European Network For Smoking Prevention -ENSP

Second-Hand Smoke (SHS) Markers

Review of methods for monitoring exposure levels

WITH THE SUPPORT OF
THE EUROPEAN COMMISSION

CENTRO
PER LO STUDIO
E LA PREVENZIONE
ONCOLOGICA

Istituto Scientifico
della Regione Toscana

Servizio
Sanitario
della
Toscana

SECOND-HAND SMOKE (SHS) MARKERS: REVIEW OF METHODS FOR MONITORING EXPOSURE LEVELS

Giuseppe Gorini *, Antonio Gasparrini *, Maria Cristina Fondelli *, and Giovanni Invernizzi §

* Environmental and Occupational Epidemiology Unit - Centre for Study and Prevention of Cancer (CSPO) - Tuscan Cancer Institute - Florence, Italy

§ Tobacco Control Unit – National Cancer Institute – SIMG Italian College GPs - Milan, Italy

1. Introduction **3**
1.1 Health effects of SHS 3
1.2 SHS exposure 4
1.3 Physical and chemical properties 5
1.4 Measuring exposure 6
1.5 Aim of the study 8

2. Questionnaires **9**

3. Environmental markers **13**

3.1 Gas-phase markers 16
3.1.1 Nicotine in air 16
3.1.2 3-ethenylpyridine 17
3.1.4 Myosmine 18
3.1.5 Carbon monoxide (CO) 19
3.1.6 Volatile Organic Compounds (VOCs) 20

3.2 Solid-phase markers 21
3.2.1 Scopoletin 21
3.2.2 Solanesol-PM 21
3.2.3 Particulate matter (PM) 22

4. Biomarkers **25**

4.1 Non carcinogenic biomarkers 29
4.1.1 Cotinine 29
4.1.2 Nicotine and cotinine in hair 32
4.1.3 Carbon monoxide and carboxyhemoglobin 34
4.1.4 Thiocyanate 34

4.2 Carcinogenic biomarkers 36
4.2.1 Polycyclic aromatic hydrocarbons (PAH) 37
4.2.2 Aromatic amines 39
4.2.3 N-nitrosamines 39
4.2.4 Benzene 41
4.2.5 Others 42

5. Conclusion **45**

Tables **48**

References **52**

1. INTRODUCTION

1.1 Health effects of SHS

Indoor tobacco smoke or second-hand smoke (SHS) has been a growing concern in public health policies since the early 1980s. Authoritative reviews over the past two decades have presented scientific evidence linking SHS exposure to a number of adverse health outcomes. Two early reports noted several adverse effects: the first (U.S. DHEW, 1979) described respiratory outcomes in children and adults, as well as some acute cardiovascular effects associated with involuntary exposure to tobacco smoke. The second (U.S. DHHS, 1982), which focused on the carcinogenic effects of active smoking, raised the concern that involuntary smoking may cause lung cancer. The extensive series of epidemiological investigations following the publication of those reports provided compelling evidence of a causal relationship. Subsequently three important reviews (U.S. DHHS, 1986; NRC, 1986; U.S. EPA, 1992) concluded that SHS exposure causes lung cancer and is associated with lower respiratory tract illnesses in young children, as well as with other adverse respiratory affects.

Evidence on overall SHS-related effects has expanded considerably since those publications, and a comprehensive review (NCI, 1999), recently updated (Cal-EPA, 2005), resumed the last results. Recently, involuntary smoking has been certified as a human carcinogen by the International Agency for Research on Cancer (IARC, 2002). These findings are summarized in Table 1. SHS exposure is causally associated with a number of health effects, including effects on infants and children. SHS has a number of serious impacts on children's health, including sudden infant death syndrome (SIDS), exacerbation of asthma, increased respiratory tract infections, increased middle ear infections, and also causes developmental

toxicity resulting in low birth weight, impaired lung function growth, and other developmental impacts. Effects for which there is sufficient evidence of a causal relationship are fatal outcomes, such as sudden infant death syndrome and heart disease mortality, cancers of the lung and other organs, as well as serious chronic diseases such as childhood asthma. There are, in addition, effects for which evidence suggests an association, but further research is required to confirm this. These include spontaneous abortion, cervical cancer, decreased female fertility, and chronic respiratory symptoms in adults. Finally, on the basis of current evidence it is not possible to judge the impact of SHS on a number of endpoints, including congenital malformations, male reproductive effects and rare childhood cancers.

1.2 SHS exposure

Because the various health endpoints reviewed above may be the result of either acute or chronic exposure, both present and past patterns of exposure are of interest and should be taken into account. Measuring the prevalence of SHS exposure should be considered representative only of the general time periods and the specific populations covered by the studies. Smoking prevalence, smoking behaviours and other factors contributing to exposure to SHS have continued to change, as smoking customs have changed worldwide, with a number of important changes occurring within the past few years. Thus, it is expected that the number of individuals exposed to SHS and the patterns of exposure have also changed over time (NCI, 1999).

Although overall trends in smoking prevalence and other factors suggest that SHS exposure is decreasing in industrialized countries, this may not be true for all population sub-groups and the rate of decline may differ among different groups. Patterns of cigarette smoking in several countries depending on sex, race, educational, and socio-economic groups have

shifted over the years (U.S. DHHS, 1989; Fiore *et al*, 1989), with differential impacts on SHS exposure of non-smokers.

Because of the multiple sources of SHS exposure and the different characteristics of population sub-groups, overall personal exposure, and consequently the related health effects, are difficult to estimate. Therefore, many studies have focused on specific settings and specific sub-populations, so any generalization about the results of these studies should be viewed with caution.

Exposure assessment studies have been performed in many countries, especially in occupational settings. However, studies about the health risks of SHS are difficult to carry out, because of the long-term exposure that many related diseases are associated with, and the misclassification and confounding factors encountered in tracing exposure over a large period of time.

Accurate and precise exposure assessment is crucial, since the health effects of SHS are likely to be relatively small in magnitude. Appropriate exposure assessment is also needed to infer causality and for risk assessment. In addition, exposure assessment is obviously necessary to develop preventive strategies (Jaakkola and Jaakkola, 1997).

1.3 Physical and chemical properties

SHS is a complex mixture of thousands of gases and particulate matter emitted by the combustion of tobacco products and from smoke exhaled by the smokers (NRC, 1986). Tobacco smoke contains over 4,500 compounds found both in vapour and particle phases. These compounds include over 50 chemicals recognized as known and probable human carcinogens, other animal carcinogens and many toxic and irritant agents (U.S. EPA, 1992).

Cigarette smoke is constituted by two main components, mainstream and side-stream smoke. Mainstream smoke is material that is drawn through the

mouthpiece of a burning cigarette, and contributes to nearly all of the vapour-phase constituents and over half of the solid-phase compounds. Side-stream smoke is material that is emitted from a burning cigarette between puffs. SHS is a combination of mainstream smoke exhaled by smokers, side-stream smoke, and compounds that diffuse through the cigarette paper (NCI, 1999).

Mainstream and side-stream smoke are qualitatively similar in chemical composition, but the quantities of constituents are different, due to differences in the heat of combustion, tobacco content, additives present, and the type of filter material used. The cigarette burns at a higher temperature during inhalation, leading to more complete combustion in mainstream smoke. Thus, side-stream smoke contains considerably higher concentrations of many carcinogenic and toxic substances.

1.4 Measuring exposure

Since environmental tobacco smoke is a combination of gases and particulate matter, SHS exposure is in reality composed of numerous concentration-time curves of different compounds. Relatively little is known about the importance of individual constituents in causing adverse health effects, and interactions between different compounds may also play an important role. Therefore, assessment of exposure to the entire SHS mixture is relevant.

The first step in exposure assessment is to identify an indicator (or a marker) of SHS, which can be measured and represents the magnitude, duration and frequency of SHS exposure (U.S. EPA, 1992). It is more feasible to measure one indicator compound than several or all compounds. Furthermore, by selecting a good indicator, it is possible to assess overall exposure to the complex mixture. Ideally, an indicator of SHS should vary

with the source strength, should be easily and accurately measured at an affordable cost, and should be representative of SHS as a whole (NRC, 1986).

In order to provide a quantitative assessment of SHS exposure, it is important to estimate the magnitude of pollutant concentration and the duration of exposure in the different microenvironments. Moreover, it is essential to define the time-pattern that characterizes the exposure over time experienced by an individual through time and space. Studies focussing on health-related effects of SHS exposure should also take into account the latency period from exposure to manifestation of the effects, time-profile of exposure as well as individual characteristics.

Exposure assessment can be carried out prospectively or retrospectively, with important implications for validity issues. Prospective assessment takes place before the manifestation of the health outcome. This is usually preferable, since potential misclassification of exposure by prospective assessment is likely to be non-differential rather than differential. Unfortunately, prospective assessment is often unfeasible, especially for diseases with a long latency period. Retrospective exposure assessment takes place after the manifestation of the health outcome. This type of assessment is more likely to present problems regarding the validity and precision of exposure estimates. Differential misclassification is the major validity concern. Recall bias may be a problem in retrospective assessment: diseased subjects (or their surrogates) may be more prone to recall exposure than non-diseased ones (Jaakkola and Jaakkola, 1997).

Although the measure of nicotine in air and cotinine in biological samples has often been presented as a comparison in many environmental and personal exposure assessment studies, there is no method or marker that matches the criteria of a gold standard, for sensibility, specificity, validity

and reliability. Therefore, validation is based on comparing results obtained with one method to those obtained with other "non-ideal" methods.

The majority of markers used in SHS exposure assessment is borrowed from previous studies on active smoke. The amount of exposure experienced in this new context and, consequently, the quantification of the related risk is dramatically lower. So, the use of excellent markers for measuring active smoke could face serious problems with regard to limits of detection, sensitivity and specificity when used for the purpose of measuring SHS.

1.5 Aim of the study

The aim of the study is to review the SHS markers in order to better understand the available methods for measuring SHS exposure, and to choose which marker (or which combination of markers) is the most suitable for monitoring SHS exposure in different settings.

The aim of this report is, therefore, to discuss the specific characteristics of different methods to measure the presence of SHS and to compare their practicability, user-friendly characteristics, rapidity of response, reproducibility of results, capacity to impact with people’s understanding and economic burden.

Articles about SHS markers were found in Pubmed, using the following search terms, in different combinations: “marker”, “biomarker”, “environmental tobacco smoke”, “second-hand smoke”, “exposure assessment”. Specific terms were added for specific markers, and further indications were provided by references of topic papers. The review was updated to July 2005.

2. QUESTIONNAIRES

Questionnaires are the most frequently used method to assess exposure to SHS in both retrospective and prospective studies of acute and chronic effects. They are the least expensive method to obtain SHS exposure information for large populations, because it is not always feasible to collect and analyse a sufficiently large number of subjects to derive an objective index of exposure on the basis of environmental or biological markers.

In any case, memory-based proxy reports may be questioned because of limitations in the reporter's ability to estimate accurately the duration, proximity or frequency of exposure, and to accurately report potentially important physical characteristics of the exposure setting, such as air ventilation or air conditioning (Matt *et al*, 1999). Misclassification errors may arise due to the respondents' lack of knowledge, biased recall, memory failure and intentional alteration (Chen *et al*, 2002). Nevertheless, memory-based reports of SHS exposure may frequently be the only available and affordable source of information about SHS exposure for epidemiological research and clinical practice.

There are many methods to test the reliability of questionnaire responses on SHS exposure: some studies employing a "test-retest" design, with more interviews to the same subjects at different times (Pron *et al*, 1988; Coultas *et al*, 1990). Conformity of responses was generally good, but information provided by the subjects on the amounts smoked (i.e. number of cigarettes smoked or hours spent smoking per day) was found to be less reliable.

A number of studies have examined the quality of information provided by surrogate respondents (Sandler and Shore, 1986; McLaughlin *et al*, 1987). Use of surrogate respondents occurs frequently in studies of SHS exposure.

Studies examining the effects of exposure to spousal or household smoking often ask subjects to report on the smoking habits of members of their households. In retrospective studies of adult health risks from exposures occurring early in life, subjects who are now adults are questioned concerning parental smoking habits. In summary, the results of these studies indicate that information on childhood exposure to SHS provided by individuals who are now adults is of good quality, particularly with regard to qualitative information. Similarly, qualitative information on spousal smoking is of good quality. However, in both cases, quantitative information about the number of years of smoking, the years of beginning or the number of cigarettes smoked per day is sometimes less reliable.

Nevertheless, the most accurate way to assess the validity of self-reported exposure to SHS is biochemical verification using a highly sensitive method. Cotinine, the major metabolite of nicotine, has been considered the "gold standard" for assessing questionnaires' reliability (Chen *et al*, 2002). Memory-based recall normally covers the previous 48 hours and the previous week. The 48-hour time frame is particularly appropriate to validate against urine cotinine measures because of the metabolite's half-life (40–60 hours). Although biochemical verification provides empirical support for the validity of short-term exposure, reflecting recent exposure to tobacco smoke for non-smokers, it does not reflect the validity of longer-term exposure (Matt *et al*, 1999).

In many studies of active smokers there was a high correspondence between self-reported tobacco use and amount assessed by cotinine (Post *et al*, 2005; Vartiainen *et al*, 2002; Eisner *et al*, 2001). Indeed, the correspondence was higher for self-reported non-users than for users. Overall, the correlation between the biological marker and the reported patterns of use (frequency, recentness and intensity) was very good. A considerable discrepancy was found among subjects reporting non-daily

tobacco use: half of the weekly users, for example, were classified as non-users by the biological test. The discrepancy is probably explained by the very unstable patterns of tobacco use in these subjects, for which recent consumption does not imply intensive or regular use.

These findings could explain the low correlation between self-reported consumption and biomarker levels found in several studies of SHS exposure (Chen *et al*, 2002; Hecht, 1999; Emmons *et al*, 1996; O'Connor *et al*, 1995; Delfino *et al*, 1993; Coultas *et al*, 1990), with large individual variability and short half-life of biomarkers such as cotinine. Biomarker studies have shown that a proportion of subjects reporting no SHS exposure have measurable biomarker concentrations, indicating that subjects either forgot or were not aware of their SHS exposure. Indeed, in many cases it is impossible to distinguish non-exposed from SHS-exposed subjects (Kemmeren *et al*, 1994). However, other authors reported a good correlation and a clear dose-response relationship between the two measures (Sexton *et al*, 2004; Willemsen *et al*, 1997; Haley *et al*, 1989; van Vunakis *et al*, 1989).

