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Biomarkers of Environmental Tobacco Smoke Exposure

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Biomarkers are desirable for quantitating human exposure to environmental tobacco smoke (ETS) and for predicting potential health risks for exposed individuals. A number of biomarkers of ETS have been proposed. At present cotinine, measured in blood, saliva, or urine, appears to be the most specific and the most sensitive biomarker. In nonsmokers with significant exposure to ETS, cotinine levels in the body are derived primarily from tobacco smoke, can be measured with extremely high sensitivity, and reflect exposure to a variety of types of cigarettes independent of machine-determined yield. Under conditions of sustained exposure to ETS (i.e., over hours or days), cotinine levels reflect exposure to other components of ETS. Supporting the validity of cotinine as a biomarker, cotinine levels have been positively correlated to the risks of some ETS-related health complications in children who are not cigarette smokers. — *Environ Health Perspect* 107(Suppl 2):349–355 (1999). *http://ehpnet1.niehs.nih.gov/docs/1999/Suppl-2/349-355benowitz/abstract.html*

Key words: environmental tobacco smoke, passive smoking biomarkers, cotinine, cigarettes, tobacco

A biomarker is desirable in quantitating systemic exposure of nonsmokers to constituents of environmental tobacco smoke (ETS). Self-report measures such as hours per day exposed to ETS by nonsmokers are likely to be imprecise indicators of intake of tobacco smoke because of variations in the number of cigarettes smoked, proximity of nonsmokers to smokers, room ventilation, and other environmental characteristics, as well as individual differences in sensitivity to and concern about adverse effects of ETS. The optimal assessment of exposure to tobacco smoke would be by analysis of the concentrations of a component of smoke, a biologic marker or biomarker, in body fluids of an exposed individual.

Two broad questions must be considered in assessing the validity of a biomarker of tobacco smoke exposure. The first is, How well does the concentration of a marker chemical in the air reflect exposure to toxic constituents of smoke that are of concern with respect to health? The second is, How well does a concentration of a particular chemical in a biologic fluid reflect an individual's intake of that chemical (or a related chemical) from tobacco smoke?

The National Research Council (*1*) has proposed criteria for a valid marker of ETS in the air as follows. The markers *a*) should be unique or nearly unique for ETS so that other sources are minor in comparison, *b*) should be easily detectable, *c*) should be emitted at similar rates for a variety of tobacco products, and *d*) should have a fairly constant ratio to other ETS components of interest under a range of environmental conditions encountered. Furthermore, the validity of a biomarker depends on the accuracy of the biologic fluid measurement in quantitating the intake of the marker chemical, which in turn may be influenced by individual differences in rates or patterns of metabolism or excretion, the presence of other sources (such as diet) of the chemical, and sensitivity and specificity of the analytic methods used to measure the chemical.

Other issues of interest in assessing the risks of exposure to ETS are how well the biomarker indicates long-term exposure to ETS as well as whether a biomarker predicts the likelihood of ETS-related disease and if so how well.

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Abbreviations used: 4-ABP–Hb, 4-aminobiphenyl–hemoglobin; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNAL-GLUC, glucurononide of NNAL; NNK, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone; PAH, polycyclic aromatic hydrocarbon; RSP, respirable suspended particles.

Potential Biomarkers of Environmental Tobacco Smoke

A variety of biomarkers of tobacco smoke exposure are proposed (Table 1). The measurement of cotinine concentrations in biologic fluids has been used most widely by scientists to evaluate ETS exposure because cotinine reflects exposure to nicotine, which is almost specific to tobacco. Chemicals in tobacco smoke such as carbon monoxide or cyanide (the latter metabolized in the body to thiocyanate) can be measured in blood. However, the levels of these chemicals are nonspecific, i.e., there are significant sources of carbon monoxide and cyanide, including the body's own metabolism, other than ETS. Thus, these markers are both nonspecific and insensitive markers of ETS exposure. Other markers that have been proposed to quantitate tobacco exposure include adducts of 4-aminobiphenyl to hemoglobin in red blood cells (*2–4*), adducts of benzo[*a*]pyrene and other potential carcinogens to DNA in white blood cells (*5–8*), adducts of polycyclic aromatic hydrocarbons (PAHs) to plasma albumin (*9*), urinary excretion of nicotine-derived nitrosoamines (*10*), urinary hydroxyproline or *n*–nitrosoproline excretion (*11*), and urinary mutagenicity (*2,12*). Also, solanesol has recently been proposed as perhaps the best marker of particle exposure in the air (*13,14*). Unfortunately, solanesol is extensively metabolized in people and levels are quite low, making quantitation difficult. Levels of 4-aminobiphenyl–hemoglobin (4-ABP–Hb) adducts have been shown to be elevated in nonsmoking ETS-exposed adults compared to non-ETS-exposed adults (*2–4*). Adduct levels showed a significant dose response with increasing history of ETS exposure in a study by Hammond et al. (*3*). However, 4-ABP–Hb levels in nonexposed nonsmokers were substantial, and there was considerable overlap between levels for exposed and nonexposed nonsmokers.

