

# Biomarkers of Polycyclic Aromatic Hydrocarbon-DNA Damage and Cigarette Smoke Exposures in Paired Maternal and Newborn Blood Samples as a Measure of Differential Susceptibility<sup>1</sup>

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## Abstract

Human and experimental evidence indicates that the developing fetus may be more susceptible than the adult to the effects of certain carcinogens, including some polycyclic aromatic hydrocarbons (PAHs). Factors that can modulate susceptibility include proliferation rates, detoxification capabilities, and DNA repair capacity. Biomarkers can facilitate quantification of age-related susceptibility among human populations. In this study, we report on three biomarkers measured in paired blood samples collected at birth from 160 Polish mothers and newborns: 70 pairs from Krakow (a city with high air pollution including PAHs) and 90 pairs from Limanowa (an area with lower ambient pollution but greater indoor coal use). Biomarkers were: WBC aromatic-DNA adducts by <sup>32</sup>P-postlabeling and PAH-DNA adducts by ELISA (as indicators of DNA damage from PAHs and other aromatics) and plasma cotinine (as an internal dosimeter of cigarette smoke). Correlations were assessed by Spearman's rank test, and differences in biomarker levels were assessed by the Wilcoxon signed-ranks test. A significant correlation between paired newborn/maternal samples was seen for aromatic-DNA adduct levels ( $r = 0.3$ ;  $P < 0.001$ ) and plasma cotinine ( $r = 0.8$ ;  $P < 0.001$ ) but not PAH-DNA adduct levels ( $r = 0.14$ ;  $P = 0.13$ ). Among the total cohort, levels of the three biomarkers were higher in newborn samples compared with paired maternal samples. The difference was significant for

aromatic-DNA adduct levels ( $16.6 \pm 12.5$  versus  $14.21 \pm 15.4/10^8$  nucleotides;  $P = 0.002$ ) and plasma cotinine ( $14.2 \pm 35.5$  versus  $8.3 \pm 24.5$  ng/ml;  $P < 0.001$ ) but not for PAH-DNA adduct levels ( $7.9 \pm 9.9$  versus  $5.9 \pm 8.2/10^8$  nucleotides;  $P = 0.13$ ). When analyses were restricted to the 80 mother/newborn pairs from whom the blood sample was drawn concurrently (within 1 h of each other), levels of all of the three biomarkers were significantly higher in the newborn compared with paired maternal blood samples ( $P < 0.05$ ). Results suggest reduced detoxification capabilities and increased susceptibility of the fetus to DNA damage, especially in light of experimental evidence that transplacental exposures to PAHs are 10-fold lower than paired maternal exposures. The results have implications for risk assessment, which currently does not adequately account for sensitive subsets of the population.

## Introduction

Experimental and human evidence indicates that the developing fetus and neonate can differ in susceptibility to chemical carcinogenesis compared with the adult (reviewed in Refs. 1, 2). Age-related factors that may modulate susceptibility include differing rates of cell proliferation, abilities to activate and detoxify carcinogens, DNA repair capacity, and the number of target cells at risk (1, 2). Bioassays have shown the young to be both more susceptible and more resistant, depending on the carcinogen tested, the species evaluated, the age at first exposure, and the target organ (1–5). A number of the PAHs<sup>3</sup> are transplacental carcinogens in experimental bioassays, producing tumors in the liver, lung, lymphatic tissues, and nervous system of the offspring (4–6). Cancer incidence at these sites has been shown to be greater if the PAH exposure begins prenatally or early postnatally compared with later in life, at comparable doses (4, 7–10). However, human data on age-related susceptibility to PAH tumorigenesis are not available (1). Biomarkers can facilitate evaluation of factors modulating susceptibility to carcinogens among human populations (11–13). As an indication of DNA damage, carcinogen-DNA adducts represent a critical step on the carcinogenic pathway and are an informative biomarker of age-related susceptibility (11). Therefore, we have compared levels of two biomarkers of DNA damage from PAHs and other aromatics in paired maternal and newborn blood samples: WBC aromatic-DNA adducts by <sup>32</sup>P-postlabeling and PAH-DNA adducts by ELISA. Plasma cotinine levels were also compared as an internal dosimeter of cigarette smoke exposure.

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<sup>3</sup> The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

The biological basis for measuring DNA adducts derives from extensive experimental data supporting their role in the initiation and possibly in the progression of cancer (14). An association between PAH-DNA adduct levels and cancer risk has been seen previously (14–17) in both epidemiological and experimental research. PAHs such as benzo(a)pyrene readily cross the placenta. Experimental studies (18–20) using radio-labeled PAHs indicate that the dose to the fetus is generally an order of magnitude or more lower than the dose to paired maternal tissues. Adducts formed between DNA and various PAHs have been measured in multiple fetal tissues in experimental rodent bioassays (21–23). Fetal levels are generally lower than maternal levels but higher than expected given the maternal/fetal dose differential. Levels of genotoxic damage (micronuclei formation and DNA single strand breaks) after transplacental PAH exposure have been shown experimentally to be higher in fetal than paired maternal tissues (23–25). One prior molecular epidemiological study has measured PAH-DNA adducts in maternal and cord blood samples (26). When measured by ELISA with fluorescence detection, PAH-DNA adducts were detected in 18 of 27 and 13 of 21 of the maternal and newborn WBCs, respectively. For mothers and newborns with detectable adducts, mean levels were similar (26).