The sensitivity and specificity of self-reports have been calculated with measures of different biomarkers as standards in smoker subjects (Dolcini *et al*, 2003). When the self-report items were compatible with the half-life of the biological indicator, and values were adjusted for smokeless tobacco use, sensitivity varied widely. The range for specificity was more constrained. The biological indicator examined and the time-frame of questions on smoking affected the sensitivity of self-report. The biological indicator examined and use of other tobacco products influenced the specificity of self-report. Overall, sensitivity and specificity were highest when recent smoking was assessed, when cotinine was the standard and smokeless tobacco use was considered.

In SHS exposure, self-reported measures show a good specificity but low sensitivity (Kemmeren *et al*, 1994), probably because of the difficulty of non-smoker subjects to identify sources, periods and amounts of exposure.

By way of conclusion, questionnaires provide useful information about SHS exposure and are relatively easy and inexpensive to obtain and analyse, even if they do require more accurate measures, such as biomarkers, in order to assure sensitivity and reliability. They are the only method that permits tracing of the exposure pattern in retrospective studies.

3. ENVIRONMENTAL MARKERS

The extent of exposure to SHS by environmental measures could be assessed by different ways. First, direct methods are represented by the use of personal monitors, which measure concentrations of SHS constituents at or near the breathing zone. They can be worn by individuals to assess exposure occurring at a specific location or accumulated throughout the day, thus providing an integrated measure of short-term exposure. They are often used in conjunction with biomarkers in order to compare or validate assessment of exposure. Indirect methods include measurements of indoor air concentrations of SHS constituents and the time that an individual spends in a specific environment. Models based on mass balance are another means of indirectly assessing SHS exposure. Although it has been argued that predictions derived from these models are too situation-specific to be generalized relating to the overall population (NCI, 1999), several recent studies have taken steps toward designing models with greater general applicability, thereby allowing even greater ability to predict population-wide patterns (Klepeis, 1999).

Given the complex chemical composition of SHS, air concentrations are typically assessed by measuring individual SHS constituents referred to as tracers or proxy compounds. Gas-phase (nicotine, carbon monoxide, volatile organic compounds, 3-ethenylpyridine, scopoletin) and solid-phase markers (particulate matter, solanesol) of SHS are currently used to monitor tobacco smoke pollution (Hodgson *et al*, 1996; Jenkins *et al*, 1996). Although at least one marker in each phase should be measured to describe adequately SHS load (Daisey, 1999), most of the field studies that have marked our present culture about SHS prevention have relied on single markers, which have

gained widespread reliability among researchers. Ambient SHS markers can be further sub-divided into specific (generated only by tobacco combustion) and non-specific (common to other kinds of combustion, but useful for SHS field studies).

Personal monitoring of relevant tobacco smoke components can be considered as the most direct method to assess SHS exposure. Personal monitoring measures the contact of an individual with different concentrations of a pollutant in the course of normal activities, using samplers worn for several hours to several days (NRC, 1986). Personal monitoring usually measures an integrated exposure across a variety of environments occupied by the person over time. Continuously recording instrumentation with data-logging capacity has also been developed. The samplers can be passive, working on the principle of diffusion, or active pumps, that collect and concentrate the air contaminant for further analysis or draw air through a direct-reading detector. There are severe limitations of personal monitoring methods to assess SHS exposure: first, they can only be used for relatively short time periods, and this time period during which monitoring is carried out may not be representative of an individual's normal activities in the long run. Moreover, they are currently not available for all components of SHS. Finally, they are time-consuming and expensive, and thus, may not be feasible in studies with large samples (Jaakkola and Jaakkola 1997).

Fixed location measurements of air concentrations of SHS constituents indicate the presence of SHS and allow an estimation of the contribution of SHS to indoor air contaminant levels, but do not constitute a direct measure of an individual’s total SHS exposure. During the course of a single day, an individual spends varying amounts of time in a number of different environments; for that individual, the total exposure is the sum of the concentration at each location multiplied by the time spent at that location.

Further, for different individuals exposed to the same levels of SHS constituents in the same room, the actual dose will vary as a function of a number of factors, including gender, age, specific activity level and breathing rate at the time of exposure (NCI, 1999). Moreover, the measured concentrations of individual constituents in homes and other indoor environments show marked spatial and temporal variation as a result of the complex interaction of factors related to the introduction, removal and dispersion of SHS constituents. These factors include the rate of tobacco consumption, room size, the location at which smoking occurs, the placement of air monitors, the ventilation and infiltration rate, air mixing and removal of contaminants by air filters or deposition (NRC 1986). In order to provide a quantitative assessment of exposure, several variables should be taken into account, like the magnitude of the pollutant concentration and the duration and the time-specificity of exposure (Jaakkola and Jaakkola, 1997). If concentrations of a specified tobacco smoke component are measured in all microenvironments occupied by an individual and this information is combined with detailed time-activity information to assess total exposure of that individual, the stationary monitoring method approaches direct personal monitoring of exposure.

Subsequently, we present the principal environmental biomarkers used in previous studies in order to evaluate exposure to SHS.

3.1 Gas-phase markers

3.1.1 Nicotine in air

Vapour-phase nicotine is the most commonly measured indoor air indicator of SHS, and it is used both in personal sampling methods and in fixed-site indirect measurements (Nebot *et al,* 2005; Daisey, 1999; LaKind *et al,* 1999). Several other components have been studied as markers for SHS, but nicotine has been most widely used because its only source is tobacco smoke (Hammond *et al*, 1987).

Nicotine is emitted in the side-stream smoke particle phase and evaporates when it is diluted. It is suitable as an indicator of SHS, since it is specific to tobacco combustion and is emitted in large quantities in SHS (U.S. EPA 1992). Nicotine can be collected on a fibre back-up filter treated with sodium bisulphate and analysed by gas chromatography, which is a fairly simple and inexpensive method (Henderson *et al*, 1989; Hammond *et al*, 1987), and can also be measured by means of personal badge monitor (Nebot *et al,* 2005; LaKind *et al,* 1996; Phillips *et al,* 1996) or in indoor dust and household surfaces (Matt *et al,* 2004). Ambient nicotine measurement has been used to monitor SHS pollution in private buildings, public places, workplaces and transport facilities, to assess personal exposure, and to estimate the overall exposure of non-smokers to SHS.

In the case of air nicotine one must distinguish between exposed and non-exposed, and a clear dose-response relationship with number of cigarettes smoked and period of exposure (Özkaynak *et al,* 1996).

This kind of marker has been widely used for several years in many studies (NCI, 1999) with very similar methods, providing references about

levels of exposure and estimates in different settings or different countries (Nebot *et al,* 2005; Hammond*,* 1999; Jenkins *et al,* 1996).

Levels of air nicotine, measured by fixed-site or personal methods, show a good correlation with memory-based proxy reports (O'Connor *et al,* 1995; Coghlin *et al,* 1989), particulate matter (Daisey*,* 1999; Leaderer and Hammond*,* 1991) and urinary or salivary cotinine (LaKind *et al*, 1999; Jenkins and Counts*,* 1999; Marbury *et al,* 1993; Henderson *et al,* 1989).

Measurements taken in a wide variety of indoor environments indicate that most average concentrations of nicotine range about 100-fold, from 0.3 to 30 $\mu g/m^3$ (NCI, 1999), even if in some settings levels up to 500 $\mu g/m^3$ have been found (Nebot *et al,* 2005).

A dose-response relationship was found between air nicotine and health effects in occupational settings, and specific models about levels, years of exposure, and risk of lung cancer have been carried out (Hammond *et al*, 1995; Repace and Lowrey, 1993).

A drawback of vapour phase nicotine as an SHS indicator is its high adsorption rate to indoor surfaces and a tendency to be re-emitted even in the absence of active smoking (Jaakkola and Jaakkola, 1997). This source of exposure (residual SHS when non-smoker is present after smoker finishes) accounted for a larger fraction of exposure for nicotine and other sorbing compounds versus non-sorbing SHS components. Indirect routes accounted for about 50 percent of potential nicotine exposure during the non-smoking periods (Singer *et al,* 2003). This property can change its concentration relative to other SHS constituents.

3.1.2 3-ethenylpyridine

Ambient nicotine was originally considered to be an ideal tracer for SHS concentrations because it is unique to tobacco plants. However, its chemical structure confers adsorptive tendencies and unpredictable decay rates. Thus

nicotine measurements may underestimate SHS because of adsorption on building materials and room furnishings, so that it can disappear from SHS at a faster rate than other components. Another property of nicotine is desorption from the surfaces where it deposited initially, causing a possible overestimation of SHS from the slow desorption of nicotine over time. The presence of ambient nicotine is a strong indication that smoking has occurred. However, it does not necessarily indicate the presence of other SHS components. The unpredictable decay characteristics of SHS nicotine can be addressed by providing for simultaneous determination of 3-ethenylpyridine (3-EP), the principal product of nicotine pyrolysis, by a gas chromatographic method (Vainiotalo *et al,* 2001). However, analytical standards for 3-EP are not as readily available as for nicotine.

Several field studies have been carried out by measuring 3-EP along with ambient nicotine (Bi *et al,* 2005; Kim *et al,* 2001; Hyvarinen *et al,* 2000; Phillips *et al,* 1998). 3-EP bears optimal correlation and relatively constant relationship to the initial SHS nicotine concentration as well as the same uniqueness of source in indoor environments (LaKind *et al*, 1999). Unlike nicotine, 3-EP is more volatile and less adsorptive than nicotine, so that this marker has decay characteristics more representative than other SHS vapour phase constituents (Jenkins *et al,* 2000). However, some reports observed that in real world measurements 3-EP does not behave entirely as previously described in experimental chambers, especially when there are low ventilation levels (Bi *et al,* 2005; Scherer *et al,* 2000).

3.1.4 Myosmine

Myosmine is an alkaloid compound present in tobacco leaves. It is regarded as a specific marker of SHS, even though myosmine can also be found in nuts (U.S. EPA, 1992). Myosmine is easily nitrosated giving rise to DNA adducts. Even if myosmine shows a good correlation with nicotine

levels (LaKind *et al*, 1999), it is present only in 2-7% of the gas-phase nicotine concentration, and measurements of environmental exposure could sometimes be under the limit of detection (Benner *et al*, 1989). It is rarely used as an SHS marker.

3.1.5 Carbon monoxide (CO)

CO is a non-specific marker of combustion, very useful to monitor both outdoor and indoor air. It is a highly toxic poison, as CO has a strong reaction with hemoglobin to form carboxy-hemoglobin (COHb). The affinity of hemoglobin for CO is 200-250 times that for oxygen, so that this binding reduces the oxygen-carrying capacity of the blood and impairs the release of oxygen to extravascular tissues (Tomaszewski, 1999). COHb concentrations depend on CO level in inhaled air, duration of exposure and lung ventilation.

Cigarette smoking is a major source of indoor CO contamination. The CO yields of different brands of cigarettes have been studied for a long time, and were reported to increase with puff volume and tobacco moisture, and to decrease with increased paper porosity, with broad differences among the various brands (Calafat *et al,* 2004; Robinson and Forbes, 1975). Ambient CO measurements have been carried out often in association with other markers in many field studies to evaluate SHS pollution at home, at the workplace and in public places (De Bruin *et al,* 2004; Jo *et al,* 2004; Klepeis *et al,* 1999; Siegel, 1993). It should to be noted that it is quite common to find smoky places with CO levels above the 8-hour average concentration limit of 10 mg/m^3 set for outdoor air (Akbar-Khanzadeh and Greco, 1996; Seppanen, 1977). Measuring CO with electrochemical analysers is affordable and reliable, but since CO is highly diffusible and many domestic and vehicular sources are possible sources of this gas, CO monitoring for SHS assessment should be carried out only together with other markers.

3.1.6 Volatile Organic Compounds (VOCs)

VOCs, such as benzene, styrene, formaldehyde, butadiene, acetone, toluene, ethyl benzene, xylene, N-nitrosamines and many other volatile compounds, constitute a major proportion of the organic mass of SHS, and many of the compounds in these fractions are known to be biologically active as carcinogens in animals.

There have been only a few field studies of the contribution of SHS to VOC exposure at the workplace and in the home (Kim *et al,* 2001; Daisey*,* 1999; Hodgson *et al,* 1996). In any case, it does not seem that these compounds could compete in sensitivity, laboratory techniques and user-friendly characteristics with the previous environmental markers.

3.2 Solid-phase markers

3.2.1 Scopoletin

Scopoletin (6-methoxy-7-hydroxy-coumarin) is another compound present in tobacco leaves, which has been identified as a possible particulate-associated SHS tracer molecule (Douce *et al,* 2001). Scopoletin is extracted into methanol from particulate material collected on Teflon impregnated glass fibre filters. Quantitative analysis can be achieved using reverse-phase liquid chromatography (RP-HPLC) and fluorescence detection (Risner*,* 1994). Some field studies have been carried out for scopoletin to trace SHS-specific particulates (Douce *et al,* 2001; LaKind *et al,* 1999).

3.2.2 Solanesol-PM

Solanesol is a naturally occurring terpene component in tobacco, present in the particulate phase of SHS. Due to this property, solanesol is also known as "SHS-specific particle" or "Sol-PM", and can be measured using gas chromatographic techniques (Singer *et al,* 2002). It has been shown to be an important precursor of the carcinogenic polynuclear aromatic hydrocarbons of smoke (Severson *et al,* 1977). Due to its high molecular weight, solanesol is very involatile and is found entirely associated with the particulate emissions of SHS. Its primary use in SHS research was to calculate the fraction of SHS-derived particulates to total particulates present in ambient air, which has been found to be up to 92% (Daisey*,* 1999; Ogden and Maiolo*,* 1989). Several field studies have been carried out using solanesol-PM as a specific marker of SHS-derived particulates in association with other markers, confirming the high contribution of SHS to indoor particulate matter (Gee *et al,* 2005, Zhou *et al,* 2000).

