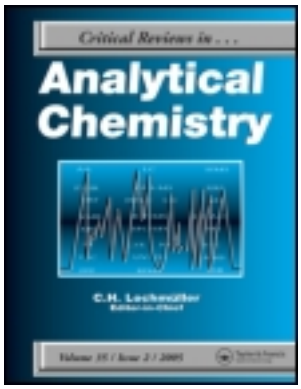

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Analysis of Markers of Exposure to Constituents of Environmental Tobacco Smoke (ETS)

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Tobacco smoke is a complex mixture of more than 4000 chemical compounds, many of which are harmful to human health. These compounds belong to various chemical classes, including amides, imides, lactams, carboxylic acids, aldehydes, ketones, alcohols, phenols, amines, hydrocarbons, ethers, and inorganic compounds. There are three types of tobacco smoke streams: the mainstream, the sidestream, and environmental tobacco smoke (ETS). In view of the threat to human health resulting from exposure to either of these tobacco smoke streams or to ETS, it has become necessary to monitor levels of substances characteristic of tobacco smoke, i.e., "biomarkers," The biomonitoring of toxic substances involves the analysis of biological materials taken from human subjects (urine, saliva, placenta, sweat, blood) for the presence of selected biomarkers. This article presents information on sample preparation techniques and analytical methodologies applicable in the analysis of biological materials for the presence of biomarkers and also on the levels of biomarkers present in samples of biological material taken from people exposed to various degrees to the harmful actions of substances present in tobacco smoke.

Keywords tobacco smoke, environmental tobacco smoke, constituents of tobacco smoke, analytical methods, biomarkers, biological fluids

INTRODUCTION

Tobacco is the only product legally available on the market that is harmful to every person exposed to its action (World Health Organization, 2008). Tobacco smoking is a habit caused by the pharmacological addiction to nicotine and dependence on psychological, environmental, and social factors that influence people (Kośmicki, 2001). At present there are about a billion smokers worldwide (DeMarini, 2004).

In 1959 some 400 compounds were known to be present in tobacco leaves and tobacco smoke; today, this figure has risen to 4000 (Stedman, 1968). Belonging to different classes of chemical compounds, these constituents may be in gaseous form or bonded to suspended particulate matter. They include inorganic compounds, ethers, hydrocarbons, amines, phenols, alcohols, ketones, aldehydes, carboxylic acids, and amides (Counts et al., 2004; Rummenie et al., 2008; Satici et al., 2003; Baker, 2006; Torikaiu et al., 2005; Nusbaum et al., 2000; Avşar et al., 2009). Most of them are hazardous to human health, affecting different organs (Rummenie et al., 2008). During smoking a complex mixture of compounds is inhaled into the respiratory system (Borgerding and Klus, 2005).

Experts from the Centers for Disease Control and Prevention (CDC) estimated that between the years 2000 and 2004 at least 443,000 deaths were attributable to cigarette smoking or exposure to second-hand smoke (Miller et al., 2010). Cigarette smoking is globally prevalent and is increasing, especially in developing countries. It is estimated that by 2015, 6.4 million deaths worldwide will be due to smoking (Man et al., 2009). Other data indicate that as a consequence of smoking, 3 million people die per year and that in the next 30–40 years, this number will grow to 10 million per year. The International Agency for Research on Cancer has identified tobacco smoke as a factor inducing cancer in a greater number of human organs than other carcinogens (DeMarini, 2004).

But exposure to environmental tobacco smoke (ETS) can cause disease, even if people have never smoked. Depending on how much smoke is present, the type of ventilation, contact with indoor surfaces, and a host of other environmental conditions, ETS can contain some 60 different carcinogens (Howard and Sanderow, 2008). People are exposed to tobacco smoke at the workplace and in their leisure pursuits (at home, and in bars and restaurants) (Collier and Pritsos, 2003). The term "passive smoking" implies the involuntary inhalation of tobacco smoke

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present in the environment, which is formed as a result of the burning of a cigarette (diluted sidestream, 57–85%) or the inhalation of smoke by a smoker (diluted mainstream, 15–43%) (Krzemieniecki, 2001; Henderson, 2009; Frost-Pineda et al., 2008). This is particularly dangerous in view of the fact that some constituents of this smoke are present in concentrations far higher in the smoke stream forming as a result of the burning of a cigarette (Krzemieniecki, 2001); for example, the concentration of carbon monoxide is 3–5 times, the ammonia level approximately 40–170 times, nitrogen oxides 4–10 times, and hydrogen cyanide 4–10 times greater in the sidestream.

BIOMONITORING AND BIOMARKERS

The smoke generated by a burning cigarette can be divided into:

- Mainstream smoke (MS), which emerges from the mouth end of the cigarette during puffing,
- Sidestream smoke (SS), which emerges into the environment from the lit end of the cigarette between puffs, and
- Environmental tobacco smoke (ETS), which is a mixture of sidestream smoke and exhaled mainstream smoke that diffuses into the atmosphere, becomes diluted by ambient air, and undergoes various physical and chemical changes, including reactions with chemical substances not generated by tobacco. Another name for ETS is secondhand smoke (Borgerding and Klus, 2005; Chung et al., 2005; Miller et al., 2005).

