Biomarkers, January–February 2007; 12(1): 38–46

informa
healthcare

Biomarkers of exposure to environmental tobacco smoke in infants

M. SØRENSEN[1], H. BISGAARD[2], M. STAGE[2], & S. LOFT[1]

[1]*Institute of Public Health, University of Copenhagen, Copenhagen, Denmark and* [2]*Danish Pediatrics Asthma Center, Copenhagen University Hospital, Copenhagen, Denmark*

Abstract
Non-invasive biomonitoring of exposure to environmental tobacco smoke (ETS) by means of hair is attractive in children, although systematic evaluation is required in infants. The objective was to compare nicotine and cotinine concentrations in hair and plasma and parentally reported exposure to ETS in a birth cohort of 411 infants. Plasma was collected from 356 six-month-old infants and hair samples were collected from 368 one-year-old infants. Concentrations of nicotine and cotinine were measured by an optimized gas chromatography-mass spectrometry (GC/MS)-based method requiring 4 mg hair or 200 μl plasma. Information was obtained on the number of days with ETS exposure during the first year of life, the smoking habits of the parents, and the number of cigarettes smoked per day in the home. All three parentally reported indices of ETS exposure were significantly associated with the biomarkers, with clear dose–response relationships. There was a significant association between days with ETS exposure and nicotine in hair at relatively low exposure levels (10–99 days per year), whereas the other biomarkers only showed significant increases at higher exposure levels. In conclusion, nicotine in hair appears to be the biomarker most strongly associated with parental reports on exposure to ETS in infants.

Keywords: *Nicotine, cotinine, environmental tobacco smoke, birth cohort*

(Received 15 February 2006; returned 27 July 2006; accepted 1 August 2006)

Introduction

Exposure to environmental tobacco smoke (ETS) is a major environmental risk factor for respiratory illness in infants and children (Cook & Strachan 1999, DiFranza et al. 2004). ETS exposure has typically been assessed by questionnaires with the inherent problems of recall bias and underreporting. Furthermore, questionnaires often lack sensitivity to quantify low concentrations of ETS exposure.

Exposure to ETS can be assessed by means of biomarkers. Nicotine and its metabolites in biological material reflect exposure to ETS (Eliopoulos et al. 1996, Al Delaimy 2002). Nicotine in ETS is rapidly adsorbed through the lungs and widely distributed throughout the body (Feyerabend et al. 1985, Jarvis et al. 1988). Due to rapid elimination, plasma and urine levels of both nicotine and cotinine mainly reflect the recent exposure (24 h). Nicotine and cotinine are incorporated in the hair as long

Correspondence: S. Loft, Institute of Public Health, University of Copenhagen, Øster Farimagsgade 5A, DK-1014 Copenhagen K, Denmark. Tel: 45-3532-7649. Fax: 45-3532-7686. E-mail: s.loft@pubhealth.ku.dk

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.1080/13547500600943148

as they are present in the circulation. Therefore, the concentrations of nicotine and cotinine in hair represent a cumulative dose collected gradually over a period of exposure and appear to be valid and reliable biomarkers of long-term exposure (Nafstad et al. 1995, Al Delaimy 2002, Al Delaimy et al. 2002). However, the association between ETS exposure and nicotine/cotinine concentrations in hair, plasma and urine are disputed and there are very limited data from infants (Kintz & Mangin 1993, Nafstad et al. 1995, 1998, Eliopoulos et al. 1996, Klein & Koren 1999, Sovik et al. 1999, Al Delaimy et al. 2000, 2002).

The aim of this paper was to compare two biomarkers for ETS exposure in terms of concentrations of nicotine and cotinine in hair and plasma and parentally reported exposure to ETS, including assessment of dose–response relationships, in infants. We developed gas chromatography-mass spectrometry (GC/MS)-based assays for low concentrations of nicotine and cotinine in small samples of hair and plasma from infants. We studied ETS exposure in a birth cohort of 411 infants by means of interviews and questionnaires at the age of 1.0–1.5 years and measurement of the concentration of nicotine and cotinine in plasma samples obtained at the age of 6 months and hair samples obtained at the age of 1 year.

