

## Exposure to Environmental Tobacco Smoke in Pregnant Women: The Association between Self-Report and Serum Cotinine

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**The risk of delivering a low-birth-weight infant as the result of exposing a nonsmoking pregnant woman to environmental tobacco smoke (ETS) is not well defined. The method of ascertaining ETS exposure during pregnancy may explain the lack of consistent study findings. In a large sample of pregnant women, we compared distributions between two methods of ETS exposure: self-report and cotinine, a nicotine metabolite, from serum. At delivery, subjects were asked about duration and location of exposure to ETS during their second trimester. A single cotinine measurement was assayed from serum collected at 15–19 weeks gestation (limit of detection = 0.05 ng/mL). Self-reported (hours per day) ETS exposure was correlated ( $r=0.38$ ) with cotinine concentration. Regression analysis revealed that while self-reported ETS was significantly associated with (log) cotinine, it did not explain a large amount of total variation. While 72% of subjects reported no exposure to ETS, almost all had measurable levels of cotinine. Studies of pregnant women based upon an hours per day ETS question have likely misclassified a sizable portion of ETS-exposed women as “unexposed.” Since there is recent evidence that low levels of ETS exposure result in unfavorable pregnancy outcomes, these studies have underestimated the effect of ETS.** © 2002 Elsevier Science (USA)

**Key Words:** cotinine; environmental tobacco smoke pollution; pregnancy; validity.

### INTRODUCTION

A woman who actively smokes during pregnancy substantially increases her risk of delivering a low-birth-weight infant (Meredith, 1975). Women who do not smoke during pregnancy may still be exposed to environmental tobacco smoke (ETS). While the

propensity of studies has shown diminished birth weight with exposure to ETS, the size of the effect is variable (National Cancer Institute, 1999). Studies that have ascertained mother's ETS exposure during pregnancy through self-report of father's smoking behavior (Yerushalmy, 1971; Borlee *et al.*, 1978; Ruben *et al.*, 1986; Zhang and Ratcliffe, 1983; Martinez *et al.*, 1994), number of smokers in household (Mathai *et al.*, 1992; Ahluwalia *et al.*, 1997), and duration and intensity of exposure in different settings (Martin and Bracken, 1986; Lazzaroni *et al.*, 1990; Ahlborg and Bodin, 1991; Sadler *et al.*, 1999), have reported a range of differences in mean birth weight for ETS-exposed vs ETS-unexposed mothers of  $-9$  to  $-228$  g. Other studies have utilized a more objective measurement of ETS exposure through analysis of the biomarker, cotinine (Haddow *et al.*, 1988; Mathai *et al.*, 1990; Eskenazi *et al.*, 1995; Peacock *et al.*, 1998), and found differences in mean birth weight ranging from  $-43$  to  $-107$  g for the most highly exposed women. A recent investigation, using the most sensitive measurement of serum cotinine level currently available (Bernert *et al.*, 1997), has found a statistically significant dose-dependent 106g decrement in mean birth weight across the range of cotinine values (Kharrazi *et al.*, 2001). Studies that have employed less sensitive measurements of cotinine in physiological fluid may have underestimated the effect of prenatal ETS exposure on birth weight by misclassifying women as unexposed.

A lack of consistent findings could be related to different methods of assessing ETS exposure. Self-reports of exposure to ETS may lack precision, especially when detailed reporting of duration, dose (number of cigarettes smoked per day by others), and intensity of exposure (e.g., proximity to smokers, ventilation) are not collected (Benowitz,

1996). Quantification of urine, salivary, or serum cotinine concentration is correlated with levels of ETS exposure within 72 h of specimen collection (Benowitz, 1996). However, cotinine analysis can be expensive. Although questionnaire-obtained information is the more economical way to assess ETS exposure in large populations, response to ETS questions may not accurately estimate the amount of exposure during pregnancy. Thus, it is important to validate ETS questions by comparison with biomarker analysis.

Since the mid-1980s the proportion of nonsmoking women in the United States who are exposed to ETS during pregnancy has been estimated by a few self-report and biomarker studies with varying results. In population-based studies conducted in the United States, 22–49% of nonsmoking female study subjects reported being exposed to ETS during pregnancy (Ahluwalia *et al.*, 1997; Martin and Bracken, 1986; Pierce *et al.*, 1994; Sadler *et al.*, 1999; O'Connor *et al.*, 1995). Outside the United States, studies among pregnant women found that as high as 80% of nonsmoking women reported ETS exposure during pregnancy (Rebagliato *et al.*, 1995). By comparison, self-reported ETS exposure among all women in the United States has been estimated at 33% and as high as 62% among Hispanic-American women (Pirkle *et al.*, 1996; Pletsch, 1994). For biomarker-measured exposure, Haddow and colleagues (1988) found that 70% of pregnant women participating in a prenatal screening program had detectable levels of cotinine in serum (mean = 0.71 ng/mL). In the Yale Pregnancy Outcome Study, 52% of nonsmoking women had detectable levels of cotinine in urine (mean = 2.3 ng/mL) (O'Connor *et al.*, 1995). Pirkle and colleagues (1996) observed in a national sample, conducted between 1988 and 1991, that 88% of all nonsmokers, female and male, had detectable levels of cotinine in serum (geometric mean for females = 0.198 ng/mL).