Cotinine is the major proximal metabolite of nicotine but has a longer half-life in sera (15–40 h *versus* 1–3 h for nicotine), and serum concentrations of cotinine are 10-fold higher than they are for nicotine (27–31). It is less biologically active than nicotine (30, 31) but has been widely used as a marker of exposure to tobacco smoke (29, 32, 33), which contains a myriad of carcinogens, including the nicotine-derived carcinogens, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N'*-nitrosonornicotine (34, 35), as well as carcinogenic PAHs (27). Nicotine readily crosses the placenta, and both nicotine and cotinine have been measured in fetal tissues (30, 36–38). Studies (39–43) comparing paired maternal/fetal cotinine levels are few and have yielded conflicting results. Both higher and lower concentrations in maternal samples compared with fetal samples have been reported.

This study extends prior evaluations in the current cohort of 70 mother/newborn pairs from Krakow, Poland (an industrial city with elevated ambient air pollution including PAHs from coal-burning for industry and residential heating) and 90 pairs from Limanowa (a small town located 70 km southeast of Krakow with lower ambient pollution levels but 2-fold more frequent use of coal-stoves for indoor home heating (44)). We have reported previously (45, 46) that a significant association was seen between ambient particulate levels of  $<10 \mu\text{m}$  ( $\text{PM}_{10}$ ) and PAH-DNA but not aromatic-DNA adduct levels in both maternal and newborn WBCs. Maternal active and passive cigarette smoking status was significantly associated with maternal PAH-DNA but not aromatic-DNA adduct levels (45, 46). However, adduct levels in maternal WBCs did not differ significantly between ex-smokers and nonsmokers (45, 46). Nor was any association seen between maternal smoking status and either measure of DNA adducts in newborn WBCs, possibly because the activity of placental enzymes that are induced by maternal smoking reduced the biologically effective dose to the fetus (47, 48). Newborn PAH-DNA adduct levels were significantly inversely associated with birth weight, length, and head circumference (49). Newborn plasma cotinine was inversely associated with birth weight and length (49). The present report extends the research to compare levels of three biomarkers in mother/newborn pairs.

## Materials and Methods

Field studies were conducted in Poland during January–March, 1992 under the direction of Dr. Wieslaw Jedrychowski (Jagiellonian University, Krakow, Poland) in accordance with current guidelines for human subjects. Enrollment was restricted to women who had resided in Krakow or Limanowa for at least 1 year and was limited to vaginal deliveries. In all of the cases, samples of umbilical cord blood (20–60 ml) were collected at delivery, and a maternal blood sample was collected within an average of  $8.7 \pm 11.6$  h after delivery (range, 5 h before delivery to 66 h after delivery). For a subset of 80 of these mother/newborn pairs, the maternal blood sample was drawn within 1 h of delivery (either before or after delivery). Samples were processed as described previously (44).

A detailed, validated questionnaire administered to the mother within 2 days postpartum included information on smoking (active and passive), residential and employment histories, use of coal stoves for residential heating, and other environmental exposures as described previously (44).

**Aromatic-DNA Adducts.** The  $^{32}\text{P}$ -postlabeling TLC assay was carried out at the Karolinska Institute as described previously (50, 51). In brief, DNA was extracted from the crude nuclei using organic solvents after degrading RNAs and proteins; 5  $\mu\text{g}$  of DNA was digested by micrococcal nuclease and spleen phosphodiesterase to 3' nucleotides. Adducts were then enriched by nuclease P1 treatment. A postlabeling reaction was carried out and applied on a TLC plate for adduct separation in three dimensions. After autoradiography, the adduct spots were excised from the successfully developed TLC plate for counting of radioactivity as uniform areas covering most of the radioactive fractions of the plate (51). Two to five assays were carried out for each sample, and the mean of all of the assay results were calculated. The detection limit of the assay was 0.07 adducts/ $10^8$  nucleotides. The assay detects multiple PAHs and other aromatic compounds bound to DNA (52). Aromatic-DNA adduct levels were determined for 122 mother/newborn pairs.

**PAH-DNA Adducts.** PAH-DNA adducts were measured by a competitive ELISA with fluorescence end-point detection essentially as described previously (53). The detection limit of the assay is two adducts/ $10^8$  nucleotides. Samples were assayed in triplicate at 50  $\mu\text{g}$  of DNA/well. The median values were used to determine the percentage of inhibition. When sufficient DNA was available (63% of samples), the assay was repeated. Laboratory personnel were blinded to subject status. The antiserum was elicited against benzo(a)pyrene-diol-epoxide-DNA but recognizes other structurally related PAH diol-epoxide-DNA adducts, including those formed by benz[a]anthracene and chrysene (54). Thus, positive reaction with the antiserum may indicate the presence of multiple PAH-DNA adducts in the sample. Values are expressed as the amount of BPDE-DNA that would cause a similar inhibition in the assay. PAH-DNA adduct levels were determined for 112 mother/newborn pairs.

