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by Husgafvel-Pursiainen K

Affiliation: Finnish Institute of Occupational Health, Department of Industrial Hygiene and Toxicology, Topeliuksenkatu 41 aA, FIN-00250 Helsinki, Finland. Kirsti.Husgafvel-Pursiainen@ttl.fi]

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by Kirsti Husgafvel-Pursiainen, PhD [1]

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Environmental tobacco smoke is one of the most widespread carcinogenic exposures. Given the substantial numbers daily exposed to this substance and the great amount of scientific data on its association with chronic diseases, accurate measurements of its exposure, intake, and biological effects are needed. In fact, studies exploiting various kinds of biomarkers are crucial in increasing the understanding of the biological processes and mechanisms of the adverse health effects related to exposure, as well as in adding biological plausibility to the existing epidemiologic evidence. This paper summarizes data on known biomarkers currently in use in human population studies for detecting exposure, the biologically effective dose, the biological effects, or the disease processes related to environmental tobacco smoke. Of the biomarkers discussed, cotinine is currently well suited for assessing the exposure and intake of environmental tobacco smoke, while urinary metabolites of tobacco-specific nitrosamines appear to serve as sensitive markers for the uptake and metabolism of carcinogenic constituents of environmental tobacco smoke.

Key terms 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, cotinine, genotoxicity, involuntary smoking, metabolism, passive smoking, review, second-hand smoke.

The number of people daily exposed to environmental tobacco smoke in various environmental settings, including public places, restaurants and cafés, workplaces, and homes, is very large. In Finland, from a population of about 5 million, it has been calculated that around 600 000 people are currently exposed to environmental tobacco smoke. Of this number, more than 300 000 are exposed at work, either occasionally or regularly (1, 2). In the European Union, the number of people significantly exposed to environmental tobacco smoke at work is estimated to be 7.5 million (3). Given the substantial numbers of exposed persons and the large amount of scientific data demonstrating an association between exposure to environmental tobacco smoke and chronic diseases, such as such as lung cancer (4, 5) and cardiovascular diseases (6, 7), it is clear that accurate measurement of exposure, uptake, and biological effects are needed. Epidemiologic studies traditionally rely on estimates of exposure based on self-reports. Such measures only reflect external exposure and may be prone to bias from, for example, misclassification and recall. Biomarker-based measurements of exposure, including the biologically effective dose and effect, represent a promising approach for future epidemiologic studies. In fact, studies exploiting various kinds of biomarkers have a crucial role in increasing our understanding of the biological processes and mechanisms of the chronic diseases associated with exposure to environmental tobacco smoke, as well as in adding biological plausibility to the existing epidemiologic evidence (8, 9).

Biomarkers of exposure and intake

Many of the various compounds used as biomarkers of smoking, such as carbon monoxide bound to hemoglobin or thiocyanate in plasma or urine samples, have a limited capacity to reflect exposure to environmental tobacco smoke, which is characteristically low-dose exposure. The lack of sensitivity of some of the biomarkers for environmental tobacco smoke is often related to

1 Finnish Institute of Occupational Health, Department of Industrial Hygiene and Toxicology, Helsinki, Finland.

Reprint requests to: Dr Kirsti Husgafvel-Pursiainen, Finnish Institute of Occupational Health, Department of Industrial Hygiene and Toxicology, Topeliuksenkatu 41 aA, FIN-00250 Helsinki, Finland. [E-mail: Kirsti.Husgafvel-Pursiainen@ttl.fi]

exposure to the same or related chemical substances through diet, other life-style factors, or ambient air (10–13). Currently, nicotine and its main biotransformation product, cotinine, are the most widely used intake markers for environmental tobacco smoke (10–12).