Researchers have explored the relation between biochemical and self-reported ETS exposure methods in the general population (Coults *et al.*, 1990; Haley *et al.*, 1989; Delfino *et al.*, 1993; Wagenknecht *et al.*, 1993; Kemmeren *et al.*, 1994; Emmons *et al.*, 1996). These studies employed plasma/saliva cotinine samples to compare with self-reports. Estimates of association were highly variable (correlation coefficients,  $r$ , 0.22 to 0.89 in some studies;  $R^2$  values from linear regression models, 0.06 to 0.98 in other studies). Fewer studies have been conducted among women only (Becher *et al.*, 1992; Riboli *et al.*, 1995) or where pregnant women have been selected exclusively (O'Connor *et al.*, 1995; Rebagliato *et al.*, 1995).

Among nonsmoking pregnant women attending a prenatal clinic (Valencia, Spain), Rebagliato and colleagues (1995) found that self-reported hours of exposure to ETS over 3 days prior to interview were positively correlated with saliva cotinine ( $r = 0.52$ ). In the Yale Passive Smoking and Pregnancy Study, nonsmoking pregnant women reported source and length of time exposed to ETS (O'Connor *et al.*, 1995). Self-reports were compared to urine cotinine levels and air nicotine levels from personal monitors. Self-reported ETS information showed little relation to urine cotinine concentrations ( $r = 0.03$  for all weekly exposures to ETS). Differing results from only two studies conducted during pregnancy strongly indicate the need for further investigation. Moreover, both studies were clinic-based investigations, leaving open the question of how generalizable results are to the population of pregnant women at large.

In this population-based study of nonsmoking pregnant women, we investigated the association between self-reported hours per day of exposure to ETS in three different settings and serum cotinine samples obtained during pregnancy. Since we expected that levels of exposure will be low, we have employed the most sensitive assay method currently available for the analysis of serum cotinine (Bernert *et al.*, 1997). We will demonstrate that a large percentage of women who said that they were exposed to zero hours of ETS had quantifiable concentrations of cotinine from sera obtained during mid-pregnancy.

## MATERIALS AND METHODS

### *Study Population*

The eligible study population included 1560 pregnant women who enrolled in the California state-wide Maternal Serum Alpha-Fetoprotein (MSAFP) prenatal screening program in April 1992 in four Central Valley California Counties. For this program, women provided a blood specimen between the 15th and 19th weeks of gestation. After MSAFP analyses were completed, the remaining specimen was stored for up to 4 years at  $-20^{\circ}\text{C}$ , until it was sent to the Centers for Disease Control and Prevention (CDC) for cotinine quantification.

For purposes of this analysis, prenatal screening program records were linked with vital records of live birth/fetal death occurring between July and October 1992 (92% match rate). During birth registration women were asked to complete a supplemental questionnaire. For the study sample we excluded women who did not have a banked blood

specimen ( $n = 229$ ), women who did not receive the supplemental questionnaire ( $n = 66$ ), women who did not complete the questionnaire ( $n = 176$ ), or women who were not linked to vital records ( $n = 51$ ). Demographic (e.g., mother's age, ethnicity, education) and health care payer information was obtained from vital records. Moreover, women whose vital records could not be linked may have terminated the pregnancy or given birth outside of the study area. Of the remaining 1038 eligible participants, 181 women had incomplete responses to the study question concerning number of hours per day of exposure to ETS. An additional 177 women were excluded if they responded affirmatively to actively smoking during the 3 months before pregnancy or while pregnant, quit smoking, or had a serum cotinine measurement above 10 ng/mL. This left a study sample of 680 nonsmoking women.

The 181 women who did not answer the study question were more likely to be born in Mexico, to have less than a high school education, and to receive payment for prenatal care from government programs, compared to the 857 women who answered the study question. Nonresponders were also more likely to complete the questionnaire in Spanish. They had higher mean cotinine levels than the responders. However, after excluding women who reported smoking at any time during pregnancy or the 3 months before pregnancy or who had serum cotinine concentrations greater than 10 ng/mL, there was no difference in mean cotinine concentrations between nonresponders and responders.

### Questionnaire

At the time of birth registration, a one-page supplemental questionnaire was administered in either English or Spanish by the hospital birth recorder. The 22-item questionnaire had two questions on ETS exposure, including the question of interest in the present study: "How many hours per day were you indoors with other people who were smoking, during the fourth and fifth months of your pregnancy: in your home? at work/school? in other places?" An additional ETS question asked about the number of household members who smoke cigarettes; while it is considered in our analyses, it is not the main focus of this intensive investigation and has been evaluated elsewhere (Kaufman *et al.*, 2002).