As for its specificity, solanesol can be found in many plants of the Solanaceae family, to which the Nicotiana genus belongs. Other members of the family contain solanesol, like potato plants, tomato plants, pepper plants and aubergines. Therefore, certain cooking emissions represent sources of solanesol, other than SHS. However, this kind of interference is thought to be of only minor relevance (Ogden and Maiolo, 1989).

Indeed, solanesol is thought to degrade when exposed to ultraviolet light and hence would not be a good marker for SHS outdoors. Also, as solanesol air concentrations may be too low to measure (Jenkins *et al,* 2000), there is no steady correlation with other markers nor is it consistent across different tobacco products (LaKind *et al,* 1999).

3.2.3 Particulate matter (PM)

Particulate matter is defined as particles of solid and/or liquid nature of such dimensions and morphology as to remain suspended in the atmosphere for a certain time depending on their size, form, specific weight and air turbulence. Larger particles sediment quickly due to gravitational attraction, while smaller particles can remain suspended for hours and even days and can be transported very far from the generation point by winds and turbulences.

In contrast to nicotine, particulate matter is not specific to SHS and thus measurements in environments where smoking occurs must be compared to concentrations in comparable environments where smoking does not occur. Like nicotine, measured concentrations of SHS-associated particulate range about 100-fold, from 5 to 500 $\mu g/m^3$, over a wide variety of indoor environments (NCI, 1999).

Outdoor particulate matter pollution measurements were consistently smaller than exposure by personal monitoring, indicating the strong influence of indoor environment on personal exposure (Spengler *et al,*

1985). Correlations between home indoor particulate matter concentrations and personal exposure were high. SHS was a substantial contributor to personal exposure in indoor environments, the average personal particulate matter exposure being higher in subjects exposed to SHS, compared to those not exposed.

Although the PM is not a specific marker of SHS, the amount of pollution generated can be extremely high in indoor environments: pioneering works identified SHS as a major source of indoor air pollution, the greatest source of population exposure to respirable particulate air pollution (RSP), and developed equations for its prediction (Repace and Lowrey, 1980).

Particulate matter can be detected and measured by instruments using several different methods: gravimetric and optical to detect the mass or the number of particles, fluorescence (FPM) and ultraviolet (UVPM) adsorption to measure the particle bound hydrocarbons (U.S. EPA, 1992; NRC, 1986). These different measures show a good relationship in experimental conditions (Rando et al, 1992), but only a weak correlation in field studies (LaKind *et al*, 1999)

Measurements have shown concentrations of PM2.5 (class of particulate matter including particles with aerodynamic diameters up to 2.5 micrometers) and/or RSP (class of particulate matter including particles with aerodynamic diameters up to 3.5 micrometers) in the range of 10-20 or even more as the maximum allowed concentrations for outdoor pollution. Many field studies on SHS pollution have been carried out with PM determinations, both with stationary sampling (Bi *et al,* 2005; Invernizzi *et al,* 2002; Liu *et al,* 2001; Scherer *et al,* 1990) and with personal samplers (Georgiadis *et al,* 2001; Jenkins *et al,* 2001,1996; LaKind *et al,* 1999; Phillips *et al,* 1998, 1996).

The novelty in PM monitoring is the availability of portable, user-friendly, affordable instruments capable of real-time monitoring (one measurement

every 1-5 minutes) (Invernizzi *et al,* 2002). These instruments allow multiple assessments of indoor air quality each day and are suited to check compliance with smoking policy rules. Moreover, the results of the measurements can be shared instantly with bartenders, patrons and customers, thus representing an educational opportunity. Real-time analysers can also compare outdoor and indoor air quality instantly, often a shocking experience for lay people who are accustomed to considering pollution mainly as an outdoor problem (Invernizzi *et al,* 2004). The principal drawback of this marker is its poor specificity to SHS: in order to collect reliable information about exposure, it could be useful to link PM measures with other specific markers.

4. BIOMARKERS

Personal monitoring of exposure and analysis of the respiratory environment make it possible to estimate the level of toxic agents for individuals exposed to SHS. On the other hand, studies on the uptake of smoke constituents by individuals and on the metabolic fate of such compounds can provide deeper information relative to epidemiological observations and the real exposure levels of different populations.

Exposure to SHS may depend on several factors, including the number of smokers in an enclosed area, the size and nature of the area, and the degree of ventilation. Moreover, the uptake depends on breathing rate, mouth versus nose breathing, airway geometry and other respiratory factors, and thus modifies the dose received by the human body (Jaakkola and Jaakkola 1997). Individual processes taking place after uptake, including metabolism and elimination of the compound, determine the biologically effective dose. Thus, optimal assessment of exposure should be done by analysing the physiological fluids of exposed persons, rather than by analysing the respiratory environment. The development of new biochemical methods allows us to obtain measurements of exposure to SHS by determining the uptake of specific agents in body fluids and calculating the risk relative to that of the exposure of active smokers. The uptake of individual agents from SHS can be determined by biochemical measures that have been developed to assess active smoking behaviour, as long as these measures are sensitive and specific enough to quantify exposure to such agents in non-smokers.

Biomarkers can be classified in three different categories: biomarkers of internal exposure, biomarkers estimating the biologically effective dose, and

biomarkers of potential harm (IOM, 2001). The latter two biomarkers report the consequences of exposure at the cellular level and can provide information about the effects of individual chemical components which enter the body due to tobacco smoke and from other sources (i.e. tobacco, diet, air) (Shields, 2002). Given the large number of available potential biomarkers, and the numerous ones under development with new technologies, a framework is needed to assess the importance of a biomarker. When developing new tests, it is often helpful to have complementary markers that can measure the same effect or a surrogate for the effect. Studies of any biomarker should include determination of the replicability (i.e., coefficient of variation), inter-laboratory variability, intra-individual variation, and inter-individual variation. Laboratories should have quality control and quality assurance procedures (Shields, 2002).

Biomarkers of exposure include any test of a body fluid (including exhaled air) or tissue that measures a constituent or constituent metabolite of tobacco smoke. It is not a measurement of how the constituents interact with body functions or macromolecules to cause harm.

The biologically effective dose represents the net effect of tobacco smoke constituents (single or complex) on a cellular macromolecule (e.g., protein or DNA) following metabolic activation to reactive intermediates, decreased rate of detoxification, decreased repair capacity, and decreased rates of cell death (Perera*,* 1987). A common way to assess the biologically effective dose is to measure carcinogen–DNA adducts. It reflects integrated measure of external exposure, smoking behaviour, metabolic activation, DNA repair capacity, cell cycle control, and apoptosis capacity. These experiments are generally challenging due to the technological limitations in measuring low levels in human tissues.

Biomarkers of potential harm can range from non-functional effects on cells that serve as surrogate markers for actual harm to pre-clinical and

clinical disease. These markers may reflect early biological and genetic effects, or alterations in morphology, structure, or function. These experiments are typically expensive, but new opportunities exist with newer technologies. Specificity for smoking exposure is usually not possible (Shields, 2002). As biomarkers of effective dose, these are best measured in target tissue but sometimes that tissue, or an adequate sample of it, is difficult to obtain.

Two broad questions must be taken into account 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 biological fluid reflect an individual’s intake of that chemical (or a related chemical) from tobacco smoke (Benowitz, 1999)?

Criteria for a valid marker of SHS in the air have been proposed (NRC, 1986). First, the marker should be unique or nearly unique for SHS so that other sources are minor in comparison. Then, it should be easily detectable and emitted at similar rates for a variety of tobacco products. Finally, it should have a fairly constant ratio to other SHS components of interest under a range of environmental conditions encountered. On the other hand, the validity of a biomarker depends on the accuracy of the biologic fluid measurement in quantifying 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 (NCI, 1999). Other issues of interest in assessing the risks of exposure to SHS are how well the biomarker indicates long-term exposure to SHS as well as whether a biomarker predicts the likelihood of SHS-related disease.

Genetic variations of xenobiotic metabolism are currently widely studied and largely applied as markers of susceptibility in molecular epidemiology studies on cancer (Vineis *et al*, 1999; Perera, 1997). As a reflection of such variation in biotransformation, also levels of many biomarkers may be affected in the exposed persons. Even if many studies have been carried out about the role of polymorphisms in tobacco product metabolisms, especially cytochrome P-450 and glutathione-S-transferase genes (Perera *et al*, 2002; Hecht *et al*, 2002; Smith GB *et al*, 2001; Heudorf and Angerer, 2001), the influence of genetic factors is to be estimated in the long run. Thus, confounding and modification effects using biomarkers cannot be ruled out.

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 non-specific (i.e. high baseline values even in non-exposed non-smokers or environmental sources other than tobacco smoke) and/or insensitive (i.e., the increment due to SHS exposure is small compared to baseline values) for use in quantification of levels of smoke exposure to which most non-smokers are exposed.

The principal non-carcinogenic biomarkers used in exposure assessment studies are presented in the following.

4.1 Non carcinogenic biomarkers

4.1.1 Cotinine

The advantages of nicotine and cotinine in body fluids as biomarkers of SHS include their relatively high sensitivity and specificity for tobacco combustion and the availability of accurate measurement methods at low concentrations (U.S. EPA, 1992).

Seventy-five percent or more of the nicotine that is emitted from a cigarette is emitted into the air as side-stream smoke, which contributes substantially to SHS (NRC, 1986); on average, 70-80% of adsorbed nicotine is converted into cotinine, which is transformed to a high degree into other metabolites (Benowitz and Jacob, 1993). Cotinine in urine or in saliva is the most widely used biomarker of tobacco exposure, and the ability to measure the exposure to SHS, with a clear dose-response relationship, is undoubted (Husgafvel-Pursiainen, 2002; Jaakkola and Jaakkola, 1997; Benowitz, 1996). Levels in urine or saliva are highly correlated with blood specimens (Benowitz, 1996), and they are preferred because of their easier collection. The average half-life of cotinine in different body fluids (plasma, saliva and urine) is about the same, approximately 15 to 19 hours (Jarvis *et al,* 1988; Benowitz and Jacob, 1994), making it a good indicator of the integrated SHS exposure over the previous two to three days. The half-life is typically longer in infants and children, averaging approximately 65 hours in neonates, 60 hours in infants less than 18 months, and 40 hours in children over 18 months (U.S. EPA, 1992).

Nicotine measured in body fluids is not considered a good biomarker, considering that its half-life averages only 2-3 hours, compared to the 17

hours of cotinine, which, moreover, remains relatively constant throughout the day (Benowitz and Jacob*,* 1993).

Despite large cotinine utilization, some aspects should be taken into account. The low half-life of this biomarker makes it useful only for assessing short-term exposure. Besides, urinary cotinine shows a high inter-individual variability in conversion from nicotine (usual range 55-92 percent), depending on renal function, urinary flow rate, and urinary pH (Benowitz and Jacob*,* 1993). Urinary results may be expressed as nanograms of cotinine per milligram of creatinine in order to compensate, in part, for differences in dilution effects. Because the amount of endogenous creatinine produced is a function of muscle mass, age, sex and inter-individual excretion rates of creatinine are also variable. In particular, cotinine/creatinine ratios may not be appropriate for comparisons between males and females. In addition, low levels of creatinine in infants compared to adults may result in cotinine to creatinine ratios for infants that fall into the range reported for active smokers (Watts *et al,* 1990). In general, it is preferable to collect urine over 24 hours, although is impracticable for most studies. Nevertheless, levels in large groups of subjects would be expected to accurately reflect average group exposure to SHS.

In general, the presence of nicotine or its metabolites in physiological fluids can be attributed only to exposure to tobacco smoke. The few exceptions include occupational exposure to tobacco leaves (Gehlback *et al,* 1975) and nicotine products, use of smokeless tobacco products, chewing of nicotine gum, and use of nicotine patches or other aids for smoking cessation. Low levels of nicotine have been found in tea and in edible solanaceous plants including aubergines, green peppers and tomatoes (Sheen*,* 1988; Davis *et al,* 1991; Domino *et al,* 1993). While some authors have claimed that dietary intake of nicotine may be of practical importance in the use of nicotine and cotinine as biomarkers of SHS exposure (Domino

et al, 1993), others dispute this assertion (Jarvis*,* 1994; Repace*,* 1994; Benowitz, 1996; Pirkle *et al,* 1996). In general, the levels of nicotine and its metabolites in physiological fluids resulting from the ingestion of foods has not been found to significantly impact the levels resulting from exposure to nicotine from tobacco sources.

Many studies are available which report concentrations of cotinine in the physiological fluids of smokers and non-smokers (NCI, 1999). The levels of SHS encountered by exposed non-smokers during their daily activities are sufficiently high for nicotine and cotinine to be detected in their urine, blood and saliva. The physiological concentrations of cotinine detected in saliva and plasma of non-smokers typically range from 0.5 ng/ml to 10 or 15 ng/ml (U.S. EPA, 1992; Guerin *et al,* 1992), and urinary concentrations range to 50 or more ng/ml. However, it is important to realize that some of the differences in cotinine levels reported here could be explained by the different analytical methods used. Thus, in comparing cotinine levels reported in various studies, it is important to consider the analytical method employed and what components were being measured. There are two methods to detect cotinine and nicotine in biological specimens: gas chromatography (GC) and radioimmunoassay (RIA). Both techniques reliably gauge nicotine and cotinine in urine and serum samples and are capable of discriminating between smokers and non- smokers (NCI, 1999).

A significant correlation was found between cotinine levels and acute biologic effects of SHS, as middle ear effusion (Strachan *et al,* 1989) and wheezing bronchitis (Rylander *et al,* 1995) in children, or coronary heart disease (Whincup *et al,* 2004; Tunstall-Pedoe *et al,* 1995). Other studies, reported that SHS measured by cotinine has been correlated to adverse effects on fœtal growth as well as child growth and development (Perera, Tang *et al,* 2004; Yolton *et al,* 2005; Eskenazi *et al,* 1995; NRC, 1986). This

fact further supports the idea that cotinine could be used as a quantitative marker of SHS exposure.

Measures of cotinine in biologic fluid have been widely used to enhance the reliability of questionnaire-derived assessments of SHS exposure. Several studies show a weak correlation between self-reported exposure and cotinine levels, even in distinguishing exposed and unexposed (Emmons *et al,* 1994; Kemmeren *et al,* 1994; Delfino *et al,* 1993; Coultas *et al,* 1990; Haley *et al,* 1989). Stronger relations between ambient air nicotine and urinary or salivary cotinine levels of non-smokers have been reported (Jenkins and Counts, 1999; Matt *et al*, 1999; Marbury *et al,* 1993; Henderson *et al,* 1989). Those findings emphasize that the validation of exposure status with a biomarker is an essential prerequisite for epidemiological studies investigating passive smoking.