**Plasma Cotinine.** The method involved liquid/liquid extraction of plasma, followed by gas chromatographic separation using a 30-m 0.25  $\mu$  DB WAX megabore column and a nitrogen phosphorus detector operated in the nitrogen mode as described previously (44). An internal standard, *N*-methyl cotinine, was added to the plasma before extraction. Five-point standard curves were generated for each analytical run, and low- and high-quality control samples were processed each day. The method requires cold trapping injection followed by temperature programming to achieve optimal separation resulting in an analysis time of 18 min/sample. To facilitate productivity, an autosampler and online automatic data reduction were used

Table 1 Number of mother/newborn pairs,<sup>a</sup> maternal age,<sup>b</sup> and smoking status<sup>a</sup> of mothers

Mother/newborn pairs	160
Mother's age (yr)	26.3 ± 4.8
Current smokers	16 (10%)
Ex-smokers	38 (24%)
Nonsmokers	106 (66%)

<sup>a</sup> Number of subjects (%).

<sup>b</sup> Mean ± SD.

so that samples could be processed during the evening or overnight, as needed. Cotinine levels were determined on 158 mother/newborn pairs, including 80 pairs from whom blood samples were drawn within 1 h of each other (concurrent samples).

**Statistical Analyses.** Means and SDs for biomarker levels in paired maternal and newborn samples are presented. Statistical analyses were undertaken using nonparametric statistics because of the distributional properties of the biomarkers and the large number of samples that were below the limit of detection. Correlations between biomarkers in paired maternal and newborn samples were assessed by Spearman's rank. The differences in biomarker levels in mother/newborn pairs were assessed by the Wilcoxon signed-ranks test. Analyses were first undertaken involving all of the mother/newborn pairs (total sample) and then were restricted to the 80 mother/newborn pairs from whom blood samples were drawn within 1 h of each other (concurrent samples). Associations were considered significant at  $P \leq 0.05$ .

## Results

Data on demographic variables and smoking status are summarized in Table 1. Fig. 1 and Fig. 2 show the correlation between biomarkers in paired maternal and newborn blood samples for all of the mother/newborn pairs. The correlation between WBC PAH-DNA adduct levels in paired maternal/newborn samples was not significant (Fig. 1A;  $r = 0.14$ ;  $P = 0.13$ ). WBC aromatic-DNA adduct levels were moderately but significantly correlated (Fig. 1B;  $r = 0.3$ ;  $P < 0.001$ ). Plasma cotinine levels were highly correlated (Fig. 2A;  $r = 0.8$ ;  $P < 0.001$ ). When analyses were restricted to the mother/newborn pairs from whom the blood samples were drawn concurrently, the correlations between the biomarkers in paired samples remained essentially unchanged. Specifically, WBC PAH-DNA adduct levels in paired samples were not correlated ( $r = 0.08$ ;  $P = 0.53$ ;  $n = 60$ ). WBC aromatic-DNA adduct levels in paired samples were moderately correlated ( $r = 0.3$ ;  $P = 0.01$ ;  $n = 61$ ). Plasma cotinine levels in paired samples were highly correlated ( $r = 0.8$ ;  $P < 0.001$ ;  $n = 80$ ; data not shown).

Tables 2–4 compare the biomarker levels in mother/newborn pairs and provide results of the Wilcoxon signed-ranks test. Analyses are provided both for the total cohort and for the mother/newborn pairs from whom the blood samples were drawn concurrently.

As seen from Table 2, aromatic-DNA adduct levels ( $/10^8$  nucleotides) were higher in newborn than in paired maternal WBCs. Specifically, when analyses involved all of the mother/newborn pairs ( $n = 122$ ), newborn aromatic-DNA adduct levels exceeded paired maternal levels approximately twice as frequently because they were less than paired maternal levels (see ranks for mother/newborn pairs; Table 2), a difference that was statistically significant ( $P = 0.002$ ). After stratifying by

smoking status, newborn adduct levels again exceeded maternal levels among both nonsmokers and ex-smokers, but the difference was significant only among the nonsmokers ( $P = 0.005$ ) and was not significant among the ex-smokers ( $P = 0.2$ ). By contrast, aromatic-DNA adduct levels among newborns of current smokers were similar and not significantly different from paired maternal levels ( $P = 0.79$ ; Table 2).

When analyses were restricted to mother/newborn pairs from whom blood was drawn concurrently ( $n = 61$ ), newborn aromatic-DNA adduct levels consistently exceeded maternal adduct levels, but the difference was significant only when analyses involved all of the mother/newborn pairs with concurrent blood sampling ( $P = 0.04$ ) and was not significant when analyses were stratified by smoking status ( $P \geq 0.1$ ).