### Cotinine Measurement

The serum cotinine concentration was measured at CDC using a highly sensitive isotope-dilution high-

performance liquid chromatographic/atmospheric-pressure ionization tandem mass spectrometric (LC/MS/MS) procedure. Samples were preclassified by a screening cotinine immunoassay and subdivided into nominal "high" or "low" cotinine groups. Samples were then analyzed by LC/MS/MS in runs of 50 (either all high or all low), which included two water blanks and two quality control (QC) pool sera. The low QC pool averaged  $1.77 \pm 0.094$  ng/mL ( $cv = 5.3\%$ ), and the high QC pool averaged  $202 \pm 8.5$  ng/mL ( $cv = 4.2\%$ ). Repeat analyses of random samples showed excellent agreement. Samples were analyzed over several months in two different calibration groups. Limits of detection (LOD) were calculated by the limiting standard deviation method (Taylor, 1987) and remained below the nominal method detection limit of 0.050 ng/mL in each case. Serum cotinine was log-normally distributed. One third of the specimens had cotinine values below the LOD. To avoid placing an unnaturally large proportion of the study population at a single value at the lower end of the distribution, actual cotinine values below the LOD were used instead of assigning the LOD or  $0.5 \times \text{LOD}$  to these specimens. Moreover, to retain 29 specimens with cotinine values of zero in the analyses after log transformation, the value of the lowest measured cotinine sample (0.001 ng/mL) was assigned to these specimens. The statistical analyses (described below) were rerun assigning  $0.5 \times \text{LOD}$  to specimens with values below the LOD, assigning the LOD, and excluding such specimens; the results were not meaningfully altered.

### Data Analysis

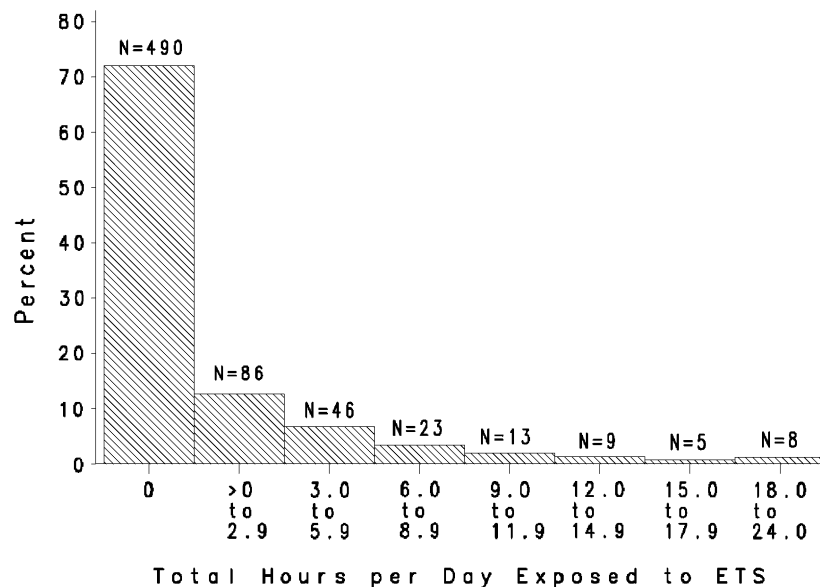
We compared and contrasted the distribution of hours per day of ETS exposure from the questionnaire with that of serum cotinine levels in respondents using histograms, scatter plots, Spearman's rank order correlation, and least squares regression, using Statistical Analysis System (SAS version 6.12). A regression curve was fit to the data in Fig. 2 by using a loess smooth (Cleveland, 1979). Because serum cotinine levels were highly skewed toward smaller values, we present geometric means. Although hours per day of ETS exposure was skewed toward zero, we decided to present arithmetic means; other measures of central tendency (medians, first and third quartiles) would have zero values at most covariate levels. Different modeling approaches were used to maximize the power of the ETS question in predicting cotinine levels. Multivariate regression was used to estimate the mean

change in (log) serum cotinine levels (ng/mL) as a linear function of several covariates including number of hours per day exposed to ETS, at all sites combined and separately at each site. We treated the number of hours per day exposed to ETS at home, at work/school, or in other places in two ways: continuous (0–24 h) and categorized as any versus 0 hours per day. Logistic regression was used to identify characteristics of respondents who reported zero hours of ETS exposure, but who had elevated cotinine levels. The following variables were considered covariates in the data analysis: number of active smokers in household (0, 1, and  $\geq 2$  smokers), maternal age (continuous), maternal education (continuous), race/ethnicity (non-Hispanic White, Black, Mexican-born Mexican-American, US-born Mexican-American, Other, Unknown), marital status (married, unmarried), payment source for prenatal care (government program versus private insurance/self-payment), language spoken at home (Spanish only, Spanish and English, English and other language, English only), tea consumption (cups/week), and total caffeine consumption (continuous). Women of Hispanic origin other than Mexican, such as Puerto Rican or South American, were classified as Other. For some analyses, hours per day of ETS exposure was summed across all three sites to create a total ETS exposure variable. Question responses in which hours per day was unanswered for one or two of the three sites of exposure were coded zero for the unanswered sites before summing to total hours per day.