Materials and methods

Study population

The Danish birth cohort entitled 'The Copenhagen Prospective Study on Asthma in Childhood' (COPSAC) includes 411 infants of asthmatic mothers (Bisgaard 2004). All the children are Caucasians. Blood was collected at the age of 6 months in ethylenediamine tetra-acetic acid (EDTA) tubes from 356 infants, after which plasma was separated and stored at -80°C. A tuft of hair was removed from the occipital region (1–3 cm behind the ear) of 368 infants aged 1 year and stored at -80°C. The average hair length was 1.4 ± 0.6 cm and all infants except one had light blond, medium blond or dark blond hair. At the 1-year clinic visit, the parents were interview with predefined questions and closed response categories. Information was gathered on the number of days the infants were exposed to ETS during the first year as well as the parents' smoking habits. Furthermore, information on the number of cigarettes smoked per day in the home was collected later by a questionnaire at age 1.0–1.5 year for 384 of the children. It was collected by daily recording of the number of cigarettes smoked in the home during a period of 7–14 days. The Copenhagen Ethics Committee approved the study (No. KF-01-189/96). Before enrolment, the parents gave informed consent. The quality control procedures assured data validity.

Extraction of cotinine and nicotine in hair

We modified and optimized a method previously described for the determination of nicotine and cotinine in plasma (Jacob et al. 1991) in order to measure nicotine and cotinine concentrations in small amounts of hair samples. Before analysis we washed 4 mg of hair from each infant with 2 ml dichloromethane for 1.5 h (on a vortex) at room temperature; every 30 min the dichloromethane was changed. The samples were dried at 50°C. Subsequently, 150 µl of 2 M NaOH, 0.2 M ammonia were added to each vial, which was then capped and incubated at 50°C overnight. After cooling we split the sample in two and 0.5 ng ($\pm$)-nicotine-d_3 and 0.5 ng DL cotinine-d_3

(Sigma-Aldrich, Copenhagen, Denmark) in 10 µl toluene were added to each sample as internal standards. At this point we prepared samples for the standard curve by adding the standard solutions (0, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 ng/10 µl) in 10 µl toluene and 10 µl internal standard to 150 µl 2 M NaOH, 0.2 M ammonia. After vortexing for 5 min, we added 300 µl toluene-1-butanol mixture (70:30). The vials were capped, vortexed for 5 min and centrifuged at 1000*g* for 10 min. Subsequently, we placed them in a dry ice–acetone bath to freeze the aqueous layer. The organic layers were poured into new glass vials containing 50 µl of 0.5 M sulphuric acid and vortexed for 5 min, centrifuged at 1000*g* for 10 min and placed in a dry ice–acetone bath. The organic layers were poured off and discarded and 50 µl of 50% w/v potassium carbonate, 0.2 M ammonia and 20 µl toluene–butanol (90:10) were added. After vortexing for 5 min and centrifugation at 1000*g* for 10 min we placed the samples in dry ice–acetone. The organic layer (approximately 25 µl) was transferred into 300 µl glass vials and 2 µl was injected into the GC/MS.

To reduce the risk of nicotine contamination of the samples, all glassware used was incubated at 200°C overnight. Furthermore, a system was installed that created high air pressure in the laboratory used for the nicotine analyses such that air from the surroundings was keep out. All personal in the laboratory were non-smokers and smoking was not allowed in the building. We examined the removal of nicotine and cotinine during the washing procedure by measuring the concentrations in washed and unwashed hair samples. We found that, depending on the sample, 30–60% of the nicotine was removed by washing, while the cotinine concentration remained constant.

Extraction of cotinine and nicotine in plasma

To 100 µl plasma we added 0.5 ng (±)-nicotine-d_3 and 0.5 ng DL cotinine-d_3 (Sigma-Aldrich) in 10 µl 0.1 M HCl to each sample as internal standards. All samples were run in duplicate. We prepared samples for the standard curve by adding the standard solutions (0, 0.05, 0.1, 0.2, 0.5, 1.0 and 2.0 ng/10 µl) in 10 µl 0.1 M HCl and 10 µl internal standard to 100 µl nicotine and cotinine free 'control' plasma. The subsequent procedure was identical to the extraction of cotinine and nicotine in hair, starting from vortexing and addition of 300 µl toluene-1-butanol mixture (70:30), except that only 1 µl was injected into the GC-MS.