4.1.2 Nicotine and cotinine in hair

Levels of nicotine or cotinine in hair have been suggested as a possible marker of long-term smoke exposure, and a review of the findings about their uses has recently been carried out (Al-Delaimy, 2002). The main advantage in using markers from hair is that, unlike other biomarkers, they can provide information about long-term (up to several months) SHS exposure. Moreover, they facilitate differentiation of the exposure in the different periods of the past months, using hair as a "tape-recorder" and distinguishing the parts by the distance from the proximal end (Uematsu, 1993).

Contrary to the urine specimens, nicotine in hair seems to be a more suitable SHS marker than cotinine. In fact, the latter is present in much lower concentrations than the former (Chetiyanukornkul *et al,* 2004; Klein *et al,* 2004), probably for the different incorporation rate into hair given by cotinine metabolism. For this reason, the detection of SHS exposure is

hardly reduced, thus explaining the poor correlation of cotinine levels to history of exposure among non-smokers (Al-Delaimy *et al,* 2000; Dimich-Ward *et al,* 1997; Nafstad *et al,* 1995).

Several studies have compared the self-reported SHS exposure with nicotine levels in hair and found significant associations, high sensitivity, steady exposure-dose ratio and good reproducibility over time (Al-Delaimy, 2002; Al-Delaimy *et al,* 2001, 2000; Dimich-Ward *et al,* 1997; Nafstad *et al,* 97, 95; Pichini *et al,* 1997; Eliopoulos *et al,* 1994; Zahlsen and Nielsen, 1994). Nicotine in hair was used as a quantitative marker to predict the health effect of SHS during pregnancy: increased risks of small-for-gestational age at birth and pre-term delivery have been demonstrated (Jaakkola *et al,* 2001; Nafstad *et al,* 1998).

Even if average nicotine in hair is less vulnerable to everyday variability of exposure, metabolism or elimination than other biomarkers, some confounding factors should be taken into account. Some authors suggest that a considerable amount of nicotine could originate from direct adsorption from environmental air (Nilsen and Nilsen, 1997; Nielsen and Zahlsen, 1994), but this route is probably secondary to the main one of systemic circulation and does not seem to affect the assessment of SHS exposure (Al-Delaimy *et al,* 2002). Cosmetics and other treatments, but not normal washing, may affect nicotine levels in hair (Pichini *et al,* 1997; Li and Cheng, 1993). Several investigations have reported that white, grey or fair hair has lower nicotine levels than black hair with similar exposure (Mizuno *et al,* 1993; Uematsu *et al,* 1995), maybe due to the high affinity of nicotine for melanin.

In a recent survey that compares urine cotinine and hair nicotine as biomarkers for SHS exposure (Al-Delaimy *et al,* 2002), the latter was found to have greater precision and accuracy, due to the lower variability of long-term exposure, and fewer sources of bias.

4.1.3 Carbon monoxide and carboxyhemoglobin

Carbon monoxide, both in exhaled alveolar air and as carboxyhemoglobin in blood, originates from endogenous processes as well as from environmental sources. In addition to cigarette smoke, common environmental sources include vehicle exhaust, gas stoves and furnaces and kerosene space heaters. Although carbon monoxide and carboxyhemoglobin have been used to distinguish smokers from non-smokers (Ohlin *et al,* 1976; Sillett *et al,* 1978; Jarvis *et al,* 1983, 1987), they are generally not good indicators of SHS exposure because they lack sensitivity and specificity. In non-smokers exposed to environments with heavy SHS pollution, elevated levels of exhaled carbon monoxide and carboxyhemoglobin in the blood were detected when measured within 30 minutes following cessation of exposure. However, several studies of more typical exposure situations did not find significant differences in the carboxyhemoglobin levels in subjects reporting no, low, or high levels of SHS exposure (Jarvis and Russell, 1984; Jarvis *et al,* 1983). It does not appear that carbon monoxide and carboxyhemoglobin could represent high-quality markers in SHS exposure assessment.

4.1.4 Thiocyanate

Present in the vapour phase of tobacco smoke, hydrogen cyanide is metabolised in the liver, yielding thiocyanate (SCN^-). Thiocyanate levels in blood, urine and saliva have been used to distinguish smokers from non-smokers, or, in combination with tests for nicotine or cotinine, to distinguish smokers from individuals using smokeless tobacco or other nicotine-containing products (Jarvis *et al,* 1987; U.S. DHHS, 1986; Hauth *et al,* 1984; Haley *et al,* 1983). Sources of thiocyanate are also present in the diet, particularly cruciferous vegetables (Haley *et al,* 1983); thus, levels of

thiocyanate in body fluids are not specific to exposure to tobacco smoke. In studies examining the use of thiocyanate as a biomarker of SHS exposure, it was not possible to distinguish between SHS-exposed and unexposed non-smokers (Hauth *et al,* 1984; Jarvis and Russell, 1984). For this reason, thiocyanate is not very useful as a biomarker of SHS and has not been widely used for monitoring SHS exposure.

4.2 Carcinogenic biomarkers

According to the International Agency for Research on Cancer, tobacco smoke contains over 60 constituents for which there is sufficient evidence of carcinogenity in either laboratory animals or humans (Hoffmann *et al,* 2001; IARC, 1986). These are included in both active and passive smoke, albeit in different concentrations: the carcinogenic mechanism is presumed to be the same. Considering the high number of carcinogenic products present in tobacco smoke and the lack in knowledge about the molecular and physiological features that link exposure and disease, it is almost impossible to describe a single and detailed carcinogenic mechanism. In spite of this fact, general principles have come to light from intensive research in the course of the past decades.

Most SHS constituents require metabolic activation to exert their carcinogenic effects, binding to DNA strains and forming permanent adducts that can lead to miscoding and mutations. This binding is critical in the carcinogenic process (Hecht*,* 2003). These adducts can be removed by repair mechanisms and, if repair does not occur, cells can be forced to apoptosis. When mutations involve critical genes such as oncogenes or suppressor genes, the cell loses its normal behaviour and the cancer process starts.

For a correct assessment of the carcinogenic risk, it is important to consider that the competition of detoxification/excretion and activation mechanisms and the repair and apoptosis processes can present a high degree of inter-individual variations, determining different susceptibility to SHS exposure.

Carcinogen-derived biomarkers used to estimate the exposure are formed during the steps illustrated above. Therefore, their use even provides information about the carcinogenic process, from exposure to disease (Shields, 2002).

Biomarkers reviewed here concern different stages of this process: most them are carcinogens or their metabolites measured in urine specimens, or DNA or protein (hemoglobin) adducts measured in blood cells.

The first group includes biomarkers of exposure, used to quantify the internal dose, even if, as we said, their measurement is affected by individual variations given by metabolic features.

DNA adducts are typical biomarkers of effective dose, and provide an integrated measure of external exposure, metabolic activation, repair capacity and cell cycle control (Shields, 2002). A protein adduct, such as hemoglobin, and to a lesser extent albumin is used as a surrogate for DNA adducts because the two parameters tend to correlate. Moreover, it is easier to collect them in large quantities and they are not subject to enzymatic repair (Hecht, 2003).

Many studies used different biomarkers in order to relate the SHS exposure with potential carcinogenic damage. These include antioxidants and metabolites related to genetic harm.

The principal groups of carcinogen-derived biomarkers used to assess exposure to environmental tobacco smoke are presented hereafter. Some studies on active smoke have been considered in order to evaluate the feasibility of each biomarker.

4.2.1 Polycyclic aromatic hydrocarbons (PAH)

This group of compounds is formed during incomplete combustion of organic matter. PAHs are found in tobacco smoke, and their contribution to the carcinogenic activity of cigarette smoke has been clearly established

(IARC, 1986). Among this class, benzo[a]pyrene (BaP) is the most extensively studied compound, and its ability to induce lung tumours in laboratory animals is well documented (Culp *et al,* 1994; IARC, 1983; IARC, vol. 3, 1972).

Airborne PAHs result mainly from the combustion of fossil fuels, tobacco products and other organic materials. Generally, emissions from motor vehicles and residential heating are the major source of PAHs in urban air, whereas SHS is a major indoor source (Perera, Rauh *et al,* 2004). This class of compounds cannot provide specific biomarkers to evaluate the exposure to SHS, because other prominent sources, such as traffic or occupational exposure, have to be considered.

1-Hydroxypyrene and hydroxy-phenanthrene are urinary metabolites widely used as biomarkers of PAH uptake. Although the parent compounds, pyrene and phenanthrene respectively, are non-carcinogenic, they represent surrogates of total PAH exposure. Despite the positive results in studies to assess the exposure in active smokers (Hecht *et al,* 2004; Hecht *et al,* 2002) and some works that reported significantly higher values in people exposed to SHS (Tsai *et al,* 2003), levels of 1-hydroxypyrene and hydroxy-phenanthrene do not seem to be increased by exposure to SHS (Saieva *et al,* 2003; Scherer *et al,* 2000; Siwinska *et al,* 1999; Van Rooij *et al,* 1994; Hoepfner *et al,* 1987). Other factors, such as environmental and occupational exposure or diet, seem to be more significant contributors of the concentration of these compounds in urine.

Prior evaluations of the effects of SHS exposure using PAHs-DNA adducts are limited and most of them show weak correlation (Georgiadis *et al,* 2001) or no correlation (Perera, Rauh *et al,* 2004; Scherer *et al,* 1992).

Metabolites of BaP and other PAHs adducts with albumin or hemoglobin are used more frequently. Some studies show an increment in the levels of these markers (Tang *et al,* 1999; Crawford *et al,* 1994), while other authors

do not find any significant differences (Scherer *et al,* 2000; Nielsen *et al,* 1996; Autrup *et al,* 1995). It is noteworthy that several different methods were used to measure the blood samples and, in some cases, values are very close to the limit of detection: this fact could explain the different results.

Finally, it does not appear that measurements of urinary metabolites or adducts of PAHs may represent a valid instrument in SHS exposure assessment, because of their low specificity and insufficient detection limit.

4.2.2 Aromatic amines

Aromatic amines were first identified as carcinogens in studies on occupational exposure. They cause tumours at a variety of sites in laboratory animals, and 2-naphtylamine and 4-aminobiphenyl are well-established human bladder carcinogens (IARC, 1973; IARC, vol. 1, 1972).

Hemoglobin adduct of 4-aminobiphenyl (ABP-Hb) is frequently used as a biomarker in SHS exposure studies. Most of them show that ABP-Hb is significantly associated with SHS, with higher values present in children (Baier *et al,* 2002; Tang *et al,* 1999), pregnant women (Hammond *et al,* 1993) or exposed volunteers (Bartsch *et al,* 1990; Maclure *et al,* 1989). By contrast, other studies showed no significant relationship between SHS exposure and adduct level (Airoldi *et al,* 2005; Richter *et al,* 2001; Branner *et al,* 1998). On the other hand, it seems that other exposure sources, like diesel exhaust, could provide a background level of aromatic amines in SHS unexposed human. Therefore, ABP-Hb could represent only an aspecific exposure indicator in SHS exposure assessment.

4.2.3 N-nitrosamines

N-nitrosamines are a large group of carcinogens, which induce cancer in a wide variety of species and tissues (IARC, 1973; IARC, vol. 1, 1972). They

are present in low concentrations in food and could be formed endogenously from amines and nitrogen oxides (Hecht*,* 2003).

4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific nitrosamine, is a systemic lung carcinogen. NNK is the most prevalent systemic lung carcinogen in tobacco products (Hoffmann *et al,* 2001; Hecht*,* 1999) and the only one that induces tumours systemically in rats, mice and hamsters irrespective of the route of administration (Hecht*,* 1998). A prominent feature of this compound is that it can be found only in tobacco products, but not in the diet or as a rule in the environment unless tobacco smoke pollution is present: its adducts or metabolites should be used as absolutely specific biomarkers of tobacco exposure.

Hemoglobin adducts of NNK and N'-nitrosonornicotine (NNN) can be hydrolysed to release 4-hydroxy-1-(3-pyridyl)-1-butanone (HBP). Even in active smokers, urinary levels of HPB are lower compared to hemoglobin adducts of other carcinogen (Atawody *et al,* 1998; Hecht *et al,* 1994; Carmella *et al,* 1990). It is also unlikely to find positive results in SHS exposure studies, as already reported (Branner *et al,* 1998).

Urinary metabolites of NNK, 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide (NNAL-Gluc) are excellent biomarkers of human uptake of this carcinogen (Hecht*,* 1999; Hecht*,* 1998). Several studies establish a positive significant correlation between SHS exposure and NNAL and NNAL-Gluc levels, in school-aged children exposed by parents (Sexton *et al,* 2004; Hecht and Carmella*,* 2001), in women exposed by spouses (Anderson *et al,* 2001), in occupational environment (Anderson *et al,* 2003; Parsons *et al,* 1998), and in amniotic fluid of fœtuses exposed by mothers (Milunsky *et al,* 2000). Levels of NNK metabolites were often tested together with other markers used in exposure assessment of active smoke or SHS: positive correlations were established first with cotinine (Sexton *et al,* 2004; Anderson *et al,* 2003, 2001; Hecht*,* 2003), but also with urinary

nicotine (Anderson *et al,* 2001; Hecht*,* 1999) and anabitine (Murphy *et al,* 2004). Some studies assessed the decrease of NNAL and NNAL-Gluc levels with tobacco use cessation or reduction (Hecht *et al,* 2002; Hecht, 2003, 1999) reporting significantly lower levels of urinary metabolites, but for most smokers, reductions were modest and transient, and slower than cotinine decrease.

The decay of NNAL and NNAL-Gluc from urine is slow after smoking cessation (average half-life 3–4 days) compared with the rapid disappearance of total cotinine (Hecht*,* 1999). Therefore, the probability of detecting NNAL or NNAL-Gluc after a given exposure to tobacco smoke may be greater than that of detecting cotinine.