## RESULTS

### *Distribution of ETS Exposure: Self-Report and Serum Cotinine*

For completion of the study question, 79% of the study sample responded to hours per day of ETS exposure at home, 72% at work/school, and 72% in other places. The distribution of responses to this question is presented in Fig. 1 and by selected demographic characteristics in Table 1. Mean values are shown for all sites combined and for exposure at home, work/school, and other places. For all sites of ETS exposure combined, the mean was 1.41 h per day (SD = 3.69); the first quartile and the median value were both zero; the third quartile value was 0.50 h per day. All demographic strata had large numbers of women who reported no hours of exposure to ETS. Mean hours of exposure increased with increasing numbers of smokers in the household, for total sites (sum of hours per day across all three sites) and individually at home and at other places. Women less than 20 years old reported the highest exposure compared to other age groups for the total of all sites; a similar pattern is observed in the home, but is not evident at work/school or other sites. Women with 9 to 11 years of education reported the highest mean hours of ETS for the total of all sites and at home compared to other education groups; women with 12 years of education reported the highest levels of exposure at work/school or other places. Black women reported the greatest mean hours for the total of all sites and at home; their mean hours of



**FIG. 1.** Distribution of hours per day exposed to ETS at all three sites combined among nonsmoking, pregnant, prenatal screening enrollees in four counties, California, 1992.

**TABLE 1**  
**Distribution of Hours per Day Exposed to ETS at Site of Exposure for Selected Demographic Characteristics of Non-smoking, Pregnant, Prenatal Screening Enrollees in Four Counties, California, 1992**

Variable	Number*		All Sites		Home		Work/school		Other	
	0**	> 0**	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Number of smokers in household										
0	432	85	0.46	1.71	0.09	1.15	0.16	1.04	0.21	0.75
1	44	78	4.18	5.62	3.70	5.50	0.12	0.79	0.41	1.34
2 +	3	24	7.25	7.62	6.52	6.68	0.27	1.35	0.46	1.06
Mother's age (years)										
< 20	69	47	2.98	5.85	2.47	5.47	0.18	1.03	0.36	1.05
20-24	153	49	0.95	3.02	0.68	2.92	0.08	0.65	0.20	0.69
25-29	121	62	1.51	3.13	0.81	2.45	0.33	1.52	0.37	1.18
30-34	119	27	0.71	2.16	0.47	1.89	0.11	0.83	0.14	0.61
35 +	28	5	1.12	4.28	1.09	4.27	0.00	0.00	0.03	0.17
Mother's education (years)										
0-8	70	23	1.46	3.74	1.38	3.70	0.04	0.32	0.04	0.24
9-11	76	44	2.47	5.45	2.01	4.95	0.16	1.26	0.34	1.01
12	161	72	1.29	3.13	0.71	2.77	0.24	1.20	0.35	0.98
> 12	183	51	0.95	2.89	0.60	2.54	0.15	0.92	0.20	0.89
Race/ethnicity										
Black	22	17	3.40	5.98	3.05	5.75	0.00	0.00	0.35	1.03
Hispanic-	113	32	1.01	3.11	0.87	2.93	0.11	1.03	0.03	0.19
Mexican-born										
US-born	111	52	1.38	3.69	0.87	3.48	0.20	1.14	0.33	0.91
White	190	73	1.44	3.74	0.89	3.20	0.24	1.18	0.33	1.08
Other	54	16	1.03	2.44	0.80	2.23	0.03	0.24	0.20	0.79
Marital status										
Married	349	110	0.95	2.55	0.51	2.09	0.20	1.15	0.24	0.89
Unmarried	140	80	2.36	5.21	2.01	4.93	0.10	0.75	0.28	0.89
Source of payment for prenatal care										
Government	253	114	1.91	4.60	1.64	4.36	0.08	0.67	0.21	0.74
Private/self pay	236	76	0.81	2.02	0.24	1.10	0.27	1.34	0.30	1.04
Parity										
1	199	80	1.56	4.04	1.14	3.69	0.24	1.17	0.20	0.71
2	144	66	1.32	3.48	0.86	3.19	0.10	0.83	0.37	1.18
3	84	20	1.34	3.82	0.89	3.34	0.19	1.27	0.26	0.93
4	34	13	1.19	2.79	0.91	2.66	0.13	0.88	0.14	0.45
5 +	29	11	1.18	2.65	1.10	2.64	0.01	0.08	0.06	0.23
Worked during pregnancy										
Not at all	223	89	1.67	4.25	1.37	3.97	0.05	0.53	0.27	0.88
Some of the time	182	75	1.27	3.24	0.79	2.94	0.28	1.34	0.21	0.74
All the time	74	23	0.75	1.91	0.22	1.03	0.22	1.21	0.31	1.22
All women	490	190	1.40	3.69	0.99	3.36	0.17	1.04	0.25	0.89

\*Number may not sum to column total because of missing information.

\*\*Hours per day of exposure to ETS.