Table 3 compares PAH-DNA adduct levels ( $/10^8$  nucleotides) in mother/newborn pairs. When analyses involved all of the mother/newborn pairs ( $n = 112$ ), PAH-DNA adduct levels were somewhat higher in newborn compared with paired maternal WBCs among the total cohort and among newborns of nonsmokers and ex-smokers (Table 3). The difference was of borderline significance among the nonsmokers ( $P = 0.05$ ; Table 3). Among the 10 current smokers, maternal adduct levels exceeded the newborn levels in 7 of the 10 pairs, although they were less than the newborn levels in 1 of the 10 pairs ( $P = 0.05$ ; Table 3). When analyses were restricted to the mother/newborn pairs from whom the blood samples were drawn concurrently ( $n = 60$ ), PAH-DNA adducts were higher in the newborn compared with paired maternal WBCs in all of the groups except current smokers. The difference was significant for the total cohort ( $P = 0.04$ ) and among nonsmokers ( $P = 0.03$ ).

Table 4 compares plasma cotinine levels (ng/ml) in maternal and newborn blood samples for all of the mother/newborn pairs and for the mother/newborn pairs from whom blood was drawn concurrently. Newborn cotinine levels consistently exceeded maternal levels, and in most cases the difference was highly significant. Specifically, as seen in Table 4, among the total cohort ( $n = 158$ ), newborn levels exceeded those in the paired maternal sample five times as frequently as maternal levels exceeded the paired newborn levels (see ranks, Table 4;  $P < 0.001$ ). Similarly, among the concurrent samples ( $n = 80$ ; Table 4), newborn cotinine levels again exceeded the paired maternal levels five times as frequently as the maternal levels exceeded the paired newborn levels, a difference that remained highly significant despite the reduced sample size ( $P < 0.001$ ).

After stratifying by smoking status, newborn cotinine levels exceeded paired maternal levels in all of the strata (nonsmokers, ex-smokers, and current smokers). This difference was significant ( $P \leq 0.005$ ) in all of the strata when analyses involved the total cohort (Table 4). When analyses were restricted to the concurrent samples (Table 4), newborn cotinine levels exceeded paired maternal levels in all of the strata (nonsmokers, ex-smokers, and current smokers), and the magnitude of the difference was similar to that seen in the total cohort. However, the difference was significant only among nonsmokers ( $P = 0.008$ ; Table 4) and was not significant among ex-smokers and current smokers, because of the reduced sample size ( $n = 16$  and  $n = 4$  pairs, respectively).

Table 5 shows the number of mother/newborn pairs with biomarker levels above and below the limit of detection. The ratio (mean ± SD, range) of newborn/maternal biomarker levels for the mother and newborn pairs in which the biomarker in both the maternal and newborn blood samples was above the limit of detection is also presented. As seen, the levels of