Similar findings were reported for PAH adducts with DNA or albumin. A study by Mooney et al. (*15*) in which these biomarkers were followed before and

Table 1. Comparison of possible biomarkers of ETS exposure.

Biomarker	Reference	Specificity	Sensitivity	Duration after exposure reflected	Comments
Cotinine	(*26*)	High	High	3–4 days	Can be measured in urine, plasma, saliva or hair
Nicotine	(*60,61*)	High	High	Hours	Short half-life indicates that results are very dependent on time of sampling; saliva nicotine can be elevated by local deposition of ETS. Plasma levels are very low. Urine levels are highly influenced by urine volume and pH. Hair measurement is promising as a long-term marker of exposure.
Carbon monoxide	(*62*)	Low	Low	Hours	Many environmental sources, CO also produced by endogenous metabolism. Only small changes in CO levels seen after ETS exposure
Thiocyanate	(*63*)	Low	Low	Weeks	Many dietary sources. Most studies show no difference between nonsmokers who are or are not exposed to ETS.
4-Aminobiphenyl-hemoglobin adduct	(*2–4*)	Moderate	Moderate	Months	Levels in nonsmokers may be 10 to 20% those of smokers. Analytical technique technically difficult.
Benzo[*a*]pyrene–DNA adduct	(*5–8*)	Low	Low	Probably months	Analysis is technically difficult. Difference between smokers and nonsmokers not found in all studies.
PAH–albumin adduct	(*9*)	Moderate	Moderate	21 days	Analysis is technically difficult.
Urinary tobacco-specific nitrosoamines	(*10*)	High	Moderate	Probably hours	Analysis is technically difficult.
Urine hydroxyproline	(*11*)	Low	Low	Probably hours	
Urine mutagenicity	(*2,12*)	Low	Low	Hours to 1 day	Influenced by dietary and other factors. Inconsistent results in comparing nonsmokers with and without exposure to ETS.

after subjects stopped smoking for 14 months illustrated the problem of nonspecificity for both 4-ABP and PAH adducts. Levels of 4-ABP–Hb and PAH–DNA adducts declined to an average of only 25 and 48%, respectively, of the baseline smoking value after subjects stopped smoking for 8 months. Thus, residual levels were still substantial, which made detection of ETS exposure difficult (Figure 1). In contrast, blood cotinine levels fell to an average of 2% that observed during baseline smoking, a level low enough to be able to detect nicotine exposure from ETS.

Nicotine-derived nitrosoamines such as 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) are specific for tobacco exposure and are metabolized to a butanol metabolite (4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol (NNAL), and its glucuronide (NNAL-GLUC) (*10*). Urine levels of NNAL + NNAL-GLUC are elevated in nonsmokers exposed to ETS, and in one small study the correlation between urine NNAL plus its glucuronide with urine cotinine concentration was quite strong ($r = 0.89$). The assay for NNAL is technically demanding; as yet very few subjects have been studied using this biomarker, so its general utility in exposure assessment is difficult to assess.

Overall, it appears that although a number of markers may reflect exposure to particular components of tobacco smoke in active smokers, most of the measures are too nonspecific (i.e., high baseline values even in nonexposed nonsmokers or environmental sources other than tobacco smoke) and/or insensitive (i.e., the increment due to ETS exposure is small compared to baseline values) for use in quantitation of levels of smoke exposure to which most nonsmokers are exposed. At present, cotinine appears to be the most specific and most sensitive biomarker for exposure to nicotine from ETS. A limitation of using cotinine, which is discussed in "Cotinine as a Biomarker," is that cotinine indicates ongoing exposure but not long-term exposure to ETS.