TABLE 2

**Distribution of Mean (Geometric) Cotinine Values by Selected Demographic Characteristics of Nonsmoking, Pregnant, Prenatal Screening Enrollees in Four Counties, California, 1992**

Variable	N*	Geometric mean	95% CI
Number of smokers in household			
0	517	0.05	0.05, 0.06
1	122	0.17	0.13, 0.22
2 +	27	0.25	0.15, 0.41
Mother's age (years)			
< 20	116	0.12	0.09, 0.16
20-24	202	0.08	0.06, 0.10
25-29	183	0.07	0.06, 0.09
30-34	146	0.04	0.03, 0.05
35 +	33	0.05	0.03, 0.09
Mother's education (years)			
0-8	93	0.06	0.05, 0.09
9-11	120	0.13	0.10, 0.17
12	233	0.08	0.06, 0.09
> 12	234	0.05	0.04, 0.06
Race/ethnicity			
Black	39	0.16	0.09, 0.26
Hispanic-Mexican Born	145	0.05	0.04, 0.07
Hispanic-US Born	163	0.07	0.06, 0.09
White	263	0.07	0.05, 0.08
Other	70	0.08	0.06, 0.13
Marital status			
Married	459	0.05	0.05, 0.06
Unmarried	220	0.13	0.10, 0.15
Source of payment for prenatal care			
Government	367	0.10	0.08, 0.11
Private/self pay	312	0.05	0.04, 0.06
Parity			
1	279	0.07	0.06, 0.09
2	210	0.07	0.06, 0.09
3	104	0.05	0.04, 0.07
4	47	0.11	0.07, 0.16
5 +	40	0.06	0.03, 0.11
Worked during pregnancy			
Not at all	312	0.08	0.07, 0.10
Some of the time	257	0.06	0.05, 0.08
All the time	97	0.05	0.04, 0.08
All women	680	0.08	0.07, 0.09

\*Number may not sum to column total because of missing information.

exposure were less at work and only slightly greater than Whites or U.S-born Mexican-Americans at other places. Those women unmarried at time of delivery reported greater exposure to ETS at all sites except work/school than married women. Women who received payment for prenatal care services through government programs (Medi-Cal) had greater mean hours of exposure for the total of all sites and at home but not at work or other places.

The distribution of mean (geometric) serum cotinine level for the same demographic characteristics was generally similar to the distribution of self-reported hours per day, although the magnitude of differences often varied (see Table 2). The mean (geometric) for all women was 0.08 ng/mL of serum; the median was 0.08 ng/mL; the first and third quartiles were 0.03 and 0.20 ng/mL, respectively. Serum cotinine levels were elevated among women < 20 years old, women with 9 to 11 years of education, unmarried women, Black women, women who received payment for prenatal care services through government programs, and women who lived with one or more smokers.

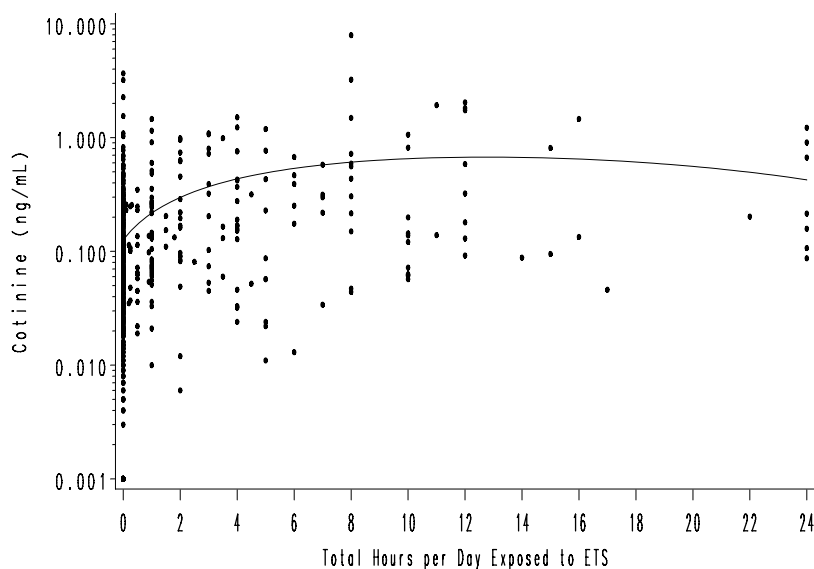
In Table 3 we observed a dose-dependent Association between hours per day of ETS exposure and mean cotinine levels.

#### *Correlation between Serum Cotinine and Self-Reported Hours per Day of ETS Exposure*

We explored the relation between cotinine levels and self-reported total hours of exposure to ETS at all sites and separately by site of exposure. Initial analysis showed a Spearman  $r = 0.38$  ( $P < 0.001$ ) between cotinine and total hours per day ETS exposure. The association between serum cotinine and hours per day ETS exposure at home (site not exclusive: women may also have been exposed elsewhere) was similar ( $r = 0.36$ ,  $P < 0.001$ ). For exposure at work/school and in other places:  $r = 0.02$  ( $P = 0.600$ ) and  $r = 0.20$  ( $P < 0.001$ ), respectively.