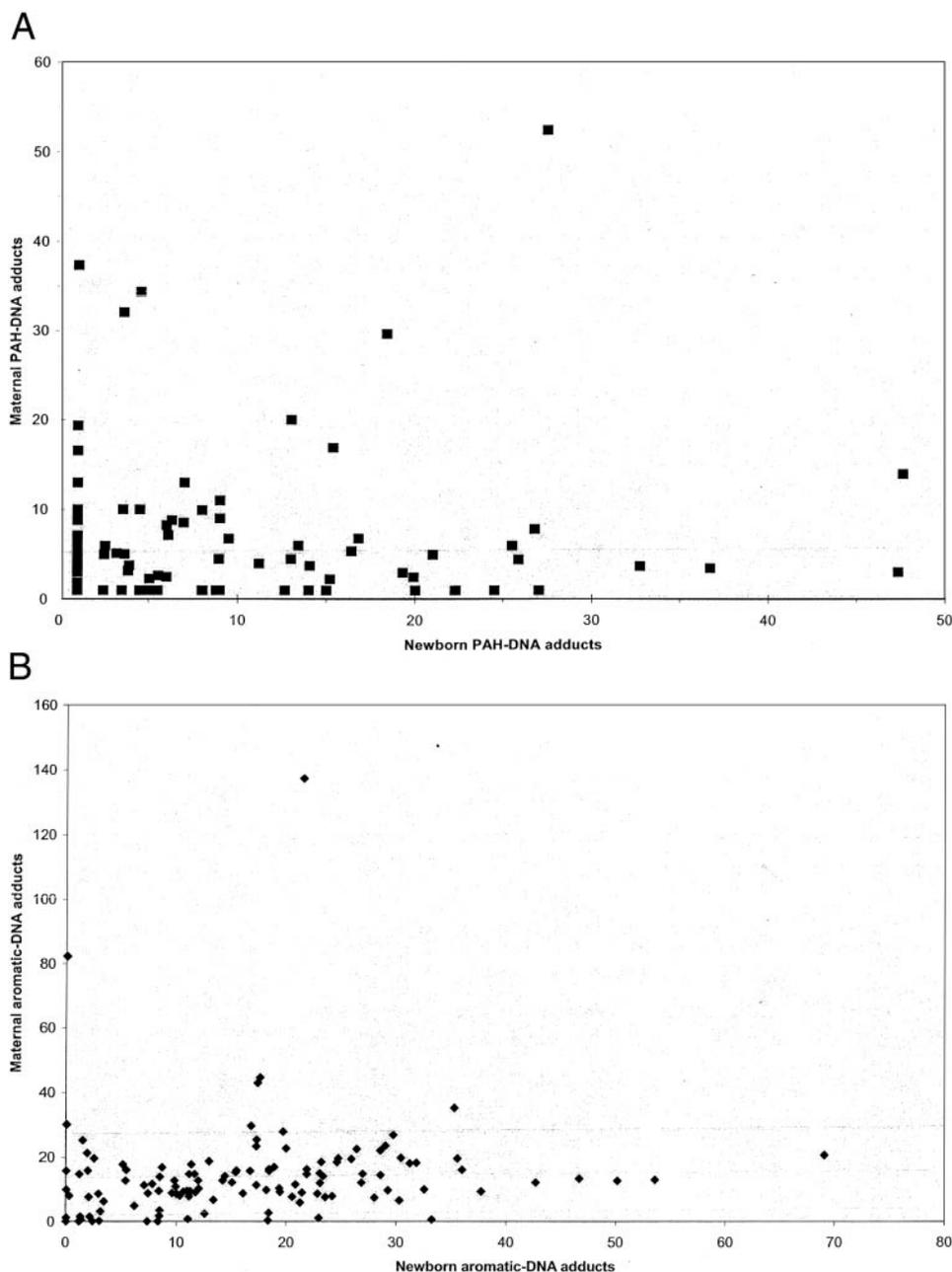


Fig. 1. Correlation between DNA adduct levels/ $10^8$  nucleotides in paired maternal and newborn WBCs. A, correlation between maternal and newborn WBC PAH-DNA adducts;  $r = 0.14$ ;  $P = 0.13$ . B, correlation between maternal and newborn WBC aromatic-DNA adduct levels;  $r = 0.3$ ;  $P < 0.001$ .

aromatic DNA-adducts were above the limit of detection in both maternal and newborn WBCs for 113 of 122 (93%) of the mother/newborn pairs. Among these pairs, newborn aromatic-DNA adduct levels averaged  $4.1 \pm 12.5$  (range, 0.04–105.3) times higher than paired maternal adduct levels.

By contrast, the levels of PAH-DNA adducts were above the limit of detection in both the maternal and newborn WBCs in only 44 of 112 (39%) of the mother/newborn pairs (Table 5). Among these mother/newborn pairs with adducts above the limit of detection, newborn PAH-DNA adduct levels averaged  $2.7 \pm 3.2$  (range, 0.11–15.5) times higher than paired maternal adduct levels. Among the 68 of 122 (61%) of the mother/newborn pairs in which the PAH-DNA adduct levels in either or both of the maternal and/or the newborn WBCs were below

the limit of detection, there was a fairly equal distribution among the pairs in which the adduct levels in both the maternal and newborn WBCs were below the limit of detection ( $n = 24$ ) compared with the pairs in which: (a) the adduct levels were above the limit of detection in the newborn WBCs and below the limit of detection in the maternal WBCs ( $n = 20$ ); and (b) the adducts were below the limit of detection in the newborn WBCs and were above the limit of detection in the maternal WBCs ( $n = 24$ ).

Levels of plasma cotinine were above the limit of detection in both the maternal and newborn blood samples in 58 of 158 (38%) of the mother/newborn pairs (Table 5). Among these pairs, newborn plasma cotinine averaged  $2.0 \pm 1.8$  (range, 0.67–9.7) times higher than paired maternal cotinine levels. For

Fig. 2. Correlation between plasma cotinine levels (ng/ml) in paired maternal and newborn blood samples. A, correlation between plasma cotinine levels in mother/newborn pairs;  $r = 0.8$ ;  $P < 0.001$ .