Because of the numerous adverse health effects due to tobacco smoking and the exposure of nonsmokers to one of the smoke streams or to ETS, the need has arisen to monitor the toxic substances present in tobacco smoke and entering an exposed person's body. This done by the biomonitoring of toxic substances in samples of biological materials taken from humans.

Biomonitoring is the application of a live organism or biological material taken from an organism in order to obtain essential information about the biosphere. Organisms used in biomonitoring are called "bioindicators." Information on the occurrence and concentration of a bioindicator substance is extremely important for quality assessment of the environment and changes taking place in it (Polkowska et al., 2004). Human biomonitoring is a means of acquiring data on the exposure of people to environmental toxicants and the effects of such exposure by means of the analysis of cells, tissues, and biological fluids. Real-time monitoring (RTM) is carried out by analyzing blood, urine, saliva, milk, and placental material; long-term monitoring (LTM) involves the analysis of hair, bones, liver, and fatty tissue (Iyengar and Rapp, 2001). The biochemical or biological variables measured during human biomonitoring are the so-called biomarkers, which define the risk of exposure, the effects of exposure, or individual susceptibility to exposure (Scherer, 2006).

Biomarkers of Exposure to ETS Constituents

A biomarker is principally an aid in the assessment of exposure and biological consequences in epidemiology, occupational medicine, and environmental protection (Scherer, 2006). A substance that is a biomarker for tobacco smoke should be specific to tobacco smoke, occur in amounts enabling its determination, and be emitted at similar levels from cigarettes of all brands (Rothberg et al., 1998). Figure 1 outlines in schematic form the concept of biomonitoring the substances contained in tobacco smoke (using as an example benzene) (Scherer, 2006).

Markers of ETS exposure are metabolites of tobacco smoke constituents that can be measured in physiological fluids or attached to DNA or proteins. They are useful in quantifying the amount of exposure to ETS. Biomarkers, which are

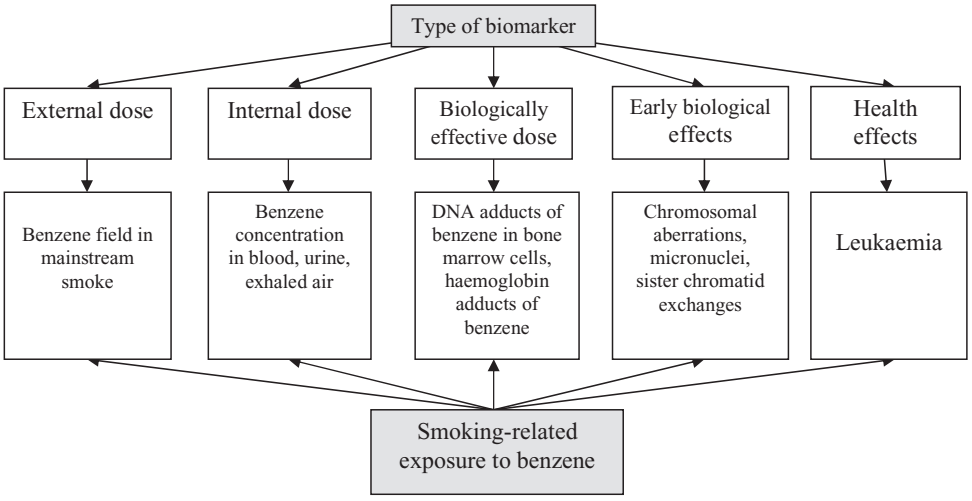


FIG. 1. The concept of biomonitoring a substance contained in tobacco smoke (based on the example of benzene).

components of ETS or their metabolites, are the best direct means of assessing ETS exposure. A difficult issue, however, is the availability of samples of human biological fluids. An alternative is the determination of biomarkers of ETS in indoor air. Markers of ETS should be unique to tobacco smoke. The following markers of exposure to tobacco smoke have been used: nicotine, cotinine, thiocyanate, carboxyhaemoglobin, hydroxyproline, aromatic amines, and also certain protein and DNA adducts. Nicotine in particular has been favored because it is specific to ETS and because, in its vapor phase, it is fairly simple and inexpensive to measure. Another biomarker used is RSP (respirable suspended particulates), but RSP is removed largely through building ventilation systems, whereas nicotine is deposited on surfaces and can later be re-emitted. RSP is used in long-term monitoring. Cotinine is a biomarker used for epidemiological purposes, as its level in biological fluids is linked to the magnitude of exposure to ETS constituents.

The appropriateness of a biomarker depends on the nature of the study and the type of exposure being assessed (RTM or LTM). The relationship between a biomarker and exposure varies as a function of environmental and physiological factors. Individual characteristics, such as rate of metabolism, absorption, and differences in distribution can also affect the biomarker concentration in body fluids. It can determine the dose derived from smoking, which is difficult in the case of exposure to the same substance from the diet or exposure from other sources (Chung et al., 2005).