These results suggest that there is a receptor for NNAL in the human body, possibly in the lung, that affects the urinary release of this carcinogen. This fact could be important using NNK metabolites as exposure biomarkers.

The previous studies show relatively wide variations of NNAL and NNAL-Gluc in exposed people. These wide ranges may have important implications for interpretation of epidemiological studies of exposure to SHS and should be taken into account in the assessment of health risks.

4.2.4 Benzene

Benzene is a volatile hydrocarbon that occurs in considerable quantities in tobacco smoke, and its capability of causing leukaemia in humans is well established (IARC, 1982; Seniori Costantini *et al,* 2003).

Trans, trans-muconic acid (ttMA) was identified as a urinary metabolite of benzene at the beginning of this century, and recently its application as a biomarker for benzene exposure has been investigated. The range of metabolic conversion of benzene to ttMA is about 2-25% and is dependent on the benzene exposure level, simultaneous exposure to toluene, and probably also to genetic factors (Scherer *et al,* 1998). Even if some studies

found somewhat higher values in SHS exposure (Yu and Weisel, 1996; Carrer *et al,* 2000) or in definite sub-cohorts of exposed subjects (Weaver *et al,* 1996; Wallace *et al,* 1987), many authors reported non-significant results (Saieva *et al,* 2003; Scherer *et al,* 1999, 1995; Ruppert *et al,* 1997). Interpretation of these findings is complicated by the considerable differences in excretion rates (Yu and Weisel, 1996) and the amount of ttMA given by metabolism of sorbate from diet (Weaver *et al,* 2000; Scherer *et al,* 1998). This may indicate that, unlike the situation with smokers who receive a substantial benzene dose from mainstream smoke, SHS makes a smaller contribution to urinary ttMA in comparison with other exposure sources.

4.2.5 Others

Several other less specific markers have been examined in studies of SHS exposure.

A study found an inverse correlation between exposure to SHS from husbands who smoke and plasma levels of antioxidant vitamins (β-carotene, retinol, L-ascorbic acid and α-Tocopherol) among non-smoking women (Farchi *et al,* 2001).

Hemoglobin adducts of ethylene oxide (N-2-hydroxyethylvaline) have been used as a biomarker of exposure to SHS. There was no difference in levels of these adducts between non-smokers who did not live or work with a smoker compared to those who did (Wu *et al,* 2004; Bono *et al,* 1999).

Some authors looked for an association between passive smoke and markers of genetic damage. Unfortunately, these biomarkers show a very low specificity for SHS, as they can be generated by the exposure to any carcinogen: for this reason, it is very difficult to predict all the possible sources and the weight of SHS contribution.

8-Hydroxydeoxyguanosine (8-OH-dG) is widely used as biomarker of oxidative damage to DNA. Some studies show a significant difference for

SHS exposure (Hong *et al,* 2001; Howard *et al,* 1998), while others do not (Smith CJ *et al,* 2001; van Zeeland *et al,* 1999; Daube *et al,* 1997). One of these studies (Hong *et al,* 2001) also establishes a positive relationship between 8-OH-dG level in neonatal urine and maternal genotype, confirming the strong influence of genetic susceptibility in carcinogen excretion.

32P-postlabelling is a technique that can estimate levels of hydrophobic DNA adducts. Significant differences between SHS-exposed and non-exposed have been found only in specific sub-groups (Georgiadis *et al,* 2001) or in induced sputum, but not in blood (Besaratinia *et al,* 2002), while other studies did not discover any effects (Daube *et al,* 1997; Binkova *et al,* 1995; Holz *et al,* 1990).

Other biomarkers, like urinary 3-ethyladenine (Kopplin *et al,* 1995), metabolites of mercapturic acid (Scherer *et al,* 1992), thioethers (Scherer *et al,* 1996, 92), urinary mutagenicity (Smith *et al,* 2000, Scherer *et al,* 1992) or epigenetic mutations (Husgafvel-Pursiainen*,* 2004; Georgiadis *et al,* 2004) have been assessed, but they demonstrated only a weak correlation or no correlation with SHS exposure.

The evaluation of the genotoxicity of urine in non-smokers with SHS exposure must consider the possibility of confounding effects, because DNA modifiers may be present in urine as a consequence of dietary intake or as a secondary result of the activity of infectious agents in the urine of the host. Nevertheless, urinary constituents may be DNA modifiers, because the inhaled agents are known or suspected mutagens or because the inhaled agents lead to the formation of such biologically active compounds.

Since 1975, the most widely used test for genotoxicity of human urine is the determination of mutagenicity in bacterial-tested strains with and without activation by enzyme-induced liver homogenate. Many studies have reported an association of urinary mutagens that are active in bacterial tester strains with cigarette smoking (IARC, 1986), but not all results from

these studies have been consistent. One reason for the divergent findings could be the influence of dietary factors on the mutagens in the urine of smokers (Sasson *et al,* 1985) and, perhaps also, non-smokers exposed to SHS.

5. CONCLUSION

The properties of SHS markers, and the results of the studies they are used in, could be useful in choosing suitable methods in specific situations. As we have demonstrated above, every marker has different features, and the choice depends on multiple factors, as outcomes of interest, degree of precision required, time-span of exposure, size and characteristics of populations studied and economic resources.

An evaluation of the markers discussed in this paper, which provide useful results concerning SHS exposure, is summarized in Table 2.

The type of exposure assessment needed for public health studies aiming to describe the distribution of exposure in populations over time differs from that required for health effect studies focusing on specific relations between SHS exposure and different health outcomes. For the latter, exposure assessment also differs depending on whether the aim is qualitative testing for an association between exposure and a health outcome, or quantitative estimation of effects of given exposure levels. Extensive study samples with less precise exposure estimates are usually preferable for qualitative studies, whereas accuracy and precision of exposure estimates are more relevant in quantitative studies (Jaakkola and Jaakkola, 1997).

Questionnaires are always recommended in order to collect information about characteristics of the population sample that could affect exposure to SHS. Moreover, this is the only feasible method that allows tracing of past exposures. Questionnaires provide useful information in studies of health outcomes with a long latency period and for studies of rare diseases requiring large study populations. However, memory-based reports should be linked with more specific markers, usually biomarkers, in order to verify

the real exposure and to prevent misclassification. These questionnaires should be designed according to the characteristics of the marker used as validation, such as the time span that it covers and the specific setting interested by exposure.

Personal monitoring of environmental markers provides good information on cumulative exposure over relatively short periods. Moreover, it provides information about individual exposure, and personal characteristics can be taken into account. Despite these advantages, these methods require heavy expense of resources, as they are expensive and time-consuming. However, they are the best approach to assess personal exposure in studies of the short-term health effects with small study samples, especially if quantitative assessment of exposure-response relation is desired.

In recent years, several biochemical methods used to measure SHS exposure have been developed. Thus, the use of biomarkers, even in exposure assessment field studies, is on the increase. Nevertheless, a majority of these new tests shows large variability in individual exposure, and our knowledge about the complex mechanisms that link exposure to health effects, such as uptake, metabolism and genetic susceptibility, should be improved. Biomarkers can add new and interesting information, in spite of high costs and sampling difficulties. Their use, depending on the study's resources, is recommended in association with complementary markers that assure the validity and reliability of measurements.

Stationary monitoring of pollutant concentrations characterizes reasonably well exposure levels in different microenvironments over time, and is suitable for overall monitoring of the presence and amount of SHS in different indoor environments. Such an approach is often suitable for the purposes of risk assessment, development of preventive strategies, and follow-up of effectiveness of risk management measures, taking into account the relatively low cost and relative ease of sampling. When combined with

time-activity data, stationary monitoring can also be used to assess an individual's exposure in studies of the relatively short-term health effects.

As already stated, the majority of markers used in SHS exposure assessment derives from previous studies on active smoke. Consequently, their levels due to SHS exposure and the related health effects are dramatically lower. Therefore, the use of specific markers is strictly recommended. Many aspecific markers, like several carcinogenic biomarkers, should be employed only in combination with more specific methods, in order to define the link between the degree of exposure and specific health effects. It is important to make a special effort to enhance the correlation between the methods, like decreasing non-SHS sources and choosing suitable markers to be measured together.

In this respect, a combination of different assessment methods is often the best alternative, if the resources are available. It is important to choose a set of markers that provides an exhaustive scenario, covering every relevant aspect of the exposure to SHS: it should be possible to fill in the gap for the others, and their results should be comparable.

TABLES

Table 1. *Effects associated with exposure to environmental tobacco smoke*

EFFECTS CAUSALLY ASSOCIATED WITH SHS EXPOSURE

Developmental effects
Fœtal growth: Low birth weight and decrease in birth weight, and pre-term delivery
Sudden Infant Death Syndrome (SIDS)

Respiratory effects
Acute lower respiratory tract infections in children (*e.g.,* bronchitis and pneumonia)
Asthma induction and exacerbation in children and adults
Chronic respiratory symptoms in children
Eye and nasal irritation in adults
Middle ear infections in children

Carcinogenic effects
Lung cancer
Nasal sinus cancer
Breast cancer

Cardiovascular effects
Heart disease mortality
Acute and chronic coronary heart disease morbidity
Altered vascular properties

EFFECTS WITH SUGGESTIVE EVIDENCE OF A CAUSAL ASSOCIATION WITH SHS EXPOSURE

Reproductive and developmental effects
Spontaneous abortion, IUGR
Adverse impact on cognition and behaviour

Allergic sensitisation
Decreased pulmonary function growth
Adverse effects on fertility or fecundability
Menstrual cycle disorders

Cardiovascular and hematological effects
Elevated risk of stroke in adults

Respiratory effects
Exacerbation of cystic fibrosis
Chronic respiratory symptoms in adults

Source: Cal-EPA (California Environmental Protection Agency). Proposed Identification of Environmental Tobacco Smoke as a Toxic Air Contaminant. June 2005.

Table 2. *Summary of properties of SHS exposure markers*

	Sensitivity	Specificity to SHS	Duration after exposure reflected	Sampling and lab qualities	References
Questionnaires	+	+++	+++	++	+++
ENVIRONMENTAL MARKERS					
Nicotine in air	+++	+++	o	++	+++
3- ethenylpyridine	+++	+++	o	++	+
Myosmine	+	+++	o	+	o
VOCs	+	+	o	+	+
CO in air	++	o	o	+++	+
Scopoletin	+	+++	o	+	+
Solanesol	+	+++	o	+	++
PM	+++	o	o	+++	+++
BIOMARKERS					
Cotinine	++	+++	+	+	+++
Hair nicotine	++	+++	++	+	++
CO and CO-Hb	+	o	o	+	+
Thiocyanate	+	+	+	+	o
PAHs in urine	o	o	+	+	+
PAH adducts	+	o	++	o	+
ABP-Hb	+	o	++	o	+
NNAL	++	+++	+	+	++
tt-MA	+	o	+	+	+

Scores: "+++" excellent; "++" good; "+" poor; "o" none

"Sensitivity" refers to the ability to provide reliable measures of low SHS exposure and the same values at a constant level of exposure.

"Specificity to SHS" is self-explanatory.

"Duration after exposure reflected" refers to the time-span of exposure covered by the marker.

"Sampling and lab qualities" informs about features of laboratory techniques (sensibility, reproducibility), cost and ease of sampling.

"References" refers to knowledge about the method's properties, and its use in previous comparable studies.

REFERENCES

1. Airoldi L, Vineis P, Colombi A et al. 4-Aminobiphenyl-Hemoglobin adducts and risk of smoking-related disease in never smokers and former smokers in the European Prospective Investigation into Cancer and Nutrition Prospective Study. Cancer Epidemiol Biomarkers Prev. 2005;14(9):2118-24.
2. Akbar-Khanzadeh F, Greco TM. Health and social concerns of restaurant/bar workers exposed to environmental tobacco smoke. Med Lav. 1996;87(2):122-32.
3. Al-Delaimy WK. Hair as a biomarker for exposure to tobacco smoke. Tob Control. 2002;11(3):176-82.
4. Al-Delaimy WK, Crane J, Woodward A. Questionnaire and hair measurement of exposure to tobacco smoke. J Expo Anal Environ Epidemiol. 2000;10:378–84.
5. Al-Delaimy WK, Fraser T, Woodward A. Nicotine in hair of bar and restaurant staff. NZ Med J. 2001;114:80–3.
6. Al-Delaimy WK, Crane J, Woodward A. Is the hair nicotine level a more accurate biomarker of environmental tobacco smoke exposure than urine cotinine? J Epidemiol Community Health. 2002;56:66–71.
7. Anderson KE, Carmella SG, Ye M, et al. Metabolites of a tobacco-specific lung carcinogen in the urine of nonsmoking women exposed to environmental tobacco smoke in their homes. J Natl Cancer Inst. 2001;93:378–81.
8. Anderson KE, Kliris J, Murphy L, Carmella SG, Han S, Link C, Bliss RL, Puumala S, Murphy SE, Hecht SS. Metabolites of a tobacco-specific lung carcinogen in non-smoking casino patrons. Cancer Epidemiol Biomarkers Prev. 2003;12:1544–46.
9. Atawodi SE, Lea S, Nyberg F, Mukeria A, Constantinescu V, Ahrens W, Brueske Hohlfeld I, Fortes C, Boffetta P, Friesen MD. 4-Hydroxy-1-(3-pyridyl)-1-butanone hemoglobin adducts as biomarkers of exposure to tobacco smoke: validation of a method to be used in multicenter studies. Cancer Epidemiol Biomarkers Prev. 1998;7(9):817-21.
10. Autrup H, Beck Vestergaard A, Okkels H. Transplacental transfer of environmental genotoxins: polycyclic aromatic hydrocarbon-albumin in non-smoking women, and the effect of maternal GSTM1 genotype. Carcinogenesis. 1995;16:1305–9.

11. Baier G, Stopper H, Kopp C, Winkler U, Zwirner-Baier I. Respiratory diseases and genotoxicity in tobacco smoke exposed children. Laryngorhinootologie. 2002;81(3):217-25.

12. Bartsch H, Caporaso N, Coda M, Kadlubar F, Malaveille C, Skipper P, Talaska G, Tannenbaum SR, Vineis P. Carcinogen hemoglobin adducts, urinary mutagenicity, and metabolic phenotype in active and passive cigarette smokers. J Natl Cancer Inst. 1990;82(23):1826-31.

