NTDs in this population.

In conclusion, higher levels of PAHs, o,p′-DDT and metabolites, and α-HCH in the placenta were associated with elevated risks of NTDs. Further research is warranted to determine whether increased maternal exposure, altered maternal metabolism, or the interactions between maternal metabolism and environmental pollutants is the underlying cause of the increased levels of the pollutants in the case placentas. Future studies may also examine placental oxidative status to provide insight into the mechanism of action exerted by these environmental pollutants.

Methods

Subjects. Subjects were recruited from four rural counties of Shanxi Province (Pingding, Xiyang, Taigu, and Zezhou), China from 2005 to 2007. Cases with a confirmed diagnosis of an NTD were ascertained through a population-based birth defects surveillance program (13). Once a fetus or a newborn with such defects was identified as a case, a healthy newborn with no congenital malformations born in the same hospital was selected as a control. The control was matched for sex, mother's county of residence, and the date of mother's last menstrual period, which was selected to be as close as possible to that of the case mother's. Information on the mother's sociodemographic characteristics, lifestyle, reproductive history, periconceptional use of folic acid supplements, smoking and passive smoking, exposure to pesticides, solvents or heavy metals, and domestic fuel use for cooking and heating was collected through face-to-face interviews, which for the most part (92%) were conducted within the first week of delivery or pregnancy termination. Placentas were collected at delivery or termination of NTD-affected pregnancies, placed in polyethylene bags, and kept at −20 °C until use for analyses. The study protocol was approved by the institutional review board of Peking University. Informed consent was obtained from the mothers before the study.

In our previous study conducted in the same population, a sample size of 35 case and 18 control mothers revealed a statistically significant difference in the blood levels of PAHs (9). In the present study, we used an empirical sample size of 35 or greater per subgroup, which would be adequate to test the existence of differences between concentrations of PAHs in the placentas of the case and the control groups, assuming that the difference in the placenta was similar to the difference in the blood. Although designed as a matched case–control study, some placentas were not available for evaluation owing to a failure to obtain consent from some women; therefore, the pairs were broken in the present study. We randomly selected 80 placentas of NTD cases (anencephaly, 36; spina bifida, 44) from a total of 155 placentas of women who had NTD-affected pregnancies, and 50 from 163 placentas of women who gave birth to singleton healthy infants as control placentas. All of the control placentas were from term deliveries. For NTD cases, 53% (40 of 75) of the placentas were collected before 28 gestational weeks, 35% (26 of 75) between 28 and 36 gestational weeks, and 12% (9 of 75) at term (5 with missing information).

Laboratory Analyses. Approximately 10 g of placental tissue was taken within 2 cm on the fetal side around the point of cord attachment. Each sample was spiked with recovery surrogate standards, homogenized in hexane/acetone solvent mixture (1:1 by volume), and extracted three times by ultrasonication and vortex mixing. Lipophilic material in the extractant was determined gravimetrically and then removed by gel permeation chromatography, followed by silica gel column chromatography. After concentration, samples were spiked with internal standards and analyzed with an Agilent 7890A-5975C gas chromatograph and mass spectrometer. PAHs, DDT and its metabolites DDD and DDE were analyzed by mass spectrometry in electron impact ionization mode, whereas all of the other chlorinated or brominated pollutants were analyzed in electron capture negative ionization mode.

One analytical procedure blank sample was prepared with each batch of seven placental samples. Median concentrations of individual pollutants in placental samples ($n = 130$) were compared with those in blank samples ($n = 19$). Pollutants were rejected if the concentration in the placental samples was not significantly different from that in the blank. For the remaining target pollutants, values in the placental samples were all more than fourfold higher than those in the blanks. The recoveries (mean ± SD) were 84% ± 7%, 88% ± 12%, 86% ± 13%, 96% ± 5%, and 93% ± 14% for the surrogate standards of deuterium-labeled phenanthrene, chrysene, perylene, α-HCH, and PCB-65, respectively. Data were expressed on a lipid weight basis and were neither blank nor recovery corrected. During sample preparation and chemical analysis, the case–control status of the placental samples was masked from those who were involved in these procedures.

Statistical Analysis. Because most of the chemicals were not normally distributed, the median with interquartile range was used to describe the skewed distributions of the chemical concentrations. Differences in proportions between groups were examined with Pearson's χ^2 test. Nonparametric analysis was used to compare the medians between the case and control groups. In dose–response analysis, concentration quartiles of a specific pollutant or the sum of a class of pollutants were used as the cutoff values. Risk of NTDs associated with pollutant exposure was estimated from the odds ratio with 95% CI. Maternal occupation, age, educational level, parity, folic acid supplementation, passive smoking, and fever and/or influenza during early pregnancy were selected as potential confounding factors on the basis of their possible relationship with both the pollutants and an increased risk for NTDs and were adjusted for using the unconditional logistic model. In addition, the matching variables, including infant sex, mother's residence, and season of conception, were also included in the multivariate model to account for possible imbalance between the case and control groups resulting from breaking the pairs. Statistical analyses were conducted using SPSS 11.5. A two-tailed P value of <0.05 was considered to indicate statistical significance.

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