GC-MS analysis

Analyses were performed on a Hewlett-Packard 6890 Series GC with 5973 mass selective detector, equipped with an autosampler (HP 7673) and a split/splitless injector. The ion source was upgraded to G2591-64710 (Agilent Technologies, Palo Alto, CA, USA). The column was a 19091B-101 ULTRA 2 capillary column (12 m × 0.2 mm fused-silica capillary column coated with a 0.33 µm film of cross-linked 5% phenyl-methyl-silicone) from Agilent Technologies. The injection of sample was made in splitless mode with use of glass injection port liners. The injection port temperature was 250°C and the carrier gas (helium) flow rate was 20 ml min^{-1}. The temperature programme for the column oven was: 70°C for 1 min, then an increase of 12°C min^{-1} in 8.5 min, followed by a 30°C increase per min for 3.7 min to 270°C, which was then kept constant for 3 min. Selective-ion monitoring was 84 *m/z* and 87 *m/z* for nicotine and the internal standard d_3-nicotine and 176 *m/z* and 179 *m/z* for cotinine and the

internal standard d_3-cotinine, respectively. Quantification was achieved by integration of the ion chromatograms and constructing seven-point standard curves of response (peak area ratio of analyte/internal standard) versus concentration, by linear regression.

The limits of quantification were 0.05 ng cotinine and 0.1 ng nicotine in 2 mg hair, and 0.2 ng cotinine ml^{-1} and 0.1 ng nicotine ml^{-1} in plasma. To be able to include measurements below the quantification limit in a logarithmic model, we gave them a value estimated according to the formula:

$$\text{Quantification limit}/\sqrt{2},$$

suggested by Hornung & Reed (1990). On the basis of ten repeated measurements of a hair sample (mean concentration: 0.74 and 0.08 ng cotinine mg^{-1}) the intraday coefficient of variation (CV) was calculated to 9.5% for nicotine and to 8.4% for cotinine. The interday CV was 10.6% for nicotine and 12.1% for cotinine (mean concentration: 3.2 and 0.10 ng cotinine mg^{-1}). Ten repeated measurements of a plasma sample (mean concentration: 5.5 and 5.3 ng cotinine ml^{-1}) resulted in an intraday CV on 4.1% for nicotine and on 4.0% for cotinine. The interday CV was 7.2% for nicotine and 4.7% for cotinine (mean concentration: 5.3 and 5.3 ng cotinine ml^{-1}).

Statistics

Correlations were calculated using Spearman's rank correlation coefficient (r_s). The nicotine and cotinine concentrations in hair and plasma were logarithmically transformed to obtain normal distribution of the residuals and variance homogeneity. Due to non-linearity of the residuals, the exposure to ETS during the child's first year of life was split into four categories, 0, 1–9, 10–99 and 100–365 days. The parental smoking habits were split into three categories: parents did not smoke, one parent smoke and both parents smoke. The daily exposure to cigarettes was split into three categories: 0, 1–9 and 10–25 cigarettes. General Linear Model analysis was used to describe the dependent variable as a function of the predictors. All statistical analyses were performed using the SAS software (version 8e).

Results

The median concentrations of cotinine and nicotine in hair and plasma are shown in Table I. Nicotine in both plasma and hair and cotinine in hair were measurable in most samples, while the cotinine plasma concentration was often below the limit of quantification.

Table I. Concentrations of nicotine and cotinine in plasma (6 month) and in hair (1 year).

	Median	Q25–Q75[a]	*n*	Above the quantification limit
Hair nicotine (ng mg^{-1})	0.74	0.33–2.45	368	366
Hair cotinine (ng mg^{-1})	0.051	0.034–0.089	368	344
Plasma nicotine (ng ml^{-1})	0.39	0.24–0.61	356	356
Plasma cotinine (ng ml^{-1})	0.42	0.00–1.58	356	232

[a]25–75% interquartile range.

Table II. Correlations between nicotine concentrations and cotinine concentrations in plasma (6 month) and hair (1 year).