Cotinine as a Biomarker

Cotinine, the major proximate metabolite of nicotine, has been widely used as a biomarker of tobacco exposure (*16,17*). Plasma cotinine concentrations correlate better to various measures of biologic effects of cigarette smoking than does self-reported cigarettes per day (*18,19*). Cotinine concentrations in plasma, urine, and saliva of nonsmokers have been used to assess population exposure to ETS to develop risk estimates for lung cancer related to ETS exposure (*20,21*). The prevalence of significant ETS exposure in control (reportedly unexposed) groups has been estimated based on cotinine measurements and used to adjust lung cancer risk estimates upward for comparison with actual unexposed controls (*1,22,23*).

The validity of using cotinine as a biomarker for ETS exposure has been questioned (*24,25*). Concerns include *a*) the concentration of nicotine in the air is not a good marker of other constituents of ETS because the ratio of nicotine to other ETS components is highly variable and depends on such factors within a space as surfaces, ventilation rate, sampling duration, time since smoking, and air distribution patterns; *b*) the ratio of nicotine emission to respirable suspended particles (RSP) emission is not constant across a wide range of cigarettes; *c*) exposure to nicotine vapor in the absence of other ETS components can occur; *d*) there is no standard method for determining nicotine or its metabolites in biologic fluids; *e*) interindividual differences in rates and patterns of nicotine and cotinine metabolism make the use of nicotine or cotinine as biomarkers of limited utility, and *f*) dietary nicotine exposure may confound low-level determinations of nicotine and cotinine in biologic fluids. A detailed discussion of these criticisms and why cotinine is a valid biomarker of ETS

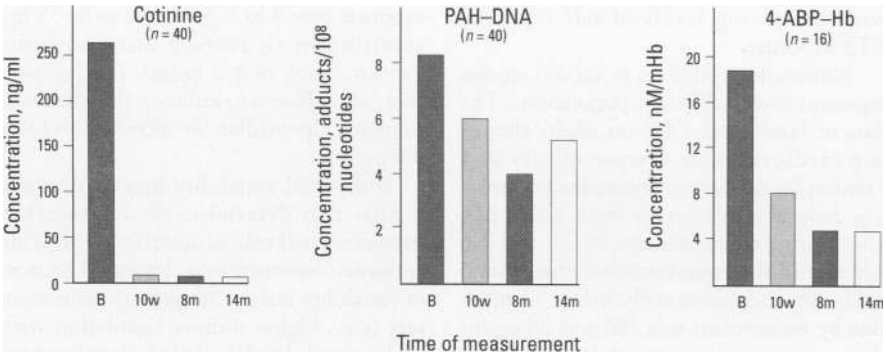

Figure 1. Biomarkers in blood following smoking cessation. Abbreviations: PAH–DNA, polycyclic aromatic hydrocarbon–DNA adducts; 4-ABP–Hb, 4-aminobiphenyl–hemoglobin adducts; B, baseline (precessation smoking condition); SD, standard deviation; 10w, 8m and 14m = 10 weeks, 8 and 14 months after smoking cessation. The number of subjects with data were cotinine, $n=40$; PAH–DNA, $n=40$; 4-ABP–Hb, $n=16$. The SD for cotinine was 114 ng/ml at baseline, and averaged 0.7 ng/ml after cessation; the SD for PAH–DNA was 8.8 adducts/10^8 nucleotides at baseline, and averaged 5.2 after cessation; the SD for 4-ABP–Hb was 8.7 nM/mHb at baseline and averaged 3.6 after cessation. Adapted from Mooney et al. (*15*).

exposure has been published recently (*26*). The following section is a summary of that discussion.