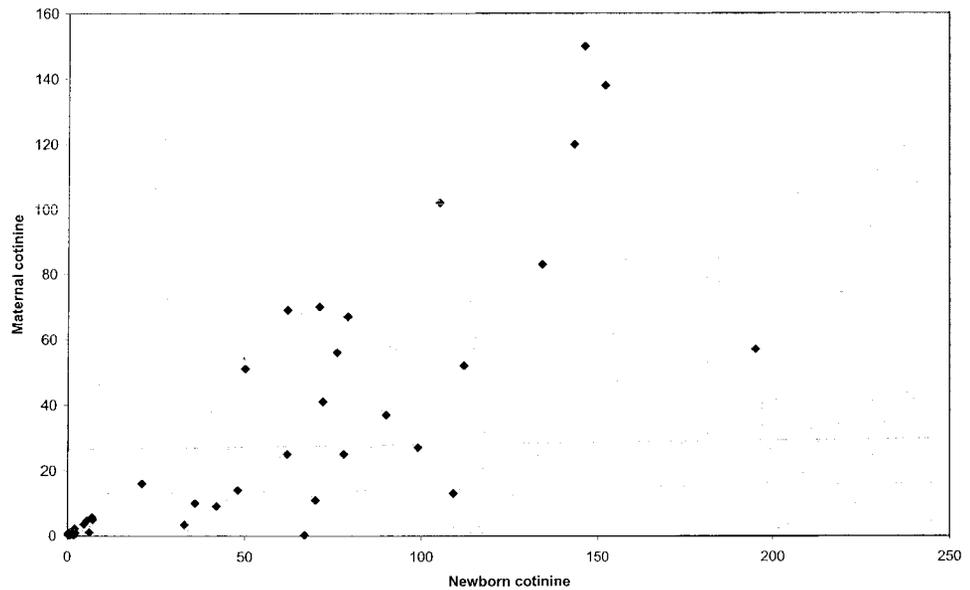


Table 2 WBC aromatic-DNA adduct levels by  $^{32}\text{P}$ -postlabeling paired maternal and newborn blood samples

	Mean $\pm$ SD/ $10^8$ nucleotides		Ranks for mother/newborn pairs <sup>a</sup>				$P^a$	
	Maternal	Newborn	Negative ranks <sup>b</sup>		Positive ranks <sup>c</sup>			Ties <sup>d</sup>
			No. <sup>e</sup>	Sum <sup>f</sup>	No. <sup>e</sup>	Sum <sup>f</sup>		No. <sup>e</sup>
A. WBC aromatic-DNA adduct levels in all of the mother/newborn pairs (total sample)								
Total cohort ( $n = 122$ )	14.2 $\pm$ 15.4	16.6 $\pm$ 12.5	41	2430	79	4830	2	$P = 0.002$
Nonsmoker ( $n = 79$ )	15.0 $\pm$ 16.3	17.1 $\pm$ 11.3	25	976	53	2104	1	$P = 0.005$
Ex-smoker ( $n = 32$ )	13.0 $\pm$ 15.6	15.7 $\pm$ 14.9	11	185	20	311	1	$P = 0.22$
Current smoker ( $n = 11$ )	12.0 $\pm$ 4.2	14.7 $\pm$ 13.7	5	30	6	36	0	$P = 0.79$
B. WBC aromatic-DNA adduct levels in mother/newborn pairs with blood drawn concurrently (concurrent samples)								
Total cohort ( $n = 61$ )	15.2 $\pm$ 17.8	17.8 $\pm$ 14.4	22	655	39	1237	0	$P = 0.04$
Nonsmoker ( $n = 46$ )	16.6 $\pm$ 20.1	17.8 $\pm$ 12.4	18	390	28	692	0	$P = 0.10$
Ex-smoker ( $n = 12$ )	10.6 $\pm$ 6.9	16.5 $\pm$ 20.4	4	29	8	49	0	$P = 0.43$
Current smoker ( $n = 3$ )	10.9 $\pm$ 2.2	23.5 $\pm$ 20.1	0	0	3	6	0	$P = 0.11$

<sup>a</sup> Wilcoxon signed-ranks test.

<sup>b</sup> Newborn adduct level < maternal adduct levels.

<sup>c</sup> Newborn adduct levels > maternal adduct levels.

<sup>d</sup> Newborn adduct levels = maternal adduct levels.

<sup>e</sup> Number of mother/newborn pairs.

<sup>f</sup> Sum of ranks.

73 of 158 (46%) of the pairs, cotinine in both maternal and newborn plasma was below the limit of detection. Among the 27 pairs in which cotinine in either the maternal or the newborn plasma was below the limit of detection, there was a higher proportion of pairs in which the cotinine level was above the limit of detection in the newborn and below the limit of detection in the mother (22 of 27) than pairs in which the cotinine was below the limit of detection in the newborn and was above the limit of detection in the mother (5 of 27).

## Discussion

This study found that the levels of DNA damage from PAHs and other aromatics were higher in newborn WBCs than in paired maternal WBCs. The difference in mean adduct levels between newborn and maternal WBCs was small and not always statistically significant; nonetheless, the results point to the possibility of increased susceptibility of the develop-

ing fetus to DNA damage from this class of carcinogens. Experimental evidence in rodents indicates that transplacental exposures to PAHs are generally an order of magnitude or more lower than paired maternal exposures (18). In light of these experimental results, our findings suggest that the amount of DNA damage/delivered dose of PAHs may be considerably higher in the fetus relative to his/her mother. This increased susceptibility could result from reduced detoxification capabilities or decreased DNA repair capacity during fetal development (reviewed in Refs. 1, 55).