Nicotine and other specific markers

The main advantage of nicotine, and, consequently, that of cotinine, is the specificity for tobacco. Nicotine has, however, some limitations as a biomarker for environmental tobacco smoke, mainly due to its short half-time of only a few hours in the body. Alternative to more traditional measures, the analysis of nicotine in hair has been suggested as a noninvasive measure of tobacco smoke exposure; it probably reflects exposure from a longer period of time (14–17). Inhaled nicotine is metabolized in the liver, where cotinine is formed as the major proximate metabolite; this oxidation reaction is mediated by cytochrome P450 enzymes, as discussed later. Apart from exposure originating from the use of tobacco products or from environmental tobacco smoke, minor quantities of nicotine can enter the body via the diet, mainly from the consumption of vegetables, fruits or tea, due to its use as a pesticide. The contribution from this source has been estimated to be negligible. It has been calculated that even very high consumption equals about 10% of the amount of nicotine typically taken up from environmental tobacco smoke (18–21).

A few alternative marker substances of high specificity exist. One is 3-ethenyl pyridine (also called 3-vinyl pyridine), a substance chemically close to nicotine and present almost exclusively in the gas phase of tobacco smoke (22–24). Another compound relatively newly adopted as an exposure marker is solanesol, a compound specific for *Solanaceae* species (including tobacco plant) and present in the particle phase of tobacco smoke (25, 26). Both of these marker compounds are applied for air measurements only (13). In addition, minor nicotine-related tobacco alkaloids, such as anabasine or anatabine, have been used as biomarkers of exposure in, for example, situations in which people undergoing nicotine replacement therapy are monitored for smoking; no data, however, are available on exposure to environmental tobacco smoke (27).

Cotinine

Cotinine concentrations can be measured in blood, urine, or saliva samples. Due to its specificity and the sensitive methods available for measurement, cotinine is currently the best-suited biomarker for tobacco smoke exposure (table 1). In contrast to nicotine, the half-time is longer, approximately 16–22 hours (12, 13, 21, 28). Cotinine concentrations therefore reflect tobacco smoke exposure during the past 2–3 days. It is, nevertheless, evident that, at best, cotinine measurements can only serve as surrogates for long-term exposure.

A multitude of studies has demonstrated that smokers have clearly higher levels of cotinine than nonsmokers. [For reviews see references 10–12.] A dose-response relationship, linear up to 20 cigarettes per day, between the daily consumption of cigarettes and the

Table 1. Examples of biomarkers used in studies assessing exposure to and the biological effects of environmental tobacco smoke.

Biomarker	Subjects	Biological sample	Biological phenomenon measured by the biomarker	Nature of exposure or effect detected	Comments	Reference
Cotinine	Healthy adults and children exposed to environmental tobacco	Serum, saliva, urine	Exposure, uptake, metabolism	Noncarcinogenic	Quantitative or semi-quantitative, short-term exposure	National Research Council, 1986 (10); US Environmental Protection Agency, 1993 (11); Thompson et al, 1990 (31); Riboli et al 1990 (32); Riboli et al, 1995 (33); Oddoze et al, 1999 (40); Hovell et al, 2000 (41)
Metabolites of tobacco-specific nitrosamines	Healthy adults and children exposed to environmental tobacco smoke	Urine	Exposure, uptake, metabolism	Carcinogenic	Quantitative or semi-quantitative, short-term exposure	Hecht et al, 1993 (51); Parsons et al, 1998 (52); Anderson et al, 2001 (53)
Protein adducts	Healthy adults and children exposed to environmental tobacco smoke	Blood	Exposure, uptake, metabolism, biological effect	Carcinogenic	Qualitative, long-term exposure	Maclure et al, 1989 (56); Bartsch et al, 1990 (57); Hammond et al, 1993 (58); Tang et al, 1999 (59); Crawford et al, 1994 (60)
p53 gene mutations	Nonsmoking lung cancer patients exposed to environmental tobacco smoke	Tumor tissue	Cancer-related biological effect	Genotoxic, carcinogenic	Qualitative, long-term exposure	Husgafvel-Pursiainen et al, 2000 (74); Vähäkangas et al, 2001 (78)

cotinine concentration in body fluids is well established for smokers. Nonsmokers exposed to environmental tobacco smoke have, according to most studies, significantly higher levels of cotinine than unexposed nonsmokers. Cotinine levels measured in nonsmokers exposed to environmental tobacco smoke commonly equal a few percent of those found in smokers (10–13, 29, 30).