Exposure to Nicotine from Environmental Tobacco Smoke

Nicotine is a chemical found in all tobacco products. The tobacco in manufactured cigarettes contains between 6 and 12 mg of nicotine (*27*). Seventy-five percent or more of nicotine emitted from a cigarette is emitted into the air as sidestream smoke, which contributes substantially to ETS (*1,22,23*). The amount of nicotine in sidestream smoke, when normalized for the generation of tar, is similar for different brands of cigarette, independent of nominal nicotine yield (*22,23,28*). For example, Rickert et al. (*28*) found an average tar-to-nicotine ratio of 5.9 in the sidestream smoke of 15 different brands of cigarettes, with a relatively low degree of variability (coefficient of variation 12.2%). They found no difference in the sidestream smoke tar-to-nicotine ratio when comparing ventilated and nonventilated cigarettes.

Because sidestream smoke is not identical to ETS, it is desirable to examine the composition of ETS generated by different brands of cigarettes. Leaderer and Hammond (*29*) confirmed in chamber studies that the air RSP-to-nicotine ratio generated by smokers smoking 10 different brands of U.S. cigarettes with widely differing machine-determined yields is similar. The average RSP-to-nicotine ratio was 14.1 (coefficient of variation 13.4%). The ratio was similar for filtered and nonfiltered cigarettes. The results of the Rickert et al. (*28*) and Leaderer and Hammond (*29*) studies are consistent, and there is little reason to believe that the composition of sidestream smoke generated by other currently marketed cigarettes, which are of similar nicotine content, should be different. However, if future cigarettes contain less nicotine but generate similar amounts of RSP and other combustion products as produced by currently marketed cigarettes, as has been proposed to make cigarettes nonaddictive (*30*), ETS nicotine would be expected to underestimate exposure to other tobacco combustion products.

In mainstream smoke—i.e., smoke taken in by the smoker—nicotine is contained in particles composed of tar, water, and other nicotinelike alkaloids. In ETS, most of the nicotine leaves the particulate phase and becomes part of the gaseous or vapor phase (*29,31,32*). Nicotine in ETS is breathed in through the nose and throat and inhaled into the lungs by nonsmokers. Nicotine is extremely soluble in water and is highly extracted from ETS within the respiratory tree (*33*).

Levels of nicotine and other chemicals in ETS decay at different rates over time so that the ratio of nicotine to other constituents of ETS may differ at various points in time after generation of the ETS (*13,34,35*). Another source of variability in the RSP-to-nicotine ratio is due to a background level of particles arising from sources other than ETS. Thus, when nicotine and particle levels for ETS decline to low levels, background particle concentrations substantially influence the ratio of RSP to nicotine. At low concentrations of ETS, this ratio becomes very large. For this reason, measuring the slope of the regression line between air nicotine concentration and respirable particle concentration is the best way to assess the degree of correlation between air RSP and nicotine (*29*). In addition, when air samples are collected over the time interval of a typical human exposure, i.e., over hours or days, RSP-to-nicotine ratios are much less variable compared with spot or brief measurements. Leaderer and Hammond (*29*) showed an RSP-to-nicotine slope of 9.8 for 47 home air samples sampled over several days. The correlation coefficient between RSP and nicotine was 0.8. Similar ratios for RSP to nicotine were reported by Miesner et al. (*36*) in workplace samples. Thus, time-averaged ratios of nicotine to RSP and presumably other ETS constituents are relatively consistent (*21*). When a person is exposed to ETS over time, the intake of nicotine reflects exposure to other constituents of ETS.

Cotinine as a Biomarker for Intake of Nicotine

The presence of cotinine in a biologic fluid indicates exposure to nicotine. There is some individual variation in the quantitative relationship between cotinine levels in the blood, saliva, or urine and the intake of nicotine. This is because different people convert different percentages of nicotine to cotinine (usual range 55–92%) and because different people metabolize cotinine at different rates (usual range of cotinine clearance, 19–75 ml/min) (*37*). The relationship between nicotine and cotinine can be expressed mathematically as follows based on steady-state exposure:

$$\text{Generation rate of } COT = \text{intake rate of } NIC \times \text{percent conversion } NIC \text{ to } COT \quad [1]$$

where COT = cotinine and NIC = nicotine.

At steady state,

$$\text{Generation rate of } COT = \text{elimination rate of } COT = CL_{COT} \times C_{B_{SS}} \quad [2]$$

where CL_{COT} is the clearance of cotinine and $C_{B_{SS}}$ is the steady-state blood cotinine concentration.