13. Benner CL, Bayona JM, Caka FM et al. Chemical composition of environmental tobacco smoke. 2. Particulate-phase compounds. Environ Sci Technol. 1989;23:688-99.

14. Benowitz NL. Biomarkers of environmental tobacco smoke exposure. Environ Health Perspect. 1999;107(Suppl 2):349–55.

15. Benowitz NL, Jacob P III. Nicotine and cotinine elimination pharmacokinetics in smokers and nonsmokers. Clin Pharmacol Ther. 1993;53:316-323.

16. Benowitz NL, Jacob P III. Metabolism of nicotine to cotinine studied by a dual stable isotope method. Clin Pharmacol Ther. 1994;56:483-493.

17. Benowitz NL. Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiol Rev. 1996;18:188-204.

18. Besaratinia A, Maas LM, Brouwer EM, et al. A molecular dosimetry approach to assess human exposure to environmental tobacco smoke in pubs. Carcinogenesis. 2002;23:1171–6.

19. Bi X, Sheng G, Feng Y, Fu J, Xie J. Gas- and particulate-phase specific tracer and toxic organic compounds in environmental tobacco smoke. Chemosphere. 2005; June 20 (published online ahead of print).

20. Binkova B, Lewtas J, Miskova I, et al. DNA adducts and personal air monitoring of carcinogenic polycyclic aromatic hydrocarbons in an environmentally exposed population. Carcinogenesis. 1995;16:1037–46.

21. Bono R, Vincenti M, Meineri V, et al. Formation of N-(2-hydroxyethyl)valine due to exposure to ethylene oxide via tobacco smoke: a risk factor for onset of cancer. Environ Res. 1999;81:62–71.

22. Branner B.; Kutzer C.; Wickenpflug W.; Scherer G.; Heller W-D.; Richter E. Haemoglobin adducts from aromatic amines and tobacco specific nitrosamines in pregnant smoking and non smoking women. Biomarkers. 1998;3(1):35-47.

23. Calafat AM, Polzin GM, Saylor J, Richter P, Ashley DL, Watson CH. Determination of tar, nicotine, and carbon monoxide yields in the mainstream smoke of selected international cigarettes. Tob Control. 2004;13(1):45-51.

24. Cal-EPA (California Environmental Protection Agency). Air Resources Board, Office of Environmental Health Hazard Assessment. Proposed Identification of Environmental Tobacco Smoke as a Toxic Air Contaminant. June, 2005. http://www.arb.ca.gov/toxics/ets/finalreport/finalreport.htm

25. Carmella SG, Kagan SS, Kagan M, et al. Mass spectrometric analysis of tobacco-specific nitrosamine hemoglobin adducts in snuff dippers, smokers, and non-smokers. Cancer Res. 1990;50:5438–45.

26. Carrer P, Maroni M, Alcini D, et al. Assessment through environmental and biological measurements of total daily exposure to volatile organic compounds of office workers in Milan, Italy. Indoor Air. 2000;10:258–68.

27. Chen R, Tavendale R, Tunstall-Pedoe H. Measurement of passive smoking in adults: self-reported questionnaire or serum cotinine? J Cancer Epidemiol Prev. 2002;7(2):85-95.

28. Chetiyanukornkul T, Toriba A, Kizu R, Kimura K, Hayakawa K. Hair analysis of nicotine and cotinine for evaluating tobacco smoke exposure by liquid chromatography-mass spectrometry. Biomed Chromatogr. 2004;18(9):655-61.

29. Coghlin J, Hammond SK, Gann PH. Development of epidemiologic tools for measuring environmental tobacco smoke exposure. Am J Epidemiol 1989;130: 696–704.

30. Coultas DB, Samet JM, McCarthy JF, Spengler JD. Variability of measures of exposure to environmental tobacco smoke in the home. Am Rev Respir Dis. 1990;142(3):602-6.

31. Crawford FG, Mayer J, Santella RM, et al. Biomarkers of environmental tobacco smoke in preschool children and their mothers. J Natl Cancer Inst. 1994;86:1398–402.

32. Culp SJ, Gaylor DW, Sheldon WG, et al. A comparison of the tumors induced by coal tar and benzo[a]pyrene in a two-year bioassay. Carcinogenesis. 1998;19:117–24.

33. Daisey J.M. Tracers for assessing exposure to environmental tobacco smoke: what are they tracing? Environ Health Perspectives. 1999;107(suppl. 2):319-327.

34. Daube H, Scherer G, Riedel K, et al. DNA adducts in human placenta in relation to tobacco smoke exposure and plasma antioxidant status. J Cancer Res Clin Oncol. 1997;123:141–51.

35. Davis RA, Stiles MF, DeBethizy JD, Reynolds JH. Dietary nicotine: a source of urinary cotinine. Food Chem Toxicol. 1991; 29: 821–827.

36. De Bruin YB, Carrer P, Jantunen M, Hanninen O, Di Marco GS, Kephalopoulos S, Cavallo D, Maroni M. Personal carbon monoxide exposure levels: contribution of local sources to exposures and

microenvironment concentrations in Milan. J Expo Anal Environ Epidemiol. 2004;14(4):312-22.

37. Delfino RJ, Ernst P, Jaakkola MS, Solomon S, Becklake MR. Questionnaire assessments of recent exposure to environmental tobacco smoke in relation to salivary cotinine. Eur Respir J. 1993;6(8):1104-8.

38. Dimich-Ward H, Gee H, Brauer M, et al. Analysis of nicotine and cotinine in the hair of hospitality workers exposed to environmental tobacco smoke. J Occup Environ Med. 1997;39:946–8.

39. Dolcini MM, Adler NE, Lee P, Bauman KE. An assessment of the validity of adolescent self-reported smoking using three biological indicators. Nicotine Tob Res. 2003;5(4):473-83.

40. Domino EF, Hornbach E, Demana T. The nicotine content of common vegetables. N Engl J Med. 1993;329:437.

41. Douce DS, Clench MR, Frost B. Variations in the estimation of the contribution of environmental tobacco smoke (ETS) to respirable (< or = 5 microns) indoor air particulates obtained by the use of different analytical methods. J Environ Monit. 2001;3(3):295-301.

42. Eisner MD, Katz PP, Yelin EH, Hammond SK, Blanc PD. Measurement of environmental tobacco smoke exposure among adults with asthma. Environ Health Perspect. 2001;109(8):809-14.

43. Eliopoulos C, Klein J, Phan MK, et al. Hair concentrations of nicotine and cotinine in women and their newborn infants. JAMA. 1994;271:621–3.

44. Emmons KM, Abrams DB, Marshall R, Marcus BH, Kane M, Novotny TE, Etzel RA. An evaluation of the relationship between self-report and biochemical measures of environmental tobacco smoke exposure. Prev Med. 1994;23(1):35-9.

45. Emmons KM, Marcus BH, Abrams DB, Marshall R, Novotny TE, Kane ME, Etzel RA. Use of a 24-hour recall diary to assess exposure to environmental tobacco smoke. Arch Environ Health. 1996;51(2):146-9.

46. Eskenazi B, Prehn AW, Christianson RE.. Passive and active maternal smoking as measured by serum cotinine: the effect on birthweight. Am J Public Health. 1995;85:395–398.

47. Farchi S, Forastiere F, Pistelli R, et al. Exposure to environmental tobacco smoke is associated with lower plasma beta-carotene levels among nonsmoking women married to a smoker. Cancer Epidemiol Biomarkers Prev. 2001;10:907–9.

48. Fiore MC, Novotny TE, Pierce JP, Hatziandreu EJ, Patel KM, Davis RM. Trends in cigarette smoking in the United States: The changing influence of gender and race. JAMA. 1989;261:49-55.

49. Gee IL, Watson AFR, Carrington J. The Contribution of Environmental Tobacco Smoke to Indoor Pollution in Pubs and Bars. Indoor and Built Environment. 2005;14:301-306.

50. Gehlbach SH, Williams WA, Perry LD, Freeman JH, Langone JJ, Peta LV, Van Vunakis H. Nicotine absorption by workers harvesting green tobacco. Lancet. 1975;1:478-480.

51. Georgiadis P, Stoikidou M, Topinka J, Kaila S, Gioka M, Katsouyanni K, Sram R, Kyrtopoulos SA. Personal exposures to PM(2.5) and polycyclic aromatic hydrocarbons and their relationship to environmental tobacco smoke at two locations in Greece. J Expo Anal Environ Epidemiol. 2001;11(3):169-83.

52. Georgiadis P, Topinka J, Stoikidou M, et al. Biomarkers of genotoxicity of air pollution (the AULIS project): bulky DNA adducts in subjects with moderate to low exposures to airborne polycyclic aromatic hydrocarbons and their relationship to environmental tobacco smoke and other parameters. Carcinogenesis. 2001;22:1447–57.

53. Georgiadis P, Demopoulos NA, Topinka J, et al. Impact of phase I or phase II enzyme polymorphisms on lymphocyte DNA adducts in subjects exposed to urban air pollution and environmental tobacco smoke. Toxicol Lett. 2004;149(1-3):269-80.

54. Grimmer G, Dettbarn G, Seidel A, Jacob J. Detection of carcinogenic aromatic amines in the urine of non-smokers. Sci Total Environ. 2000;247(1):81-90.

55. Guerin MR, Jenkins RA, Tomkins BA. The Chemistry of Environmental Tobacco Smoke: Composition and Measurement. Lewis Publishers, Boca Raton, 1992.

56. Haley NJ, Axelrad CM, Tilton KA. Validation of self-reported smoking behavior: Biochemical analyses of cotinine and thiocyanate. Am J Public Health. 1983;73:1204-1207.

57. Haley NJ, Colosimo SG, Axelrad CM, Harris R, Sepkovic DW. Biochemical validation of self-reported exposure to environmental tobacco smoke. Environ Res. 1989;49(1):127-35.

58. Hammond S.K. Exposure of U.S. Workers to environmental tobacco smoke. Environ. Health Perspectives. 1999;107(suppl. 2):329-340.

59. Hammond SK, Leaderer BP, Roche AC, Schenker M. Collection and analysis of nicotine as a marker for environmental tobacco smoke. Atmos Environ. 1987; 21: 457–462.

60. Hammond SK, Coghlin J, Gann PH, Paul M, Taghizadeh K, Skipper PL, Tannenbaum SR. Relationship between environmental tobacco smoke exposure and carcinogen-hemoglobin adduct levels in nonsmokers. J Natl Cancer Inst. 1993;85(6):474-8.

61. Hammond SK, Sorensens G, Youngstrom R, et al. Occupational exposure to environmental tobacco smoke. JAMA. 1995;274:956–60.

62. Hauth JC, Hauth J, Drawbaugh RB. Passive smoking and thiocyanate concentrations in pregnant women and newborns. Obstetr Gynecol. 1984;63:519-522.

63. Hecht SS. Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. Chem Res Toxicol. 1998;11:559–603.

64. Hecht SS. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst. 1999;91:1194–210.

65. Hecht ss. Carcinogen derived biomarkers: applications in studies of human exposure to secondhand tobacco smoke. Tobacco Control 2003;13(suppl):48-56.

66. Hecht SS, Ye M, Carmella SG, et al. Metabolites of a tobacco-specific lung carcinogen in the urine of elementary school-aged children. Cancer Epidemiol Biomarkers Prev. 2001;10:1109–16.

67. Hecht SS, Carmella SG, Murphy SE. Tobacco-specific nitrosamine hemoglobin adducts. Methods Enzymol. 1994;231:657–67.

68. Hecht SS, Carmella SG, Ye M, Le K, Jensen JA, Zimmerman CL, Hatsukami DK. Quantitation of metabolites of 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone after cessation of smokeless tobacco use. Cancer Res. 2002;62:129–134.

69. Hecht SS, Carmella SG, Le KA, Murphy SE, Li YS, Le C, Jensen J, Hatsukami DK. Effects of reduced cigarette smoking on levels of 1-hydroxypyrene in urine. Cancer Epidemiol Biomarkers Prev. 2004;13(5):834-42.

70. Henderson FW, Reid HF, Morris R, Wang OL, Hu PC, Helms RW, Forehand L, Mumford J, Lewtas J, Haley NJ, et al. Home air nicotine levels and urinary cotinine excretion in preschool children. Am Rev Respir Dis. 1989;140(1):197-201.

71. Heudorf U, Angerer J. Urinary monohydroxylated phenanthrenesand hydroxypyrene – the effects of smoking habits and changes induced by smoking on monooxygenase-mediated metabolism. Int Arch Occup Environ Health. 2001;74:177-183.

72. Hodgson AT, Daisey JM, Mahanama KR, Brinke JT, Alevantis LE. Use of volatile traces to determine the contribution of environmental tobacco smoke to concentrations of volatile organic compounds in smoking environments. Environmental International. 1996;22(3):295-307.

73. Hoepfner I, Dettbarn G, Scherer G, et al. Hydroxy-phenanthrenes in the urine of nonsmokers and smokers. Toxicol Lett. 1987;35:67–71.

74. Hoffmann D, Hoffmann I, El Bayoumy K. The less harmful cigarette: a controversial issue. A tribute to Ernst L. Wynder. Chem Res Toxicol. 2001;14:767–90.

75. Holz O, Krause T, Scherer G, et al. 32P-postlabelling analysis of DANN adducts in monocytes of smokers and passive smokers. Int Arch Occup Environ Health. 1990;62:299–303.

76. Hong YC, Kim H, Im MW, Lee KH, Woo BH, Christiani DC. Maternal Genetic Effects on Neonatal Susceptibility to Oxidative Damage From Environmental Tobacco Smoke. J Natl Cancer Inst. 2001;93:545-47.

77. Howard DJ, Ota RB, Briggs LA, Hampton M, Pritsos CA. Environmental tobacco smoke in the workplace induces oxidative stress in employees, including increased production of 8-hydroxy-2'-deoxyguanosine. Cancer Epidemiol Biomarkers Prev. 1998;7(2):141-6.

78. Husgafvel-Pursiainen K. Genotoxicity of environmental tobacco smoke: a review. Mutat Res. 2004 Nov;567(2-3):427-45.

79. Husgafvel-Pursiainen K. Biomarkers in the assessment of exposure and the biological effects of environmental tobacco smoke. Scand J Work Environ Health. 2002;28(suppl. 2):21-29.