The quantitative relationship between exposure to environmental tobacco smoke and cotinine concentrations in body fluids has been thoroughly investigated in large studies. A study that investigated close to 200 persons exposed to environmental tobacco smoke at home or at work showed that urinary cotinine concentrations depended on the duration of exposure. The background level of urinary cotinine was 5.6 ng/ml, and increasing exposure resulted in increasing concentrations. The investigators concluded that every additional 10 hours of exposure resulted in a 44% increase in the urinary cotinine concentration (31). A similar finding that mean urinary cotinine concentrations in nonsmoking women increased linearly along with increased exposure to environmental tobacco smoke was obtained in a multicenter study conducted among 1300 nonsmokers (32). In that study, the lowest concentrations were measured among women who had no environmental tobacco smoke exposure either at work or at home (mean 2.7 ng/mg creatinine), and it was highest among those with both kinds of exposure (mean 10.0 ng/mg creatinine) (32). It was demonstrated that cotinine concentrations depended on both the duration of exposure and the number of cigarettes smoked by others. As indicated by measured cotinine concentrations, the number of cigarettes smoked by the spouse is the best estimate of domestic exposure, and the duration of exposure is the best estimate of occupational exposure (32, 33).

Studies conducted among flight attendants and personnel working in restaurants, casinos, and other similar settings have clearly shown elevated levels of cotinine, the measured levels often following the levels of air concentrations of environmental tobacco smoke (1, 34–37). A similar result was found also in a recent study in the United States on waiters, waitresses, and bartenders working in restaurants and taverns, although it was clear from the measurements that living in a home where somebody smoked also contributed to cotinine levels (26). In Finland, urinary cotinine concentrations measured in the late 1990s among nonsmoking restaurant personnel varied between 0.5 and 5.6 ng/ml (0.5–12 ng/mg creatinine) in dining restaurants, 0.5–45 ng/ml (0.5–65 ng/mg creatinine) in pubs and drinking restaurants, and 0.5–24 ng/ml (0.5–26 ng/mg creatinine) in dancing restaurants and night clubs (unpublished data of Johansson et al). Earlier, nonsmoking waitresses and waiters working in pubs or drinking restaurants were measured to have somewhat higher cotinine levels, with mean urinary concentrations of 56 ng/ml (38). The decreased levels agree with other measures indicating diminished levels of environmental tobacco smoke in restaurants, bars, and many other public places (1).

Many studies on children have shown a significant correlation between the children's cotinine concentrations and the amount of parental smoking (39, 40). Interestingly, a cross-sectional survey of secondary schoolchildren conducted in 1998 found that salivary cotinine concentrations correlated with parental smoking but the concentrations had halved since the late 1980s, presumably due to less smoking at home in the presence of children in the 1990s (41).

In line with these observations, a remarkable decrease in cotinine levels was recently observed in the nonsmoking population aged 3 years and older in the United States. Cotinine serum levels among nonsmokers had decreased by more than 75%, from the median level of 0.20 ng/ml documented during the period 1988–1991 to the median level of 0.050 ng/ml measured in 1999 (42). Such a considerable decline in cotinine levels documents a dramatic reduction in exposure to environmental tobacco smoke among the general population in the United States since 1988–1991 (42).

Biomarkers related to genotoxicity and carcinogenicity

Cotinine and its parent compound nicotine have a high specificity and sensitivity for exposure to environmental tobacco smoke, but yet they do not represent carcinogenic constituents of tobacco smoke. Tobacco smoke is known to be highly genotoxic in all its forms (11, 43), and biomarkers of genotoxicity have widely been used to document smokers' exposure to carcinogenic and mutagenic components of tobacco smoke. [For reviews see references 10–12, 43–44.] Even if many of the markers available distinguish smokers from nonsmokers, they have so far had less sensitivity for detecting exposure to environmental tobacco smoke (38, 44–47).