Visual examination of the data suggested a possible quadratic or cubic function of total hours per day ETS exposure, based upon a loess smooth fit to the data in Fig. 2. A cubic polynomial was then fit, which had an adjusted  $R^2$  of 0.14 (not shown). Partial correlations generated by the regression modeling indicated that the first order covariable for ETS hours had the strongest association with (log) cotinine (partial corr = 0.10). Number of smokers in the household was added to that model, elevating the adjusted  $R^2$  to 0.18 (not shown); the regression coefficients for total hours per day ETS exposure variables were not significantly diminished. Controlling for marital status, payment source for prenatal care, language spoken at home, and tea consumption in the cubic model increased the adjusted  $R^2$  to 0.27, but did not significantly change the parameter estimates for the ETS exposure variables (not shown). Additional analyses did not reveal any interaction or effect modification between the demographic and the ETS exposure variables.

In Model 1 (Table 4) we observed that any hours per day (versus 0 h) of exposure to ETS (total for all



**FIG. 2.** Distribution of serum (log) cotinine by hours per day exposed to ETS at all three sites combined among nonsmoking, pregnant, prenatal screening enrollees in four counties, California, 1992.  $N = 680$ .

sites) was significantly related to increased levels of serum cotinine. The adjusted  $R^2$  for Model 1 is 0.14, which was similar to that of the cubic polynomial model. Since this was a single covariate model, the partial correlation was the same as the adjusted  $R^2$ . Adding number of smokers in the household to Model 1 increased the adjusted  $R^2$  to 0.18 (see Model 2), while reducing the regression coefficient for ETS exposure. In Model 3 we examined categorizing ETS exposure as any hours per day at each site, exclusively, and at two or more sites. Exposure at home and at two or more sites showed the steepest slopes. The adjusted  $R^2$  for Model 3 is 0.17; the partial correlation was highest for exposure at home. The adjusted  $R^2$  and partial correlation did not increase substantially with additional adjustment for number of smokers in the household (see Model 4); however, the regression coefficients are reduced for ETS exposure at home and at two or more sites.

Model 5 categorizes ETS hours of exposure into the four levels presented in Table 3. Change in (log) mean cotinine increases at higher intervals of ETS hours; the highest category shows the highest partial correlation. Similar to the cubic polynomial model, controlling for marital status, payment source for prenatal care, language spoken at home, and tea consumption in Models 1–6 did not significantly change the parameter estimates for the ETS exposure variables (not shown). No interaction or effect modification between the demographic and the ETS exposure variables was observed.

A reanalysis of Models 3 and 4 where site of exposure was not exclusive yielded similar results (not shown). We also fit a model where number of sites exposed to ETS (0, 1, 2, or 3 sites) was the independent variable. This model provided an adjusted  $R^2$  similar to that of Model 1 (not shown).

**TABLE 3**

**Geometric Mean of Serum Cotinine Concentrations for Total Number of Hours per Day Exposed to ETS among Nonsmoking Pregnant, Prenatal Screening Enrollees in Four Counties, California, 1992**

Number of hours exposed to ETS	$N$	Range (ng/ml)	Geometric mean cotinine (ng/ml)	95% Confidence interval
0	490	0.001–3.67	0.05	0.04, 0.06
> 0–1.9	65	0.01–1.46	0.12	0.09, 0.15
2.0–5.9	67	0.006–1.51	0.17	0.13, 0.24
6.0–24.0	58	0.013–7.96	0.27	0.19, 0.38
Total	680	0.001–7.96	0.08	0.06, 0.08

TABLE 4

**Multivariate Regression: Estimate of Mean (log) Cotinine as a Function of Number of Hours per Day Exposure to ETS, Site of Exposure, and Number of Smokers in Household, in Non-smoking, Pregnant, Prenatal Screening Enrollees in Four Counties, California, 1992**

Variable	Model 1				Model 2			
	$\beta$	SE	P value	Partial Corr*	$\beta$	SE	P value	Partial Corr*
Constant	-2.83	0.6	0.00		-2.91	0.05	0.00	
ETS: 0 vs any hours per day	1.09	0.11	0.00	0.14	0.75	0.12	0.00	0.14
No. of smokers in household:								
1 vs 0					0.72	0.13	0.00	0.03
2 + vs 0					0.85	0.24	0.00	0.02
Adj. $R^2$			0.14				0.18	
Variable	Model 3				Model 4			
	$\beta$	SE	P value	Partial Corr*	$\beta$	SE	P value	Partial Corr*
Constant	-2.83	0.05	0.00		-2.90	0.06	0.00	
ETS: 0 vs any hours per day								
Home**	1.44	0.14	0.00	0.10	0.99	0.19	0.00	0.10
Work/school**	0.33	0.26	0.19	0.00	0.24	0.26	0.35	0.00
Other Places**	0.79	0.16	0.00	0.03	0.77	0.16	0.00	0.03
$\geq 2$ Sites	1.74	0.29	0.00	0.05	1.30	0.31	0.00	0.05
No. of smokers in household:								
1 vs 0					0.56	0.15	0.00	0.02
2 + vs 0					0.59	0.28	0.03	0.01
Adj. $R^2$		0.17					0.19	
Variable	Model 5				Model 6			
	$\beta$	SE	P value	Partial Corr*	$\beta$	SE	P value	Partial Corr*
Constant	-2.83	0.06	0.00		-2.91	0.06	0.00	
ETS:								
> 0-1.9 vs 0	0.71	0.16	0.00	0.01	0.56	0.16	0.00	0.01
2.0-5.9 vs 0	1.08	0.16	0.00	0.04	0.77	0.17	0.00	0.04
6.0+ vs 0	1.53	0.17	0.00	0.11	1.07	0.19	0.00	0.11
No. of smokers in household:								
1 vs 0					0.64	0.14	0.00	0.02
2 + vs 0					0.75	0.25	0.00	0.01
Adj. $R^2$		0.15				0.18		

\*Partial correlation.