Therefore, combining Equations 1 and 2:

$$\text{Intake rate of } NIC = \frac{CL_{COT} \times C_{B_{SS}}}{\%\ \text{Conv } NIC \text{ to } COT} \quad [3]$$

Or rearranging:

Intake rate of *NIC*

$$= \left(\frac{CL_{COT}}{\%\ \text{Conv}\ NIC\ \text{to}\ COT} \right) \times C_{B_{ss}} \qquad [4]$$

$$= K \times C_{B_{ss}}$$

In adult smokers, the conversion factor (K) that on average converts a blood level of cotinine to a daily intake of nicotine has been estimated to be 0.08 $\frac{mg/24hr}{ng/ml}$ (range 0.05–0.10, coefficient of variation 21.9%) (*37*). Thus, a cotinine level of 300 ng/ml, a typical value for a smoker, corresponds to a daily intake of 24 mg nicotine. Clearance data are similar for smokers and nonsmokers (*38*), so the K factor is expected to be similar in nonsmokers.

Cotinine levels only approximate nicotine intake because of variability in the conversion factor. However, the degree of variability in the conversion factor (coefficient of variation = 21.9%) is not particularly great compared with variability in the clearance of most other drugs and is much less than the degree of variability typically observed for pharmacodynamic parameters (*39*). Even with this inevitable degree of imprecision, cotinine levels in large groups of subjects would be expected to accurately reflect average group exposure to nicotine from ETS.

Because nicotine from ETS is absorbed into the bloodstream and cotinine is generated from nicotine in the liver and released into the bloodstream, blood levels of cotinine should most closely reflect the dose of nicotine absorbed from ETS. However, most studies of ETS exposure have used saliva or urine concentrations of cotinine because these samples are easier to obtain. Saliva and blood cotinine levels are highly correlated, with a saliva-to-blood ratio of 1.1 to 1.4 (*40,41*). Therefore, saliva and blood cotinine levels can be used interchangeably. Urine concentrations are also highly correlated with blood concentrations, with urine levels about 6 times higher than those for blood.

Typical Levels of Cotinine from ETS Exposure and Estimation of Corresponding Dose of Nicotine

Cotinine levels in people exposed to ETS have been studied by many research groups (for reviews, see *1,22,23*). Most studies found increasing levels of cotinine with increasing levels of self-reported ETS exposure.

Nonsmoking subjects in various studies represent several different populations. The data of Jarvis et al. (*42*) on adults attending cardiovascular disease clinics at a London hospital are an example of estimating daily nicotine intake from ETS (*42*). Using urine concentrations of 7.7 and 1.6 ng/ml and the equations described previously, the estimated daily intake of nicotine by nonsmokers was 100 and 20 µg for those reporting exposure and no exposure to ETS, respectively. Extreme ETS exposure is likely to occur in pubs and bars where smoking is common and ventilation is often poor. Among 42 nonsmoking bar staff in London and Birmingham, England, the median saliva cotinine concentration was 7.95 ng/ml (SD 6.1) and ranged from 2.2 to 31.3 ng/ml (*43*). Using Equation 4, the median nicotine intake is estimated to be 630 µg/day. The maximal nicotine intake, corresponding to a saliva cotinine concentration of 31.3 ng/ml, is estimated to be 2.5 mg/day (nicotine intake equivalent to actively smoking 2.5 cigarettes per day).

Air Levels of Nicotine from Environmental Tobacco Smoke and Predicted Cotinine Levels in Biologic Fluids

The theoretical relationship between air levels of nicotine and cotinine levels in the urine of nonsmokers can be described and is useful in understanding potential sources of variability in that relationship. Assume a workplace level of nicotine in the air due to ETS of 20 µg/m^3 (*44*). The dose of nicotine inhaled is equal to the product of air concentration and ventilation rate. A typical ventilation rate for an adult during light activity is 1 m^3/hr. Thus, the intake of nicotine would be about 20 µg/hr. About 71% of nicotine that is inhaled is absorbed (*33*), so the systemic dose of nicotine is estimated to be about 14 µg/hr. Assuming an 8-hr workplace exposure, this would be equivalent to 112 µg/day. Using the equations described previously results in this level of intake producing an average urine cotinine concentration of 8.6 ng/ml, a value consistent with that measured in nonsmokers exposed to ETS. Air nicotine levels measured by Hammond et al. (*45*) over 9 hr at 11 Massachusetts office worksites that allowed smoking indicated a median level of 8.6 µg/m^3 (*45*). The absorption of nicotine from this level of exposure over 9 hr is predicted to be 55 µg, resulting in an average urine cotinine concentration of 4.0 ng/ml. For perspective, in office workplaces that banned smoking, the median air nicotine level was 0.3 µg/m^3.