The ratio of DNA damage in newborn WBCs compared with maternal WBCs differed by maternal smoking status. Specifically, among newborns of nonsmoking women, both aromatic-DNA adducts and PAH-DNA adducts were higher in newborn WBCs compared with paired maternal WBCs, a difference that was highly significant for aromatic-DNA adducts ( $P = 0.005$ ) and of borderline significance for PAH-DNA

Table 3 WBC PAH-DNA adduct levels by ELISA in paired maternal and newborn blood samples

	Mean $\pm$ SD/ $10^8$ nucleotides		Ranks for mother/newborn pairs <sup>a</sup>				Ties <sup>d</sup> No. <sup>e</sup>	<i>P</i> <sup>a</sup>
	Maternal	Newborn	Negative ranks <sup>b</sup>		Positive ranks <sup>c</sup>			
			No. <sup>e</sup>	Sum <sup>f</sup>	No. <sup>e</sup>	Sum <sup>f</sup>		
A. WBC PAH-DNA adduct levels in all of the mother/newborn pairs (total sample)								
Total cohort ( <i>n</i> = 112)	5.9 $\pm$ 8.2	7.9 $\pm$ 9.9	43	1557	44	2271	25	<i>P</i> = 0.13
Nonsmoker ( <i>n</i> = 70)	5.1 $\pm$ 6.3	8.1 $\pm$ 9.4	25	498	28	933	17	<i>P</i> = 0.05
Ex-smoker ( <i>n</i> = 32)	5.4 $\pm$ 6.7	8.4 $\pm$ 1.3	11	130	15	221	6	<i>P</i> = 0.25
Current smoker ( <i>n</i> = 10)	12.9 $\pm$ 17.5	4.6 $\pm$ 8.4	7	32	1	4	2	<i>P</i> = 0.05
B. WBC PAH-DNA adduct levels in mother/newborn pairs with blood drawn concurrently (concurrent samples)								
Total cohort ( <i>n</i> = 60)	5.7 $\pm$ 7.4	9.4 $\pm$ 11.0	21	382	27	794	12	<i>P</i> = 0.04
Nonsmoker ( <i>n</i> = 45)	4.9 $\pm$ 6.8	9.1 $\pm$ 10.2	15	191	21	475	9	<i>P</i> = 0.03
Ex-smoker ( <i>n</i> = 12)	9.4 $\pm$ 9.5	12.7 $\pm$ 14.0	5	24	6	42	1	<i>P</i> = 0.42
Current smoker ( <i>n</i> = 3)	3.7 $\pm$ 4.6	1.0 $\pm$ 0.0	1	1	0	0	2	<i>P</i> = 0.32

<sup>a</sup> Wilcoxon signed-ranks test.<sup>b</sup> Newborn adduct level < maternal adduct levels.<sup>c</sup> Newborn adduct levels > maternal adduct levels.<sup>d</sup> Newborn adduct levels = maternal adduct levels.<sup>e</sup> Number of mother/newborn pairs.<sup>f</sup> Sum of ranks.

Table 4 Plasma cotinine levels in paired maternal and newborn blood samples

	Mean $\pm$ SD ng/ml		Ranks for mother/newborn pairs <sup>a</sup>				Ties <sup>d</sup> No. <sup>e</sup>	<i>P</i> <sup>a</sup>
	Maternal	Newborn	Negative ranks <sup>b</sup>		Positive ranks <sup>c</sup>			
			No. <sup>e</sup>	Sum <sup>f</sup>	No. <sup>e</sup>	Sum <sup>f</sup>		
A. Plasma cotinine levels in all of the mother/newborn pairs (total sample)								
Total cohort ( <i>n</i> = 158)	8.3 $\pm$ 24.5	14.2 $\pm$ 35.5	13	344	64	2659	81	<i>P</i> < 0.001
Nonsmoker ( <i>n</i> = 104)	3.2 $\pm$ 16.5	4.0 $\pm$ 18.3	11	201	33	790	60	<i>P</i> = 0.001
Ex-smoker ( <i>n</i> = 38)	11.4 $\pm$ 28.2	17.7 $\pm$ 38.3	2	21	16	150	20	<i>P</i> = 0.005
Current smoker ( <i>n</i> = 16)	34.7 $\pm$ 38.6	72.2 $\pm$ 53.8	0	0	15	120	1	<i>P</i> = 0.001
B. Plasma cotinine levels in mother/newborn pairs with blood drawn concurrently (concurrent samples)								
Total cohort ( <i>n</i> = 80)	7.2 $\pm$ 25.9	7.7 $\pm$ 27.3	8	159	33	703	39	<i>P</i> < 0.001
Nonsmoker ( <i>n</i> = 60)	3.3 $\pm$ 19.4	3.4 $\pm$ 18.9	7	104	23	362	30	<i>P</i> = 0.008
Ex-smoker ( <i>n</i> = 16)	9.4 $\pm$ 22.9	10.1 $\pm$ 23.9	1	7	7	29	8	<i>P</i> = 0.12
Current smoker ( <i>n</i> = 4)	57.0 $\pm$ 62.8	63.7 $\pm$ 71.4	0	0	3	6	1	<i>P</i> = 0.11

<sup>a</sup> Wilcoxon signed-ranks test.<sup>b</sup> Newborn cotinine levels < maternal cotinine levels.<sup>c</sup> Newborn cotinine levels > maternal cotinine levels.<sup>d</sup> Newborn cotinine levels = maternal cotinine levels.<sup>e</sup> Number of mother/newborn pairs.<sup>f</sup> Sum of ranks.