Metabolites of carcinogens

Tobacco-specific *N*-nitrosamines are a group of carcinogens derived from tobacco alkaloids. The most carcinogenic of these tobacco-specific compounds is NNK [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone], a powerful lung carcinogen in rodents that primarily induces adenocarcinoma of the lung (48). NNK, as well as the other tobacco-specific nitrosamines such as NNN (*N*'-nitrosonornicotine), are likely to play a major role in the development of lung cancer in smokers (49).

Urinary metabolites of NNK, namely NNAL [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol], a lung carcinogen in itself, and its glucuronide NNAL-Gluc, provide a biomarker for measuring the uptake and metabolism of carcinogenic compounds in tobacco smoke. Assessments of NNAL and NNAL-Gluc have demonstrated the presence of these tobacco-specific pulmonary carcinogens in urine samples of nonsmokers exposed to environmental tobacco smoke (50, 51). A recent study measured cotinine, nicotine, and NNAL plus NNAL-Gluc in urine from healthy nonsmoking women exposed from smoking by the spouse (52). The study showed that, for all the measured substances, nonsmoking women with smoking partners had statistically significantly higher mean levels than women with nonsmoking partners. The cotinine levels of the nonsmoking women, as well as of the smoking partners, were consistent with the self-reported smoking status (geometric mean for exposed women 0.037 nmol cotinine/mg creatinine and that for unexposed women 0.007 nmol/mg). Levels of cotinine, nicotine, and NNAL plus NNAL-Gluc were five- to six-fold higher in exposed women than in unexposed women. Within the studied couples, the levels of NNAL plus NNAL-Gluc in the women whose partners smoked at home averaged 5.6% of those detected in their smoking partners (52). The study concluded that nonsmoking women exposed to environmental tobacco smoke take up and metabolize the tobacco-specific, powerful lung carcinogen NNK, which could, in turn, increase their risk of lung cancer. When all these data are considered together, the assessment of urinary metabolites of NNK appears as one of the most promising biomarkers for documenting exposure to and the uptake and metabolism of carcinogens from environmental tobacco smoke.

Protein and DNA adducts

Another class of biomarkers capable of indicating exposure to genotoxic compounds constitutes adducts (ie, carcinogen-macromolecule binding products). Both protein adducts and deoxyribonucleic acid (DNA) adducts are considered markers of biologically effective dose. In addition, DNA adducts have the capacity to reflect cumulative unrepaired DNA damage and cancer risk, due to their biological nature and significance (53, 54).

Protein adducts in particular have been used to measure exposure to environmental tobacco smoke. Hemoglobin adducts of 4-aminobiphenyl, one of the human carcinogens of tobacco smoke present in especially high amounts in undiluted sidestream smoke, have been found to be elevated in nonsmokers exposed to environmental tobacco smoke (55–57). In line with this finding, a recent study demonstrated higher levels of 4-aminobiphenyl-hemoglobin adducts in children exposed to environmental tobacco smoke when they were compared with unexposed ones (58). One of the advantages of hemoglobin adducts is that they are relatively long-lived; in the absence of a repair system, the half-time is 3–4 months owing to the turnover of red blood cells. Exposure to polycyclic aromatic hydrocarbons (PAH), an important class of carcinogens in tobacco smoke, can be monitored measuring the formation of adducts to serum albumin. Preschoolchildren exposed to environmental tobacco smoke via mothers' smoking have been detected to have higher levels of PAH-albumin adducts than children of nonsmoking mothers (59).

Carcinogen-DNA adducts, including smoking-related DNA adducts detectable by the ^{32}P-postlabeling technique, are frequently used for biomonitoring DNA damage (46). Elevated levels of PAH adducts in white blood cell DNA from smokers, as compared with that of nonsmokers, have been demonstrated in some, but not all studies (60–62). The inconsistency between these observations is probably due to PAH exposure from other common sources such as diet (46). Consequently, the sensitivity of the ^{32}P-postlabeling method for detecting PAH-DNA adducts in nonsmokers in relation to environmental tobacco smoke appears limited. In addition to confounding PAH exposure, other factors may affect the level of adducts in smokers and nonsmokers, including individual variation in the metabolism of PAH, the presence and variation of DNA repair, and probably the fact that adducts are typically measured in surrogate tissue. Lung cells would be a more appropriate target for adduct analysis (46, 63, 64). Analyses of cells from target tissues would probably be particularly relevant in cases of low-level exposure, such as environmental tobacco smoke. For obvious reasons, however, studies on healthy subjects are typically restricted to the use of surrogate tissues.