	Cotinine in hair	Nicotine in plasma	Cotinine in plasma
Nicotine in hair	$r_s = 0.77$ $p < 0.0001$	$r_s = 0.36$ $p < 0.0001$	$r_s = 0.54$ $p < 0.0001$
Cotinine in hair	–	$r_s = 0.36$ $p < 0.0001$	$r_s = 0.50$ $p < 0.0001$
Nicotine in plasma	–	–	$r_s = 0.60$ $p < 0.0001$

r_s, Spearman's rank correlation coefficient.

The concentrations of nicotine and cotinine in hair and plasma were significantly correlated with each other (Table II). The correlations seemed strongest between nicotine and cotinine within the same biological material, hair or plasma. When comparing the biomarkers in hair with the biomarkers in plasma, the strongest correlations seemed to be between nicotine or cotinine in hair and cotinine in plasma (Table II). In a General Linear Model analysis with both the nicotine and the cotinine concentrations in plasma included as predictors of the nicotine concentration in hair, only the plasma cotinine concentration was significantly associated with the hair nicotine concentration ($p < 0.0001$). The same was found when we included the concentration of cotinine in hair as the dependent variable ($p < 0.0001$, plasma cotinine concentration).

There were no significant differences between nicotine or cotinine concentrations in plasma or hair in relation to the gender of the infant ($p > 0.19$; data not shown). The distribution of parentally reported exposure to ETS as days with exposure during the first year divided in four categories, parental dichotomized smoking habits divided in three categories and daily intensity at home divided in three categories are shown in Table III. These interview/questionnaire data indicated that exposure to ETS mainly occurred at home. Thus, 64 of the 70 infants who were reported to be exposed to ETS between 100 and 365 days were also registered with a daily intensity at home of one or more cigarettes, and 72 of the 74 infants with no days of ETS exposure were also reported to have no daily smoking at home.

All three parentally reported indices of exposure to ETS were significantly associated with the four biomarkers, nicotine and cotinine in plasma and hair, with clear dose–response relationships (Table III). With regard to cumulative ETS exposure (days with ETS exposure) there was a significant dose–response relationship for the association with nicotine in hair from 10–99 days of exposure per year, whereas the three other biomarkers showed significant increases with 100 or more days of exposure per year (Table III). The nicotine concentration in hair appeared to be the biomarker with the strongest association with the reported ETS exposure ($R^2 =$ 0.40 for days with ETS exposure and 0.44 for daily intensity), followed by plasma cotinine, hair cotinine and plasma nicotine (Table III). If we separated parental smoking into mothers smoking and fathers smoking, we found both variables to be significantly associated with the four biomarkers (results not shown). The strength of the associations between having a smoking mother or farther and the biomarkers in hair were similar with $R^2 = 0.31$ and 0.29 for nicotine and 0.25 and 0.24 for cotinine,

Table III. Associations between days with exposure to environmental tobacco smoke (ETS), parental smoking habits and exposure to cigarettes per day and the measurement of nicotine and cotinine concentrations in plasma (6 months) and hair (1 year).

	Hair nicotine (ng mg^{-1})[a]	Hair cotinine (ng mg^{-1})[a]	Plasma nicotine (ng ml^{-1})[a]	Plasma cotinine (ng ml^{-1})[a]
ETS exposure (days)				
0 ($n=79$)	0.97±1.41	0.054±0.054	0.41±0.21	1.41±4.55
1–9 ($n=111$)	0.99±2.16	0.058±0.058	0.37±0.21	0.66±1.61
10–99 ($n=138$)	2.25±5.42⋆	0.075±0.110	0.44±0.24	1.42±3.61
100–365 ($n=75$)	10.48±14.19⋆	0.181±0.163⋆	0.78±0.93⋆	6.31±7.06⋆
p	<0.0001	<0.0001	<0.0001	<0.0001
R^2 [b]	0.38	0.24	0.12	0.29
Number of smoking parents				
0 ($n=221$)	0.87±1.67	0.049±0.043	0.36±0.18	0.41±0.96
1 ($n=74$)	3.41±4.78⋆	0.096±0.093⋆	0.47±0.26⋆	2.78±5.31⋆
2 ($n=41$)	15.06±18.16⋆	0.260±0.221⋆	1.00±1.19⋆	9.01±8.21⋆
p	<0.0001	<0.0001	<0.0001	<0.0001
R^2 [b]	0.41	0.34	0.23	0.42
Daily exposure (cigarettes per day)				
0 ($n=301$)	1.19±2.41	0.058±0.058	0.40±0.22	0.96±3.03
1–9 ($n=56$)	5.60±6.04⋆	0.127±0.099⋆	0.61±0.51⋆	4.80±6.78⋆
10–25 ($n=27$)	21.06±20.08⋆	0.325±0.245⋆	1.05±1.32⋆	8.74±6.21⋆
p	<0.0001	<0.0001	<0.0001	<0.0001
R^2 [b]	0.44	0.34	0.16	0.37