\*\*Exposure only at this site.

#### *Characteristics of Women Reporting No Hours per Day of Exposure to ETS*

Response to the ETS question showed that 72% ( $n = 490$ ) of the study participants reported no expo-

sure to ETS. Corresponding cotinine values demonstrated that these women had a wide range of exposure to ETS (Fig. 2). If responses to the ETS question matched serum cotinine levels exactly, then it could be hypothesized that these 490 women would have

TABLE 5

**Binary Logit Model of Dichotomized Cotinine (High Zero/Low Zero) in Relation to Payment Source for Prenatal Care, Marital Status, Race/Ethnicity, Language Spoken at Home in Nonmoking, Pregnant, Prenatal Screening Enrollees in Four Counties, California, 1992**

Variable	Odds Ratio	95% CI
Payment source of prenatal care:		
Government vs nongovernment	2.70	(1.49, 4.89)
Marital status:		
Unmarried vs married	1.84	(1.05, 3.23)
Race/ethnicity:		
Non-White, non-Black and non-Hispanic vs all Others	2.51	(1.02, 6.28)
Language spoken at home:		
English only vs all others	1.99	(1.01, 3.92)

cotinine values in the lower 72% of the entire study sample ( $n = 680$ ). That range of cotinine values is between 0.001 and 0.168 ng/mL of serum. However, approximately 20% ( $n = 98$ ) of the women reporting zero hours had cotinine values above this range (maximum = 3.67 ng/mL). To better define the characteristics of this group, we dichotomized these 490 women into those who had cotinine values greater than or equal to 0.168 ng/mL, the "high zero" group, and those who had cotinine values less than 0.168 ng/mL, the "low zero" group. We explored the relation between dichotomized cotinine values ("high zero" versus "low zero") and several demographic characteristics in a logistic regression model (see Table 5). This model estimates the relative odds of being in the "high zero" group as a function of source of payment for prenatal care, marital status, race, and language spoken at home. It revealed that women in the "high zero" group were more likely to have prenatal care paid by government programs, to be unmarried, to belong to Other (non-White, non-Mexican-American, and non-Black) ethnic groups, and to speak only English at home.

## DISCUSSION

We have compared responses to an ETS exposure question (hours per day), asked around the time of delivery in a population-based sample of pregnant women, with level of serum cotinine (ng/mL) from blood specimens collected in the mid-second trimester of pregnancy. The two distributions were positively correlated with the highest occurring at home. Results from least squares regression indicated that self-reported total hours of ETS exposure per day was a significant predictor of change in (log) serum

cotinine when expressed as a function of a cubic polynomial or coded categorically as any hours per day of ETS exposure at any site. Both models explained the same amount of variation in the data. Additional improvement was gained after adjustment for number of active smokers in the household. Stratifying by site provided still further explanation of the total variation. Additional adjustment for number of active smokers in the household did not substantially increase the adjusted  $R^2$ . Since most ETS exposure occurred in the home for this population, asking about site of exposure may have explained a large proportion of the same variability obtained by asking about number of smokers in household. A third categorization of total ETS hours of exposure into four intervals revealed a dose response for (log) cotinine with increasing hours of exposure.

The mean (arithmetic) cotinine level for our study sample was 0.20 ng/mL of serum. This is lower than that observed in two cotinine-based studies involving pregnant women: 0.71 ng/mL in the Haddow *et al.* (1988) study, and 1.26 ng/mL in the Rebagliato *et al.* (1995) study; these studies did not present geometric mean cotinine levels. O'Connor *et al.* (1995) measured cotinine in urine, which cannot be compared directly to cotinine measured in serum.

Rebagliato *et al.* (1995) observed a stronger association between saliva cotinine level and hours of exposure to ETS in four different locations (for exposure at all locations combined:  $r = 0.52$ ; least squares regression model,  $R^2 = 0.38$ ). Women, at 24 and 39 weeks gestation, were asked to recall the number of hours exposed to ETS during the 2 days preceding the interview, when saliva samples were obtained. Our study interviewed women at the time of birth registration, asking about ETS exposure that occurred in the fourth and fifth months of pregnancy and compared responses to serum collected at 15 to 19 weeks gestation. The weaker association observed in our study might be attributed to asking women to recall hours per day of ETS exposure more than 3 months prior to the birth interview. Additionally, ETS exposure may not have been constant over the 2-month time period, and cotinine has a short half-life of approximately 20 h.