Mutations

Of the biomarkers related to genotoxic effects, gene mutations are among the ones closest associated with cancer, the ultimate outcome of interest. Mutation frequency of the *HPRT* (hypoxanthine-guanine phosphoribosyltransferase) gene is perhaps the most widely used reporter gene for detecting mutations in healthy human populations. An elevated frequency of *HPRT* mutant lymphocytes in healthy smokers, as compared with nonsmokers, was demonstrated in a study in which the smoking status of each group was confirmed by cotinine measures (65), while an earlier study failed to show such a difference (66). In addition, a recent study reported an increased frequency of *HPRT* mutations among smoking lung cancer patients as compared with nonsmoking cases. A comparison with healthy subjects showed that the increase did not depend on the disease status (67).

Data on gene mutations in healthy adult subjects exposed to environmental tobacco smoke are largely lacking. Albertini et al (68) reported a significant difference in the distribution of the types of *HPRT* mutation in T lymphocytes of newborns whose mothers were exposed to environmental tobacco smoke, as compared with infants born to nonsmoking mothers. Cotinine measurements confirmed the exposure of the mothers (68).

After the discovery of a central role of *p53* (also called *TP53*) gene mutations in cancer in general and in environmental cancer in particular (69), large series of studies have revealed high prevalences of *p53* mutations in many types of human cancers associated with external exposures (70, 71). Lung cancer is among the malignancies demonstrated to harbor a high *p53* mutation frequency, with about 50% of cases carrying such mutations (71, 72). Mutation frequency is higher in lung cancer patients who are smokers than in nonsmoking patients (71–73). Another feature of *p53* mutations in lung cancer is the predominance of G to T transversion mutations (69, 71, 72). DNA adducts induced in human cells by active metabolites of major tobacco carcinogens [benzo(a)pyrene and some other carcinogenic PAH] predominantly occur at sites of G to T transversion mutations as observed in human lung tumors (74–76). This phenomenon provides evidence for a direct link between carcinogen exposure and mutations in a gene, which has a central role in cancer development.

Lifetime nonsmokers carry *p53* mutations significantly less frequently than smokers or former smokers (73, 77). In a multicenter study conducted on lung cancer among nonsmokers, mutations of the *p53* gene were more common in lung tumors from female never smokers who reported exposure to spousal smoking, as compared with tumors from unexposed never smokers, with an odds ratio of 2.0 (95% confidence interval 0.5–8.7) (73). Taken together, the data from individual studies, the large international database on *p53* gene mutations in human malignancies, and the experimental work on DNA adducts induced by PAH in the *p53* gene sequence strongly suggest *p53* mutations as a biomarker for tobacco-related carcinogenesis. G to T transversion mutations of the *p53* gene may serve as a more specific molecular marker for the role of PAH compounds in the process (76).

Genetic variation in xenobiotic metabolism and environmental tobacco smoke exposure

Genetic variations of xenobiotic metabolism are currently widely studied and largely applied as markers of susceptibility in molecular epidemiology studies on cancer (78, 79). As a reflection of such variation in biotransformation, also levels of other biomarkers may be affected in the exposed persons. For example, it is known that interindividual variation in nicotine metabolism exists in humans; approximately 55–90% of nicotine is converted to cotinine (13). Differences in nicotine metabolism appear to depend to some extent on race or ethnic group (28, 80, 81). Genetically determined poor metabolism of nicotine results in deficient cotinine formation (82).