Table 5 Number of maternal/newborn pairs with biomarker levels above and below the limit of detection and ratio of newborn/maternal biomarker levels among pairs with detectable levels

	Pairs with maternal and/or newborn levels below limit of detection			Pairs with maternal and newborn levels above the limit of detection	
	Both below detection	Newborn above/maternal below	Newborn below/maternal above	Both above detection	Ratio newborn/maternal levels: mean $\pm$ SD (range)
Aromatic-DNA adduct levels	<i>N</i> = 2	<i>N</i> = 2	<i>N</i> = 5	<i>N</i> = 113	4.1 $\pm$ 12.5 (0.04–105.3)
PAH-DNA adduct levels	<i>N</i> = 24	<i>N</i> = 20	<i>N</i> = 24	<i>N</i> = 44	2.7 $\pm$ 3.2 (0.11–15.5)
Plasma cotinine	<i>N</i> = 73	<i>N</i> = 22	<i>N</i> = 5	<i>N</i> = 58	2.0 $\pm$ 1.8 (0.67–9.7)

adducts (*P* = 0.05). By contrast, among newborns of current smoking women, aromatic-DNA adducts were not significantly different from paired maternal levels (*P* = 0.8) and PAH-DNA adducts were lower (*P* = 0.05). It is possible that enzyme activity of cytochrome P4501A1 (CYP1A1) in placental tissue contributed to these differences. We and others (44, 56–59)

have shown placental CYP1A1 to be highly induced by maternal cigarette smoking. Prior evidence suggests that induction of CYP1A1 in placentas of smokers may reduce the biologically effective dose to the fetus (47, 48). As reported previously (45, 60), we also found that placental CYP1A1 was inversely, although not significantly, correlated with PAH-DNA adduct

levels in WBCs and placental tissue from newborns in the current cohort.

This study also found that cotinine levels were consistently and, in most cases, significantly higher in newborn plasma compared with paired maternal plasma. Few prior studies (39–43) have compared cotinine levels in mother/newborn pairs, and results have been conflicting, with both higher and lower concentration in the neonate reported. It is likely that methodological differences in the timing of the maternal blood collection relative to newborn blood collection have contributed to these differences, because most prior studies have not collected the maternal and newborn blood samples concurrently. Given the short half-life of cotinine in plasma, concurrent sampling is needed to differentiate the possibilities that newborn/maternal cotinine ratios reflect a true biological difference in dose and/or metabolic processing of cotinine or a difference in elapsed time between cigarette smoke exposure and newborn *versus* maternal blood collection. Our results suggest that there is a biological difference and that cotinine concentrates in the fetus. Consistent with our findings, a recent study by Nafstad *et al.* (41) also found cotinine levels in cord sera to be significantly higher than paired maternal sera levels. In that study, the maternal blood sample was collected within 2 h of the cord blood samples in 26 of 28 mother/newborn pairs.

Cotinine has low toxicity relative to nicotine (30, 31), but it is a good internal dosimeter for nicotine, although interindividual differences in the metabolism of nicotine to cotinine have been documented (29). Both experimental and epidemiological evidence indicates that nicotine also concentrates in the fetus (30, 39). This appears attributable, at least in part, to reduced clearance mechanisms during fetal development. Specifically, experimental studies (30) indicate that although nicotine rapidly reaches the fetus in concentrations approximately equal to those of the mother, the rate of disappearance is slower. Some studies in humans (reviewed in Ref. 61) have shown that the half-life of cotinine in bodily fluids is also longer in the neonate than in the adult, whereas others (62) have shown that the half-life of cotinine in blood and urine is similar in the newborn and in the adult but that the half-life of nicotine is three to four times longer. In addition, the fetus is likely to be exposed to nicotine from gastrointestinal reabsorption of nicotine in swallowed amniotic fluid, as well as through transfer from the maternal circulation (43). Nicotine levels in amniotic fluid have been shown to be 54% higher than the corresponding maternal serum levels (39). This additional source of fetal nicotine exposure relative to maternal nicotine exposure may also contribute to the higher cotinine concentration seen here in newborn plasma relative to maternal plasma.

These findings highlight the need for smoking prevention programs aimed at women of childbearing age. The adverse effects of nicotine on fetal growth are well documented (30). In addition, the nicotine-derived nitrosamine NNK is a potent transplacental carcinogen in experimental bioassays (63). Prior epidemiological research (63) has shown higher levels of NNK metabolites in urine collected immediately after delivery from newborns of smokers compared with NNK metabolite levels in urine from newborns of nonsmokers.

Our findings also suggest increased susceptibility of the fetus to DNA damage from PAHs and other aromatics, consistent with prior experimental evidence of age-related susceptibility to PAH-induced carcinogenesis during fetal and neonatal development (4, 7–10). They are of concern in light of the association seen previously (15, 64) in molecular epidemiological research between PAH-DNA adduct levels and cancer risk, as well as PAH-DNA adducts and adverse birth outcome (49).

The findings have implications for risk assessment, which currently does not adequately account for sensitive subsets of the population.

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## Biomarkers of Polycyclic Aromatic Hydrocarbon-DNA Damage and Cigarette Smoke Exposures in Paired Maternal and Newborn Blood Samples as a Measure of Differential Susceptibility

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