In the Yale Passive Smoking in Pregnancy Study, investigators observed that urine cotinine concentrations and self-reported minutes of exposure to ETS were poorly correlated ( $r = 0.03$  for total duration,  $-0.03$  for home exposure,  $-0.01$  at social occasions, and 0.14 at work) (O'Connor *et al.*, 1995). These women, interviewed at 20, 28, or 36 weeks gestation, were asked to recall any exposure to ETS

during the week preceding the study interviews. The burden of recall was similar to the Rebagliato *et al.* (1995) study (2 days preceding the interview), but the magnitude of association differs greatly. One explanation may be attributed to the different LOD calculated for each study's cotinine assay and the distribution of participants who fell below that LOD. In the Yale Study cotinine analyses had a high LOD, 2.0 ng/mL, and approximately 50% of the women had cotinine values below the LOD. The Rebagliato *et al.* study cotinine analyses had a LOD of 0.10 ng/mL, and less than 2% of their study sample fell below that level. Although our study had the lowest LOD at 0.05 ng/mL, we were also able to use actual cotinine values below the LOD (see Materials and Methods). Alternatively, it has been observed that urine cotinine levels are not as well controlled as serum or saliva and are subject to variability from differences in degree of hydration and possibly other factors (Benowitz, 1996; Watts *et al.*, 1990). Some researchers have attempted to control for urine flow by comparing cotinine levels to creatinine content (Haddow *et al.*, 1994), but that was not attempted by O'Connor *et al.* (1995).

In our study less than 30% of the respondents reported some exposure to ETS during pregnancy. In other studies a much greater proportion of women have reported exposure to ETS, from 51 to 80% (Becher *et al.*, 1992; O'Connor *et al.*, 1995; Rebagliato *et al.*, 1995). The mean duration of exposure from all sources in the Rebagliato *et al.* (1995) study was 6.75 h. Our study observed that women who reported exposure to ETS had a mean duration of 5.02 h (median = 3.0 h); mean duration was not given for the Yale Study (O'Connor *et al.*, 1995). The consequence of fewer women exposed to ETS would result in a lower correlation between ETS exposure measures.

Tobacco smoke is not the only source of nicotine exposure. It has been reported that nicotine is found in food and other nontobacco sources (Davis *et al.*, 1991; Domino *et al.*, 1993). Therefore, among the women reporting no hours of ETS exposure, most of them would be expected to have at least some minimal evidence of nicotine (and/or its metabolites) present in bodily fluids, although this amount would be trivial compared with moderate exposure to ETS (Benowitz, 1996, 1999). We did not expect to find that women who reported no ETS exposure would have cotinine values as high as 3.67 ng/mL. The results of the logistic regression analysis suggested that the reportedly "unexposed" women with higher levels of cotinine were more likely to be of lower socioeconomic status (SES) (i.e., government as

payer for prenatal care) and unmarried among other characteristics.

Our study may have been limited by asking respondents to recall information about ETS exposure several months prior to the birth interview. It may have been very challenging for some women to remember the location of ETS exposure (home, work/school, other places) and the number of hours per day exposed at each of these locations. We also did not ask about living arrangements (i.e., number of rooms and number of occupants in the household) or ventilation (e.g., "are the windows open, when someone is smoking at your home?"). In addition, cotinine as a valid biomarker for ETS exposure has been criticized (Idle, 1990). Cotinine has a short half-life and may not reflect ETS exposure more than 3 to 4 days prior to specimen collection. There may also be interindividual differences in the rates of cotinine metabolism (Benowitz, 1999).

Our investigation had several strengths compared to similar validation studies. We have provided a population-based estimate of ETS exposure prevalence during pregnancy. Our cotinine assay was very sensitive, allowing us to detect evidence of exposure at levels much lower than those of other studies. Because of this low limit of detection, the number of women who had cotinine values below that level was very small. Our study was population-based and yielded results which were more generalizable than clinic-based studies. Our study sample was ethnically diverse and included women from differing educational backgrounds, including those with little or no formal education.

## CONCLUSIONS

While duration of exposure is useful information, we observed that both dichotomous and polychotomous categorization of the ETS hours response can yield explanatory results similar to a continuous response when a large portion of the population reports no exposure. Self-reports of ETS exposure during pregnancy may be improved by limiting the time period of exposure to a few days prior to day of interview. For women who reported no exposure, the wide range of cotinine values suggests including questions that ask about additional smoking environments to prompt a more accurate response. A pregnant woman could be asked how much time she spends inside rooms or vehicles where others would smoke when she was not present (e.g., "do you smell the odor of tobacco smoke in such places?"). Another possible question could ask: "how many of your friends or relatives are smokers?" This

may help a woman to recall potential ETS exposure situations that a single question on duration/location of exposure may not provide and might be easier for women of low SES to answer accurately.

Finally, our findings suggest that studies of pregnant women which are based upon an hour per day ETS exposure question have in all likelihood misclassified a sizable portion of ETS-exposed women into the "unexposed" group, as evidenced by the detectable levels of cotinine for a high percentage of women who believed that they were not exposed to ETS during pregnancy. As we continue to accumulate evidence that ETS exposure during pregnancy, even at low levels, can result in low-birth-weight babies, we conclude that previous studies have underestimated the extent of this effect. Future studies must endeavor to remove women with lower levels of ETS exposure from the referent (no exposure) group. Otherwise, associations between this exposure and the birthweight will not be meaningful.

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