Data were analysed by one-way analysis of variance (ANOVA).
[a]Mean±standard deviation.
[b]Coefficient of determination.
⋆Significantly different from no exposure, $p<0.01$.

respectively, whereas the biomarkers in plasma seemed more strongly associated with having a smoking mother than a smoking farther with $R^2=0.21$ and 0.14 for nicotine and 0.39 and 0.26 for cotinine, respectively. Excluding the observations below the quantification level did not result in any marked changes in the mean values in Table III, whereas the correlations and conclusions based on the statistical analyses remained the same (data not shown).

Discussion

The measured biomarkers nicotine and cotinine in hair and plasma from infants showed associations with clear dose–response relationships with the information from parents on ETS exposure. In particular, the concentration of nicotine in hair collected at 1 year of age appeared to be more strongly associated with parentally reported ETS exposure, especially cumulated exposure during the first year of life, than hair cotinine and plasma nicotine and cotinine.

Previous studies that analysed nicotine and cotinine concentrations in hair by the GC-MS method used between 10 and 50 mg of hair (Kintz 1992, Nafstad et al. 1995, Dimich-Ward et al. 1997). The present study only had approximately 4 mg of hair available from each child. The method was optimized to this hair volume, which included search for the optimal method volumes of reagents, an improved washing procedure, and the use of a new and more sensitive ion source for the mass selective

detector. The quantification limits and the interday and intraday CVs of this method were similar to the quantification limits and CVs in previous studies (Kintz 1992, Nafstad et al. 1995, Dimich-Ward et al. 1997, Sovik et al. 1999, Al Delaimy et al. 2002, Chetiyanukornkul et al. 2004).

Most studies on the health consequences of ETS exposure in infants and children use interview/questionnaire-based information for the assessment of ETS exposure. However, using this information as a 'gold standard' has several limitations. First, with this information it is difficult to quantify a low ETS exposure because normally only a daily exposure is registered, typically the number of cigarettes smoked by the parents or whether or not the parents smoke. In accordance with this, it was found that only 115 of the infants in the present study had one or two smoking parents and that a daily exposure to cigarettes was reported for only 83 infants, whereas exposure to ETS for 1 day or more was reported for 310 infants. Second, this information has the inherent problems of recall bias and underreporting. Third, the reported exposure typically lacks information on the actual exposure due to, for example, a lack of information on ventilation at home and a lack of information on the distance between the individual smoking and the infant/child. Also, most studies do not have specific information on outdoor parental smoking which may contribute to the actual exposure (Matt et al. 2004), though this is not confirmed by all studies (Al Delaimy et al. 2001, Groner et al. 2005). Therefore, although the reported ETS exposure in the present study contains more details than many other studies, it still does not give a complete picture of the exposure.

The reported ETS exposure that is available in most studies are parental smoking habits and/or the daily number of cigarettes smoked at home (Nafstad et al. 1995, Al Delaimy et al. 2000, 2002). Discrepant results have been found when comparing the reported ETS exposure with nicotine/cotinine concentrations in neonatal hair, as some (Kintz & Mangin 1993, Klein & Koren 1999) but not all studies (Nafstad et al. 1998, Sovik et al. 1999) find the biomarkers associated with maternal hair nicotine or maternal smoking during pregnancy, whereas studies of older children mostly find daily parental smoking to be associated with nicotine and cotinine concentrations in hair (Nafstad et al. 1995, Al Delaimy et al. 2000, 2002). To our knowledge, the present study is the first to assess cumulated exposure by biomarkers in 1-year-old infants.