Individual variability may exist in the factors that determine the relationship between air levels of nicotine and urine cotinine concentrations. Potential sources of variability include respiratory ventilation rate (e.g., higher minute ventilation with higher work levels), extent of pulmonary retention of nicotine by the lung, timing of the sample collection versus time of exposure, sources of exposure other than that under study, percent metabolic conversion of nicotine to cotinine, total and renal clearance of cotinine, and urine flow rate. It is expected, therefore, that urine cotinine would yield only an approximate estimation of air nicotine levels.

The relationship between ambient air nicotine levels and cotinine levels in the urine or saliva of nonsmokers has been reported in three studies. Studies by Marbury (*46*) and Henderson (*47*) involving children in the home and one study by Coultas (*44*) of adults in the workplace found a reasonably strong correlation between ambient air nicotine and urine cotinine concentrations (correlation coefficients, $r = 0.81$, Marbury; $r = 0.68$, Henderson; $r = 0.60$, Coultas). These correlations are probably as high as can be expected, given the sources of variability in nicotine uptake and metabolism. In view of the multiple potential sources of individual variability, the Marbury, Henderson, and Coultas studies support the predictive value of urine cotinine concentration as a biomarker of ETS-derived nicotine exposure.

Nicotine in Food as a Source of Cotinine

Several foods contain small amounts of nicotine (*48–50*). It has been suggested that nicotine from food might falsely indicate exposure to ETS (*24,25*). Davis et al. (*49*) estimated that average daily consumption of tomatoes, potatoes, cauliflower, and black tea together could result in a daily intake of 8.8 µg nicotine. They estimated, based on a maximum consumption of all of these particular foods on the same day, that a person could ingest as much as 99.9 µg of nicotine per day from food. It should be noted that more than 50% of intake in the Davis study was based on drinking black tea; this study and others have shown that some black teas contain no nicotine. It is not known how

much nicotine is contained in the typical tea consumed by most Americans.

Also of note, the nicotine intake from tea reported by Davis et al. (*49*) necessitates drinking about 4 qt (3,840 ml) per day. Consumption of such large volumes of fluid would result in a urine output much greater than the 1000 ml these authors assume in their prediction of urine cotinine concentration. A larger urine volume would substantially reduce concentrations of cotinine in the urine below the Davis et al. estimate of 6.2 ng/ml.

Average nicotine intake (i.e., absorbed dose) from significant ETS plus dietary exposure is about 80 µg. Repace (*51*) used average American vegetable consumption data, which included 27 g of tomatoes and 75 g of potatoes, to estimate daily nicotine intake from the diet of 0.7 µg/day. As noted previously, Davis et al. calculated that daily consumption of tomatoes, potatoes, cauliflower, and black tea might result in a daily intake of 8.8 µg nicotine. Even in the latter case, the expected intake from a diet rich in nicotine-containing food is only 10% of the total nicotine exposure experienced by a person with significant ETS exposure. Conversely, an intake of 8.8 µg nicotine per day from food would be expected to yield a steady-state urine cotinine level of less than 0.7 ng/ml, which is well below the level indicating significant ETS exposure.

The impact of tea drinking on serum cotinine levels of nonsmokers in Scotland has been studied explicitly (*52*). No effect on plasma cotinine levels was observed with consumption levels of up to 10 cups or more of tea per day. In contrast, the same study showed a robust relationship between self-reported ETS exposure and plasma cotinine levels. Thus, nicotine in tea appears to contribute little to cotinine levels in most people and would be insignificant compared with nicotine levels from exposure from ETS.

I conclude that although food is a source of low-level nicotine exposure, for most people it represents an insignificant exposure compared with exposure to ETS and is likely to inflate population estimates of ETS nicotine exposure very little.