Many of the cytochrome P-450 (CYP) enzymes involved in nicotine metabolism are encoded by polymorphic genes. One such gene is *CYP2D6,* which was earlier indicated in nicotine biotransformation (83–85); current data from in vitro and population studies show however that *CYP2D6* is not likely to play a major role in oxidation reactions of nicotine (86–88). According to current knowledge, the most important and rate-limiting CYP gene in the oxidation of nicotine to cotinine is *CYP2A6* (89, 90). The *CYP2A6* gene is polymorphic with at least two inactivating variant alleles, plus an allele involving a deletion of the whole gene that results in a total lack of gene product (91–94). Deficient conversion of nicotine to cotinine has been reported in homozygous *CYP2A6* gene mutation carriers (91, 94–97). The significance of this finding is still unclear, since the prevalence of the homozygous gene deletion genotype varies among different populations. The genotype is very rare among Caucasians and Japanese, but has been reported to be more common in the Chinese population (95, 98, 99). In addition, the data published on *CYP2A6* polymorphism and smoking behavior (presumably as a consequence of altered nicotine metabolism) are presently inconsistent (100, 101). No reports have so far been published on *CYP2A6* genotypes and the levels of nicotine or cotinine in nonsmokers with exposure to environmental tobacco smoke, but the rarity of the homozygous poor-metabolizer genotype implies a minor role.

The CYP enzymes involved in the activation of the tobacco-related nitrosamines NNK, NNAL, and NNN to their carcinogenic metabolites include 2A6, 3A4, 1A2, and 2E1 (102), all with genetic polymorphisms (79). It is thus possible that metabolic activation of these compounds, and, consequently, the amount of genotoxic and carcinogenic intermediates generated, may be influenced by the polymorphisms to some extent. No direct data on such influence are, however, available for smokers or nonsmokers. A molecular epidemiology study conducted as an extension of a case-referent study on lung cancer suggested that those who carry inactivating variants of the *CYP2A6* gene may have a decreased risk of lung cancer, presumably due to impaired metabolic activation of tobacco-specific carcinogens (101). However, the molecular method used in the study was shown to give inaccurate results (92), and the possible relationship remains open.

Glutathione S-transferase (GST) genes have perhaps been studied the most in relation to cancer risk from metabolic polymorphisms. One such gene is the *GSTM1* gene encoding one of the GST isoforms that participates in detoxification reactions of carcinogenic PAH metabolites. Recent meta-analyses have indicated that the *GSTM1* null genotype, resulting in a lack of the gene product, is associated with a small excess risk of lung cancer (103, 104). In agreement with this possibility, never-smoking lung cancer patients exposed to environmental tobacco smoke were more likely to have the *GSTM1* null genotype than unexposed never smokers were (105). A European study on never smokers failed, however, to show risk modification by the *GSTM1* null genotype (106). Another recently reported European investigation found that the *GSTM1* null genotype was associated with an increased risk of lung cancer in nonsmokers as a group, but cases exposed to environmental tobacco smoke did not show an excess risk as a subgroup (107).

Concluding remarks

Biomarker studies have consistently demonstrated that nonsmokers regularly exposed to environmental tobacco smoke take up and metabolize noncarcinogenic (eg, nicotine) as well as carcinogenic (eg, NNK) constituents of tobacco smoke (table 1). Like smokers, nonsmokers exposed to environmental tobacco smoke have been found to carry protein adducts indicative of both exposure and intake. From more recent biomarkers, studies analyzing somatic *p53* mutations in lung cancer have suggested that the mutation burden among nonsmoking lung cancer patients with environmental tobacco smoke exposure may be increased in comparison with that of unexposed nonsmokers. Such observations support the view that biological processes of tobacco-related carcinogenesis may be similar in smokers and nonsmokers exposed to environmental tobacco smoke. In addition, there are some, although still inconsistent, data suggesting that nonsmoking lung cancer patients with certain genotypes of xenobiotic metabolism may form an at-risk group, as compared with those with other genotypic constitutions. This finding is also in line with data from smoking lung cancer patients.

In summary, given the overwhelming biomarker data concerning both smokers and nonsmokers, it can be concluded that the published biomarker data is well in line with the epidemiologic data pointing to an increased risk of lung cancer among nonsmokers exposed to environmental tobacco smoke. These data are also compatible with our increasing knowledge about the biological processes involved in the development of environmentally induced cancer, tobacco cancers in particular. Such knowledge will hopefully offer new strategies for secondary cancer prevention and intervention (108).

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