80. Hyvarinen MJ, Rothberg M, Kahkonen E, Mielo T, Reijula K. Nicotine and 3-ethenylpyridine concentrations as markers for environmental tobacco smoke in restaurants. Indoor Air. 2000;10(2):121-5.

81. IARC (International Agency for Research on Cancer). Some inorganic substances, chlorinated hydrocarbons, aromatic amines, N-nitroso compounds, and natural products IARC Monographs on the evaluation of the carcinogenic risks of chemicals to human, vol. 1. Lyon, France: IARC, 1972.

82. IARC (International Agency for Research on Cancer). Certain polycyclic aromatic hydrocarbons and heterocyclic compounds. IARC Monographs on the evaluation of the carcinogenic risks of chemicals to man, vol. 3. Lyon, France: IARC, 1972:45–268.

83. IARC (International Agency for Research on Cancer). Some aromatic amines, hydrazine and related substances, N-nitroso compounds and miscellaneous alkylating agents. IARC Monographs on the evaluation of the carcinogenic risks of chemicals to man, vol. 4. Lyon, France: IARC, 1973:127–36.

84. IARC (International Agency for Research on Cancer). Some Industrial Chemicals and Dyestuffs. IARC Monographs on the evaluation of the carcinogenic risks of chemicals to human, vol 29. Lyon, France: IARC, 1982.

85. IARC (International Agency for Research on Cancer). Polynuclear aromatic compounds, part 1. Chemical, environmental, and experimental data. IARC Monographs on the evaluation of the

carcinogenic risks of chemicals to humans, vol. 32. Lyon, France: IARC, 1983:33–91.

86. IARC (International Agency for Research on Cancer). Tobacco smoking. IARC Monographs on the evaluation of the carcinogenic risks of chemicals to human, vol. 38. Lyon, France: IARC, 1986.

87. IARC (International Agency for Research on Cancer). Tobacco Smoke and Involuntary Smoking. IARC Monographs on the Evaluation of Carcinogenic Risks to Human, vol. 83. Lyon, France: IARC, 2002.

88. Invernizzi G, Ruprecht A, Mazza R, Majno E, Rossetti E, Paredi P, Boffi R. Real-time measurement of indoor particulate matter originating from environmental tobacco smoke: a pilot study. Epidemiol Prev. 2002;26(1):30-4.

89. Invernizzi G, Ruprecht A, Mazza R, Rossetti E, Sasco A, Nardini S, Boffi R. Particulate matter from tobacco versus diesel car exhaust: an educational perspective. Tobacco Control. 2004;13:305-307.

90. IOM (Institute of Medicine), Committee to Assess the Science Base for Tobacco Harm Reduction, Board on Health Promotion and Disease Prevention. Clearing the smoke: assessing the science base for tobacco harm reduction. Washington (DC): National Academy Press; 2001.

91. Jaakkola MS, Jaakkola JJK. Assessment of exposure to environmental tobacco smoke. Eur Respir J. 1997;10:2384–2397.

92. Jaakkola JJK, Jaakkola N, Zahlsen K. Fetal Growth and Length of Gestation in Relation to prenatal exposure to environmental tobacco smoke assessed by hair nicotine concentration. Environ Health Perspect. 2001;109:557–561.

93. Jarvis MJ. ...unless subjects eat 90 kg tomatoes a day. Br Med J. 1994;308:62.

94. Jarvis MJ, Russell MAH, Feyerabend C. Absorption of nicotine and carbon monoxide from passive smoking under natural conditions of exposure. Thorax. 1983;38:829-833.

95. Jarvis MJ, Russell MAH. Measurement and estimation of smoke dosage to non-smokers from environmental tobacco smoke. Eur J Respir Dis. 1984;133(Suppl):68-75.

96. Jarvis M, Tunstall-Pedoe H, Feyerabend C, Vesey C, Salloojee Y. Comparison of tests used to distinguish smokers from nonsmokers. Am J Public Health. 1987;77:1435-1438.

97. Jarvis M, Russell MAH, Benowitz NL, Feyerabend C. Elimination of cotinine from body fluids: implications for noninvasive measurements of tobacco smoke exposure. Am J Public Health. 1988;78:696-698.

98. Jenkins RA, Palausky A, Counts RW, Bayne C.K, Dindal AB, Guerin MR. Exposure to environmental tobacco smoke in sixteen cities in the

United States as determined by personal breathing zone air sampling. J Expo Anal Environ Epidemiol. 1996; 6(4):473- 501.

99. Jenkins RA, Counts RW. Personal exposure to environmental tobacco smoke: salivary cotinine, airborne nicotine, and nonsmoker misclassification. J Expo Anal Environ Epidemiol. 1999;9(4):352-63.

100. Jenkins RA, Guerin MR, Tomkins BA. The Chemistry of Environmental Tobacco Smoke: Composition and Measurement. 2nd edition. Lewis Publishers, Boca Rotan, 2000.

101. Jenkins RA, Maskarinec MP, Counts RW, Caton JE, Tomkins BA, Ilgner RH. Environmental tobacco smoke in an unrestricted smoking workplace: area and personal exposure monitoring. J Expo Anal Environ Epidemiol. 2001;11(5):369-80.

102. Jo WK, Oh JW, Dong JI. Evaluation of exposure to carbon monoxide associated with passive smoking. Environ Res. 2004;94(3):309-18.

103. Kemmeren JM, van Poppel G, Verhoef P, Jarvis MJ. Plasma cotinine: stability in smokers and validation of self-reported smoke exposure in nonsmokers. Environ Res. 1994;66(2):235-43.

104. Kim YM, Harrad S, Harrison RM. Concentrations and sources of VOCs in urban domestic and public microenvironments. Environ Sci Technol. 2001;35(6):997-1004.

105. Klein J, Blanchette P, Koren G. Assessing nicotine metabolism in pregnancy--a novel approach using hair analysis. Forensic Sci Int. 2004;145(2-3):191-4.

106. Klepeis N.E. An introduction to the indirect exposure assessment approach: modeling human exposure using microenvironmental measurements and the recent National Human Activity Pattern Survey. Environ Health Perspect. 1999;107(suppl. 2):365-374.

107. Klepeis NE, Ott WR, Repace JL. The effect of cigar smoking on indoor levels of carbon monoxide and particles. J Expo Anal Environ Epidemiol. 1999;9(6):622-35.

108. Kopplin A, Eberle-Adamkiewicz G, Glu¨senkamp KH, et al. Urinary excretion of 3-methyladenine and 3-ethyladenine after controlled exposure to tobacco smoke. Carcinogenesis. 1995;16:2637–41. 1995;29:2311–6.

109. Kriek E, Rojas M, Alexandrov K, Bartsch H. Polycyclic aromatic hydrocarbon-DNA adducts in humans: relevance as biomarkers for exposure and cancer risk. Mutat Res. 1998;400(1-2):215-31.

110. LaKind JS, Ginevan ME, Naiman DQ, James AC, Jenkins RA, Dourson ML, Felter SP, Graves CG, Tardiff RG. Distribution of exposure concentrations and doses for constituents of environmental tobacco smoke. Risk Anal. 1999;19(3):375-90.

111. LaKind JS, Jenkins RA, Naiman DQ, Ginevan ME, Graves CG, Tardiff RG. Use of environmental tobacco smoke constituents as markers for exposure. Risk Anal. 1999;19:359-373.

112. Leaderer BP, Hammond SK. Evaluation of vapour-phase nicotine and respirable suspended particle mass as markers for environmental tobacco smoke. Environ Sci Technol 1991;25:770–777.

113. Li Y, Cheng L. Analysis of nicotine in hair as a biological marker for its exposure. Proceedings of Indoor Air '93. 1993;1:687–92.

114. Liu KS, Alevantis LE, Offermann FJ. A survey of environmental tobacco smoke controls in California office buildings. Indoor Air. 2001;11(1):26-34.

115. Maclure M, Katz RB, Bryant MS, Skipper PL, Tannenbaum SR. Elevated blood levels of carcinogens in passive smokers. Am J Public Health. 1989;79(10):1381-4.

116. Marbury MC, Hammond SK, Haley NJ. Measuring exposure to environmental tobacco smoke in studies of acute health effects. Am J Epidemiol. 1993;137(10):1089-97.

117. Matt GE, Wahlgren DR, Hovell MF et al. Measuring environmental tobacco smoke exposure in infants and young children through urine cotinine and memory-based parental reports: empirical findings and discussion. Tob Control. 1999;8(3):282-9.

118. Matt GE, Quintana PJ, Hovell MF, Bernert JT, Song S, Novianti N, Juarez T, Floro J, Gehrman C, Garcia M, Larson S. Households contaminated by environmental tobacco smoke: sources of infant exposures. Tob Control. 2004;13(1):29-37.

119. McLaughlin JK, Dietz MS, Mehl ES, Blot WJ. Reliability of surrogate information on cigarette smoking by type of informant. Am J Epidemiol. 1987;126:144-146.

120. Mizuno A, Uematsu T, Oshima A, et al. Analysis of nicotine content of hair for assessing individual cigarette-smoking behavior. Ther Drug Monit. 1993;15:99–104.

121. Murphy SE, Link CA, Jensen J, Le C, Puumala SS, Hecht SS, Carmella SG, Losey L, Hatsukami DK. A comparison of urinary biomarkers of tobacco and carcinogen exposure in smokers. Cancer Epidemiol Biomarkers Prev. 2004;13(10):1617-23.

122. Nafstad P, Botten G, Hagen JA, et al. Comparison of three methods for estimating environmental tobacco smoke exposure among children aged between 12 and 36 months. Int J Epidemiol. 1995;24:88–94.

123. Nafstad P, Jaakkola JJK, Hagen JA, et al. Hair nicotine concentrations in mothers and children in relation to parental smoking. J Expo Anal Environ Epidemiol. 1997;7:235–9.

124. Nafstad P, Fugelseth DL, Qvigstad E, et al. Nicotine concentration in the hair of nonsmoking mothers and size of offspring. Am J Public Health. 1998;88:120–4.

125. NCI (National Cancer Institutes). Health effects of exposure to environmental tobacco smoke, Report of the California Environmental Protection Agency. Monograph 10, 1999.

126. Nebot M., Lopez MJ., Gorini G. et al. Environmental tobacco smoke exposure in public places of European cities. Tob Control. 2005;14(1):60-3.

127. Nielsen PS, Okkels H, Sigsgaard T, et al. Exposure to urban and rural air pollution: DNA and protein adducts and effect of glutathione-S-transferase genotype on adduct levels. Int Arch Occup Environ Health. 1996;68:170–6.

128. Nilsen T, Nilsen OG. Accumulation of nicotine in human hair during long-term controlled exposure to a low concentration of nicotine vapour. Pharmacol Toxicol. 1997;81:48–52.

129. NRC (National Reasearch Council). Environmetal tobacco smoke: measuring exposures and assessing health effects. Washington (DC): National Academy Press, 1986.

130. O'Connor TZ, Holford TR, Leaderer BP, Hammond SK, Bracken MB. Measurement of exposure to environmental tobacco smoke in pregnant women. Am J Epidemiol. 1995;142:1315–1321.

131. Ogden, M. W., Maiolo, K. C. Collection and determination of solanesol as a tracer of environmental tobacco smoke in indoor air. Environ Sci Technol. 1989;23:1148-54.

132. Ohlin P, Lundh B, Westling H. Carbon monoxide blood levels and reported cessation of sampling. Psychopharmacology. 1976;49:263-265.

133. Özkaynak H, Xue J, Spengler J, Wallace L, Pellizzari E, Jenkins P. Personal exposure to airborne particles and metals: results from the Particle TEAM study in Riverside, California. J Expo Anal Environ Epidemiol. 1996;6:57-78.

134. Parsons WD, Carmella SG, Akerkar S, et al. A metabolite of the tobacco specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in the urine of hospital workers exposed to environmental tobacco smoke. Cancer Epidemiol Biomarkers Prev. 1998;7:257–60.

135. Perera FP. Molecular cancer epidemiology: a new tool in cancer prevention. J Natl Cancer Inst. 1987;78:887–98.

136. Perera FP. Environment and cancer: who are susceptible? Science. 1997;278:1068-73.

137. Perera FP, Mooney LA, Stampfer M et al. Associations between carcinogen–DNA damage, glutathione S-transferase genotypes, and risk of lung cancer in the prospective Physicians' Health Cohort Study. Carcinogenesis. 2002;23:1641-46.

138. Perera FP, Rauh V, Whyatt RM et al. Molecular evidence of an interaction between prenatal environmental exposures and birth outcomes in a multiethnic population. Environ Health Perspect. 2004;112:626–630.

139. Perera FP, Tang D, Tu YH et al. Biomarkers in maternal and newborn blood indicate heightened fetal susceptibility to procarcinogenic DNA damage. Environ Health Perspect. 2004;112:1133–1136.

140. Phillips K, Bentley MC, Howard DA, Alvan G. Assessment of air quality in Stockholm by personal monitoring of nonsmokers for respirable suspended particles and environmental tobacco smoke. Scand J Work Environ Health. 1996;22(Suppl 1):1-24.

141. Phillips K, Bentley MC, Howard DA, Alvan G. Assessment of environmental tobacco smoke and respirable suspended particle exposures for nonsmokers in Prague using personal monitoring. Int Arch Occup Environ Health. 1998;71(6):379-90.

142. Pichini S, Altieri I, Pellegrini M, et al. The analysis of nicotine in infants' hair for measuring exposure to environmental tobacco smoke. Forensic Sci Int. 1997;84:253–8.

143. Pichini S, Altieri I, Pellegrini M, Pacifici R, Zuccaro P. Hair analysis for nicotine and cotinine: evaluation of extraction procedures, hair treatments, and development of reference material. Forensic Sci Int. 1997;84(1-3):243-52.

144. Pirkle JL, Flegal KM, Bernert JT, Brody DJ, Etzel RA, Maurer KR. Exposure of the US Population to Environmental Tobacco Smoke. The Third National Health and Nutrition Examination Survey, 1988 to 1991. JAMA. 1996;275:1233-1240.