Nicotine Emissions from the Environment as a Source of Human Exposure

Nicotine from ETS deposits on room surfaces, such as walls and carpets, and may contaminate house dust. Nicotine emissions from surfaces or dust in the air may result in measurable levels of nicotine in the air that persist after the last cigarette was smoked in a room (*53*). Similarly, nicotine can be emitted from the clothes of smokers even when they are not smoking in the room. Thus, it has been suggested that nonsmokers may be exposed to nicotine not only through direct exposure to ETS but also by exposure to air in a room in which smoking has occurred in the past or to air in a room shared by people whose clothes have been contaminated by tobacco smoke (*53*). Although concentrations of nicotine have been measured in the air under these conditions, levels of nicotine are quite low compared to those from ETS. For example, house dust in a nonsmoker's home in which cigarettes were smoked on one occasion was reported to contribute to nicotine levels in the air of 0.2 to 0.7 µg/m^3 over the next few days (*53*). Using calculations described previously assuming a ventilation rate of 1 m^3/hr, and assuming an 8-hr exposure would produce a daily nicotine intake of 1.1 to 4.0 µg and result in a urine cotinine concentration of 0.1 to 0.3 ng/ml. These values are trivial compared to those derived from ETS exposure.

Health and Other Biologic Effects That Validate Cotinine as a Biomarker of ETS Exposure

A significant relationship between biologic effects of ETS and cotinine levels of biologic fluids would further support cotinine as a quantitative marker of ETS exposure. Several studies support this concept. Matsunga et al. (*54*) studied the effects of ETS exposure on the metabolic clearance of theophylline, a drug whose metabolism is known to be increased in nonsmokers by the presence of cigarette smoke. In 14 nonsmokers, significant correlations were found between plasma cotinine ($r = 0.72$) or urinary cotinine ($r = 0.79$) and the clearance of theophylline. Strachan et al. (*55*) studied 736 seven-year-old school children and found a positive correlation between the quintile of salivary cotinine levels and the risk of middle ear effusion. In another study of a group of 770 children, Strachan et al. (*56*) reported a significant inverse correlation between salivary cotinine and various tests of lung function. Similar results were reported among a group of 2,511 children in a study by Cook et al. (*57*), and Rylander et al. (*58*) found that the risk of wheezing bronchitis in children 18 months of age or younger increased as urinary cotinine excretion increased. Finally, and perhaps most relevant to concerns about occupational exposure in adults, Tunstall-Pedoe et al. (*59*) found a gradient of risk of diagnosed coronary heart disease that increased with increasing serum cotinine in nonsmokers. Thus, several different biologic effects of ETS were shown to be quantitatively related to cotinine levels, supporting the idea that cotinine levels reflect ETS exposure and effects.

Conclusion

To summarize, the National Research Council criteria for a valid marker of ETS exposure include: *a*) should be unique or nearly unique for ETS so that other sources are minor in comparison; *b*) should be easily detectable; *c*) should be emitted at similar rates for a variety of tobacco products; and *d*) should have a fairly constant ratio to other ETS components of interest under a range of environmental conditions encountered (*1*). Nicotine in the air and measurement of its metabolite cotinine in biologic fluids meet these criteria reasonably well. Interindividual variability exists among any set of biologic measurements. Such variability may limit the value of predictions based on measurements in individuals, but this variability is compensated for in studies of large numbers of subjects, as in epidemiologic studies. Supporting this conclusion is the observation that cotinine levels in nonsmokers have been positively correlated with the risks of some ETS-related health complications in children. The evidence presented in this review indicates that cotinine levels provide valid and quantitative measures of average ongoing human ETS exposure over time. The main limitation in using cotinine is that it does not provide a measure of long-term ETS exposure, a measure that would be most useful for epidemiologic studies.

Other biomarkers, particularly 4-ABP–Hb adducts, PAH–DNA or albumin adducts, and urine levels of metabolites of the nicotine-derived nitrosoamine NNK more directly reflect exposure to carcinogens in tobacco smoke than cotinine. Some of these biomarkers are better markers of long-term exposure, but available data indicate that they lack adequate sensitivity or specificity to be useful as quantitative biomarkers of ETS exposure. Whether levels of these biomarkers are useful in predicting disease risk remains to be determined.

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