The finding of a significantly increased concentration of nicotine in hair after low levels of exposure to ETS (10–99 days per year) indicates that this measurement could be a more sensitive biomarker than the three other measured biomarkers, and that it can detect even a non-daily cumulated exposure to ETS. It also indicates that the registration of days with exposure to ETS in infancy adds exposure information to the commonly used interview/questionnaire information about smoking intensity and parental smoking, and that this could be a valid way to register non-daily exposure in future studies. Nicotine in hair was also the biomarker that was most strongly associated with the number of cigarettes smoked at home and similar to plasma cotinine for detection of parental smoking, again suggesting that this biomarker could be a more sensitive biomarker of ETS exposure than the other measured biomarkers. The collection of hair is non-invasive, which is of advantage in studies of infants and children. This biomarker could, thus, prove to be a very important tool in future studies on the relationship between even low and cumulated exposures to ETS and the health of children.

The concentration of cotinine in hair was also increased with increased reported exposure to ETS, though this was not significant at low reported levels of exposure. This is similar to previous studies that found the concentration of nicotine in hair to be more strongly associated with the reported ETS exposure than cotinine in hair (Dimich-Ward et al. 1997, Al Delaimy et al. 2000). Cotinine in hair may have one advantage as a biomarker compared with nicotine in hair because cotinine is only generated in the body and not found in the external environment. However, most authors argue that hair mainly takes up nicotine from the systemic circulation and not from the ambient environment (summarized in Al Delaimy 2002). Moreover, hair samples are washed thoroughly in solvent before analysis.

The association between nicotine and cotinine in hair and nicotine and cotinine in blood is only sparsely investigated. A study of 36 adults reported no correlation between plasma nicotine and hair nicotine and only a weak correlation between plasma cotinine and hair cotinine (Eliopoulos et al. 1996). That study, furthermore, found that plasma nicotine and cotinine seemed more strongly associated with the reported smoking exposure than hair nicotine and cotinine (Eliopoulos et al. 1996). In contrast, the present paper found significant correlations between nicotine and cotinine in hair on one hand and nicotine and cotinine in plasma on the other hand, although sampling was separated by 6 months in time. The plasma levels depend on the instant the blood sample was drawn in relation to the most recent exposure, whereas the nicotine and cotinine concentration in hair with its slow growth rate is a long-term marker of cumulated exposure. Most likely, the finding could be explained by a rather consistent exposure over the 6-month period. Plasma nicotine was poorer related to the biomarkers in hair and to the parentally reported exposures than plasma cotinine, which could be explained by the fact that the half-life of nicotine in blood is only 2 h compared with 17 h for plasma cotinine. However, it cannot be excluded that the close temporal association between collection of parental information on ETS exposure at 12 or 12–18 months of age and hair sampling at 12 months of age as compared with collection of plasma at 6 months of age contributed to the differences between the associations

In conclusion, concurrence was found between the concentrations of nicotine and cotinine in small samples of hair and plasma from infants and information from parents on ETS exposure with clear dose–response relationships. The concentrations of nicotine from 1-year-old infants showed a particularly strong dose-dependent association with the reported information on days with ETS exposure in the previous year, indicating that these measures of non-daily cumulated exposure could become important tools in studies of health in infants.

Acknowledgements

The authors would like to thank the parents and infants who participated in the COPSAC cohort. This study was funded by the Research Centre for Environmental Health, Danish Ministry of the Interior and Health.

References

Al Delaimy WK. 2002. Hair as a biomarker for exposure to tobacco smoke. Tobacco Control 11:176–182.

Al Delaimy WK, Crane J, Woodward A. 2000. Questionnaire and hair measurement of exposure to tobacco smoke. Journal of Exposure Analysis and Environmental Epidemiology 10:378–384.

Al Delaimy WK, Crane J, Woodward A. 2001. Passive smoking in children: effect of avoidance strategies, at home as measured by hair nicotine levels. Archives in Environmental Health 56:117–122.