145. Post A, Gilljam H, Rosendahl I, Meurling L, Bremberg S, Galanti MR. Validity of self reports in a cohort of Swedish adolescent smokers and smokeless tobacco (snus) users. Tob Control. 2005;14(2):114-7.

146. Pron GE, Burch JD, Howe GR, Miller AB. The reliability of passive smoking histories reported in a case-control study of lung cancer. Am J Epidemiol. 1988;127:267-273.

147. Rando RJ, Menon PK, Poovey HG, Lehrer SB. Assessment of multiple markers of environmental tobacco smoke (ETS) in controlled, steady-state atmospheres in a dynamic test chamber. Am Ind Hyg Assoc J. 1992;53:699-704.

148. Repace JL. Dietary nicotine won't mislead on passive smoking... Br Med J. 1994;308:61-62.

149. Repace JL, Lowrey AH. Indoor Air Pollution, Tobacco Smoke, and Public Health. Science. 1980;208: 464-474.

150. Repace JL, Lowrey AH. An enforceable indoor air quality standard for environmental tobacco smoke in the workplace. Risk Anal. 1993;13:463–75.

151. Richter E, Rosler S, Scherer G, Gostomzyk JG, Grubl A, Kramer U, Behrendt H. Haemoglobin adducts from aromatic amines in children in relation to area of residence and exposure to environmental tobacco smoke. Int Arch Occup Environ Health. 2001;74(6):421-8.

152. Risner, CH. The determination of scopoletin in environmental tobacco smoke by high-performance liquid chromatography. J Liq Chromatogr. 1994;17:2723-2736.

153. Robinson JC, Forbes WF. The role of carbon monoxide in cigarette smoking. I. Carbon monoxide yield from cigarettes. Arch Environ Health. 1975;30(9):425-34.

154. Ruppert T, Scherer G, Tricker AR, et al. trans,trans-muconic acid as a biomarker of non-occupational environmental exposure to benzene. Int Arch Occup Environ Health. 1997;69:247–51.

155. Rylander E, Pershagen G, Eriksson M, Bermann G. Parental smoking, urinary cotinine, and wheezing bronchitis in children. Epidemiology. 1995;6(3):289-93.

156. Saieva C, Tumino R, Masala G, Frasca G, Salvini S, Giurdanella MC, Ceroti M, Perico A, Zanna I, Cordopatri G, Bavazzano P, Palli D. Urinary 1-hydroxypyrene and t,t-muconic acid as biomarkers of exposure to environmental pollutants in two areas in Italy (EPIC-Florence and Ragusa). Tumori. 2003;89(6):679-86.

157. Sandler DP, Shore DL. Quality of data on parents' smoking and drinking provided by adult offspring. Am J Epidemiol. 1986;124:768-778.

158. Sasson IM, Coleman DT, LaVoie EJ, Hoffmann ED, Wynder EL. Mutagens in human urine: Effects of cigarette smoking and diet. Mutat Res. 1985;158:149–157.

159. Scherer G, Conze C, von Meyerinck L, Sorsa M, Adlkofer F. Importance of exposure to gaseous and particulate phase components of tobacco smoke in active and passive smokers. Int Arch Occup Environ Health. 1990;62(6):459-66.

160. Scherer G, Conze C, Tricker AR, et al. Uptake of tobacco smoke constituents on exposure to environmental tobacco smoke (ETS). Clin Invest. 1992;70:352–67.

161. Scherer G, Ruppert T, Daube H, et al. Contribution of tobacco smoke to environmental benzene exposure in Germany. Environ Int. 1995;21:779–89.

162. Scherer G, Renner T, Meger M. Analysis and evaluation of trans,transmuconic acid as a biomarker for benzene exposure. J Chromatogr B Biomed Sci Appl. 1998;717:179–99.

163. Scherer G, Meger-Kossien I, Riedel K, et al. Assessment of the exposure of children to environmental tobacco smoke (ETS) by different methods. Hum Exp Toxicol. 1999;18:297–301.

164. Scherer G, Frank S, Riedel K, et al. Biomonitoring of exposure to polycyclic aromatic hydrocarbons of nonoccupationally exposed persons. Cancer Epidemiol Biomarkers Prev. 2000;9:373–80.

165. Seniori Costantini A, Quinn M, Consonni D, Zappa M. Exposure to benzene and risk of leukemia among shoe factory workers. Scand J Work Environ Health. 2003;29(1):51-9.

166. Seppanen A. Smoking in closed space and its effect on carboxyhaemoglobin saturation of smoking and nonsmoking subjects. Ann Clin Res. 1977;9(5):281-3

167. Severson RF, Ellington JJ, Schlotzhauer PF, Arrendale RF, Schepartz AI.Gas chromatographic method for the determination of free and total solanesol in tobacco. J Chromatogr. 1977;139(2):269-82.

168. Sexton K, Adgate JL, Church TR, Hecht SS, Ramachandran G, Greaves IA, Fredrickson AL, Ryan AD, Carmella SG, Geisser MS. Children's exposure to environmental tobacco smoke: using diverse exposure metrics to document ethnic/racial differences. Environ Health Perspect. 2004;112:392–397.

169. Sheen SJ. Detection of nicotine in foods and plant materials. J Food Sci. 1988;53:1572–1573.

170. Shields PJ. Tobacco smoking, harm reduction and biomarkers. J Natl Cancer Inst. 2002;94:1435–44.

171. Siegel M. Involuntary smoking in the restaurant workplace. A review of employee exposure and health effects. JAMA. 1993;270(4):490-3.

172. Sillett RW, Wilson MB, Malcolm, RE, Ball KP. Deception among smokers. Br Med J. 1978;2:1185-1186.

173. Singer B, Hodgson A, Nazaroff W. Gas-phase organics in environmental tobacco: 2. Exposure-relevant emission factors and indirect exposures from habitual smoking. Atmospheric Environment. 2003;37:5551-61.

174. Singer BC, Hodgson AT, Guevarra KS, Hawley EL, Nazaroff WW. Gas-phase organics in environmental tobacco smoke. 1. Effects of smoking rate, ventilation, and furnishing level on emission factors. Environ Sci Technol. 2002;36(5):846-53.

175. Siwinska E, Mielzynska D, Bubak A, et al. The effect of coal stoves and environmental tobacco smoke on the level of urinary 1-hydroxypyrene. Mutat Res. 1999;445:147–53.

176. Smith CJ, Bombick DW, Ryan BA, et al. Urinary mutagenicity in nonsmokers following exposure to fresh diluted sidestream cigarette smoke. Mutat Res. 2000;470:53–70.

177. Smith CJ, Fisher TH, Heavner DL, Rumple MA, Bowman DL, Brown BG, Morton MJ, Doolittle DJ. Urinary thromboxane, prostacyclin, cortisol, and 8-Hidroxy-2'-deoxyguanosine in nonsmokers exposed and not exposed to environmental tobacco smoke. Toxicol sci. 2001;59:316-23.

178. Smith GB, Harper PA, Wong JM et al. Human lung microsomial cytochrome P4501 A1 (CYP1A1) activities: impact of smoking status and CYP1A1, arylhydrocarbon receptor and glutathione-S-transferaseM1 genetic polymorphism. Cancer Epidemiol Biomarkers Prev. 2001;10:839-853.

179. Spengler JD, Treitman RD, Tosteson TD, Mage DT, Soczek ML. Personal exposures to respirable particulates and implications for air pollution epidemiology. Environ Sci Technol 1985;19:700–707.

180. Strachan DP, Jarvis MJ, Feyerabend C. Passive smoking, salivary cotinine concentrations, and middle ear effusion in 7 year old children. BMJ. 1989;298(6687):1549-52.

181. Tang D, Warburton D, Tannenbaum SR, et al. Molecular and genetic damage from environmental tobacco smoke in young children. Cancer Epidemiol Biomarkers Prev. 1999;8:427–31.

182. Tomaszewski C. Carbon monoxide poisoning. Early awareness and intervention can save lives. Postgraduate Med. 1999; 105:39-50.

183. Tsai HT, Wu MT, Hauser R, Rodrigues E, Ho CK, Liu CL, Christiani DC. Exposure to environmental tobacco smoke and urinary 1-hydroxypyrene levels in preschool children. Kaohsiung J Med Sci. 2003;19(3):97-104.

184. Tunstall-Pedoe H, Brown CA, Woodward M, Tavendale R. Passive smoking by self report and serum cotinine and the prevalence of respiratory and coronary heart disease in the Scottish heart health study. J Epidemiol Community Health. 1995;49(2):139-43.

185. U.S. DHEW (U.S. Department of Health, Education and Welfare). Smoking and Health: A Report of the Surgeon General. U.S. DHEW, Public Health Service, Office of the Assistant Secretary for Health, Office on Smoking and Health, DHEW Publication No. (PHS)79-50066, 1979.

186. U.S. DHHS (U.S. Department of Health and Human Services). The Health Consequences of Smoking: Cancer. A Report of the Surgeon General. U.S. DHHS, Public Health Service, Office on Smoking and Health, U.S. Government Printing Office, Washington, DC, 1982.

187. U.S. DHHS (U.S. Department of Health and Human Services). The Health Consequences of Involuntary Smoking: A Report of the Surgeon

General. U.S. DHHS, Public Health Service, Office on Smoking and Health, U.S. Government Printing Office, Washington, DC, 1986.

188. U.S. DHHS (U.S. Department of Health and Human Services). Reducing the Health Consequences of Smoking. 25 Years of Progress. A Report of the Surgeon General. DHHS Pub. No. (CDC) 89084-8411. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Center for Chronic Disease Prevention and Health Promotion, Office of Smoking and Health, Rockville, MD, 1989.

189. U.S. EPA (Environmental Protection Agency). Respiratory health effects of passive smoking: lung cancer and other disorders. Washington DC, US Environmental Protection Agency, Office of Health and Environmental Assessment, Office of Research and Development. EPA/600/6-90/006F, 1992.

190. Uematsu T. Utilization of hair analysis for therapeutic drug monitoring with a special reference to oflaxacin and to nicotine. Forensic Sci Int. 1993;63:261–8.

191. Uematsu T, Mizuno A, Nagashima S, et al. The axial distribution of nicotine content along hair shaft as an indicator of changes in smoking behaviour: evaluation in a smoking-cessation programme with or without the aid of nicotine chewing gum. Br J Clin Pharmacol. 1995;39:665–9.

192. Vainiotalo S, Vaaranrinta R, Tornaeus J, Aremo N, Hase T, Peltonen K. Passive monitoring method for 3-ethenylpyridine: a marker for environmental tobacco smoke. Envir. Sci Technol.2001;35(9) 1818-22.

193. Van Rooij JG, Veeger MM, Bodelier-Bade MM, et al. Smoking and dietary intake of polycyclic aromatic hydrocarbons as sources of interindividual variability in the baseline excretion of 1- hydroxypyrene in urine. Int Arch Occup Environ Health. 1994;66:55–65.

194. van Vunakis H, Tashkin DP, Rigas B, Simmons M, Gjika HB, Clark VA. Relative sensitivity and specificity of salivary and serum cotinine in identifying tobacco-smoking status of self-reported nonsmokers and smokers of tobacco and/or marijuana. Arch Environ Health. 1989;44(1):53-8.

195. van Zeeland AA, de Groot AJ, Hall J, et al. 8-Hydroxydeoxyguanosine in DNA from leukocytes of healthy adults: relationship with cigarette smoking, environmental tobacco smoke, alcohol and coffee consumption. Mutat Res. 1999;439:249–57.

196. Vartiainen E, Seppala T, Lillsunde P, Puska P. Validation of self reported smoking by serum cotinine measurement in a community-[i]based study. J Epidemiol Community Health. 2002;56(3):167-70.

197. Vineis P, Malats N, Lang M et al, editors. Metabolic polymorphisms and susceptibility to cancer. Lyon, International Agency for Research on Cancer (IARC). IARC scientific publications, no. 48, 1999.

198. Wallace L, Pellizzari E, Hartwell TD, et al. Exposures to benzene and other volatile compounds from active and passive smoking. Arch Environ Health. 1987;42:272–9.

199. Watts RR, Langone JJ, Knight GJ, Lewtas J. Cotinine analytical workshop report: consideration of analytical methods for determining cotinine in human body fluids as a measure of passive exposure to tobacco smoke. Environ Health Perspect. 1990;84:173-82.

200. Weaver VM, Davoli CT, Heller PJ, et al. Benzene exposure, assessed by urinary trans,trans-muconic acid, in urban children with elevated blood lead levels. Environ Health Perspect. 1996;104:318–23.

201. Weaver VM, Buckley T, Groopman JD. Lack of Specificity of trans,trans-Muconic Acid as a Benzene Biomarker after Ingestion of Sorbic Acid-preserved Foods. Cancer Epidemiol Biomarkers Prev. 2000;9:749–55.

202. Whincup PH, Gilg JA, Emberson JR, Jarvis MJ, Feyerabend C, Bryant A, Walker M, Cook DJ. Passive smoking and risk of coronary heart disease and stroke: prospective study with cotinine measurement. BMJ. 2004;329;200-205.

203. Willemsen MC, Brug J, Uges DR, Vos de Wael ML. Validity and reliability of self-reported exposure to environmental tobacco smoke in work offices. J Occup Environ Med. 1997;39(11):1111-4.

204. Wu KY, Chiang SY, Huang TH, Tseng YS, Chen YL, Kuo HW, Hsieh CL. Formation of N-(2-hydroxyethyl)valine in human hemoglobin-effect of lifestyle factors. Mutat Res. 2004;559(1-2):73-82.

205. Yolton K, Dietrich Auinger P, Lanphear BP, Hornung R. Exposure to environmental tobacco smoke and cognitive abilities among U.S. children and adolescents. Environ Health Perspect. 2005;113:98–103.

206. Yu R, Weisel CP. Measurement of the urinary benzene metabolite trans, trans-muconic acid from benzene exposure in humans. J Toxicol Environ Health. 1996;48:453–77.

207. Zahlsen K, Nilsen OG. Nicotine in hair of smokers and non-smokers: sampling procedure and gas chromatographic/mass spectrometric analysis. Pharmacol Toxicol. 1994;75:143–9.

208. Zhou R, Li S, Zhou Y, Haug A. Comparison of environmental tobacco smoke concentrations and mutagenicity for several indoor environments. Mutat Res. 2000;465(1-2):191-200.