Al Delaimy WK, Crane J, Woodward A. 2002. Is the hair nicotine level a more accurate biomarker of environmental tobacco smoke exposure than urine cotinine? Journal of Epidemiology and Community Health 56:66–71.

Bisgaard H. 2004. The Copenhagen Prospective Study on Asthma in Childhood (COPSAC): design, rationale, and baseline data from a longitudinal birth cohort study. Annals of Allergy and Asthma Immunology 93:381–389.

Chetiyanukornkul T, Toriba A, Kizu R, Kimura K, Hayakawa K. 2004. Hair analysis of nicotine and cotinine for evaluating tobacco smoke exposure by liquid chromatography-mass spectrometry. Biomedical Chromatography 18:655–661.

Cook DG, Strachan DP. 1999. Health effects of passive smoking — 10: Summary of effects of parental smoking on the respiratory health of children and implications for research. Thorax 54:357–366.

DiFranza JR, Aligne CA, Weitzman M. 2004. Prenatal and postnatal environmental tobacco smoke exposure and children's health. Pediatrics 113:1007–1015.

Dimich-Ward H, Gee H, Brauer M, Leung V. 1997. Analysis of nicotine and cotinine in the hair of hospitality workers exposed to environmental tobacco smoke. Journal of Occupational and Environmental Medicine 39:946–948.

Eliopoulos C, Klein J, Koren G. 1996. Validation of self-reported smoking by analysis of hair for nicotine and cotinine. Therapy and Drug Monitoring 18:532–536.

Feyerabend C, Ings RM, Russel MA. 1985. Nicotine pharmacokinetics and its application to intake from smoking. British Journal of Clinical Pharmacology 19:239–247.

Groner JA, Hoshaw-Woodard S, Koren G, Klein J, Castile R. 2005. Screening for children's exposure to environmental tobacco smoke in a pediatric primary care setting. Archives in Pediatric and Adolescent Medicine 159:450–455.

Hornung RW, Reed LD. 1990. Estimation of average concentration in the presence of nondetectable values. Applied Occupational and Environmental Hygiene 5:46–51.

Jacob P III, Yu L, Wilson M, Benowitz NL. 1991. Selected ion monitoring method for determination of nicotine, cotinine and deuterium-labeled analogs: absence of an isotope effect in the clearance of (S)-nicotine-3′,3′-d2 in humans. Biology and Mass Spectrometry 20:247–252.

Jarvis MJ, Russell MA, Benowitz NL, Feyerabend C. 1988. Elimination of cotinine from body fluids: implications for noninvasive measurement of tobacco smoke exposure. American Journal of Public Health 78:696–698.

Kintz P. 1992. Gas chromatographic analysis of nicotine and cotinine in hair. Journal of Chromatography 580:347–353.

Kintz P, Mangin P. 1993. Evidence of gestational heroin or nicotine exposure by analysis of fetal hair. Forensic Science International 63:99–104.

Klein J, Koren G. 1999. Hair analysis — a biological marker for passive smoking in pregnancy and childhood. Human Experimental Toxicology 18:279–282.

Matt GE, Quintana PJ, Hovell MF, Bernert JT, Song S, Novianti N, Juarez T, Floro J, Gehrman C, Garcia M, Larson S. 2004. Households contaminated by environmental tobacco smoke: sources of infant exposures. Tobacco Control 13:29–37.

Nafstad P, Botten G, Hagen JA, Zahlsen K, Nilsen OG, Silsand T, Kongerud J. 1995. Comparison of three methods for estimating environmental tobacco smoke exposure among children aged between 12 and 36 months. International Journal of Epidemiology 24:88–94.

Nafstad P, Fugelseth D, Qvigstad E, Zahlen K, Magnus P, Lindemann R. 1998. Nicotine concentration in the hair of nonsmoking mothers and size of offspring. American Journal of Public Health 88:120–124.

Sovik S, Lossius K, Eriksen M, Grogaard J, Walloe L. 1999. Development of oxygen sensitivity in infants of smoking and non-smoking mothers. Early Human Development 56:217–232.