The first mention of biomarkers goes back to the year 1856, when Riley observed the quickening of the pulse following the smoking of a cigarette. In 1932 Maddock and Coller recorded body temperature changes after smoking, and in 1957 the first EEGs (electroencephalograms) visualized the changes taking place in the brain during the smoking of a cigarette (Scherer, 2006). Table 1 lists the biomarkers of ETS exposure and their half-lives.

The Thiocyanate Ion

The first environmental biomarker of tobacco smoke to be discovered was the thiocyanate ion (SCN^-), which Claud Bernard (1813–1878) detected in urine, saliva, and blood. He recorded higher levels of this ion in these biological fluids in smokers than in nonsmokers (Scherer, 2006). This ion is a metabolite of cyanides (HCN). Cyanides are present, mainly in the form of glycosides, from which they are released in the gastric juice, in different food items. These compounds are contained in brassicas (like cabbage, broccoli, and cauliflower), almonds, nuts, leguminous plants, bamboo, beans, and linseed, as well as in cherry, peach, apricot, and plum stones and in apple pips. Further sources are mustard, cows' milk, and cheese. They are also formed endogenously by bacteria in the colon (Scherer, 2006). But only small quantities of cyanide ions are produced from food. Larger concentrations are produced by the metabolism of tobacco smoke constituents (Glatz et al., 2001). This metabolism takes place mainly in the mitochondria of the liver. The half-life of the SCN^- ion is 6 days, which is decidedly longer than that of other biomarkers, such as nicotine. The most precise and reliable information is supplied by analyses of thiocyanate ion levels in the serum, since these ions appear or disappear in serum more quickly than in other body fluids (Pré and Vassy, 1991). Thiocyanate ions get into the body by being absorbed by the pulmonary alveoli, the buccal mucosa, stomach, and skin (Scherer, 2006).

SCN^- levels are some 2–3 times higher in the urine or serum of smokers than of nonsmokers: the mean concentration in serum samples is 50 μmol/dm^3 in nonsmokers and 150 μmol/dm^3 in smokers. In saliva the respective levels are 1200 and 3000 μmol/dm^3, 20 times higher than in serum samples. SCN^- levels in urine samples are roughly the same as those in serum (Scherer, 2006).

The toxicity of the thiocyanate ion is due to its affinity for iron in the cytochrome C molecule in mitochondria, which blocks the final stage of electron transfer, as a result of which cells become anoxic and changes occur leading to ATP exhaustion, metabolic acidosis, and ultimately cell death (Pritchard, 2002).

Pyridine Alkaloids: Nicotine, Cotinine

Nicotine is the factor responsible for the addiction to tobacco smoking (Pellegrini et al., 2007; Chung et al., 2005). The main source of exposure to nicotine is the smoking of tobacco or the use of nicotine replacement therapy (e.g., chewing gum or patches containing nicotine) (Yildiz, 2004; Chung et al., 2005). One cigarette contains from 7 to 24 mg of nicotine (Moriya and Hashimoto, 2004). It is also present in plants like tomatoes, potatoes, and green peppers (Collier and Pritsos, 2003; Polańska et al., 2007; Yildiz, 2004) as well as tea and aubergines (Chung et al., 2005).

Nicotine's structure enables it to cross barriers like biological membranes, including the blood-brain barrier. The effects of nicotine are:

- Higher pulse rate
- Higher blood pressure
- Increase in the fatty acid content of the blood
- Mobilization of sugars present in the blood and an increase in the blood level of catecholamine (Yildiz, 2004)
- Stimulation of the autonomic system by releasing or facilitating the production of various neurotransmitters (dopamine, noradrenalin, serotonin, acetylcholine, and vasopressin) (Miller et al., 2010)
- At the cellular level exposure to nicotine is manifested by increased expression of heat shock proteins, exchange of sister chromatids, chromosomal aberrations, and attenuation of apoptosis (Yildiz, 2004)

Nicotine is absorbed through the mouth, skin, lungs, urinary bladder, and digestive tract. Absorption through the buccal

TABLE 1
List of biomarkers of ETS exposure and their half-lives

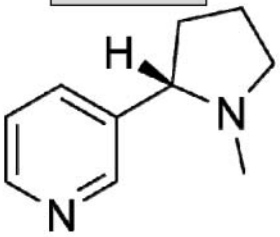
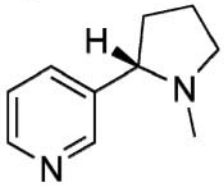
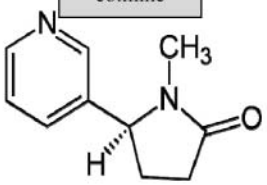
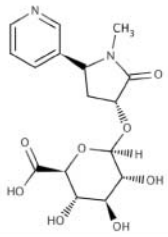
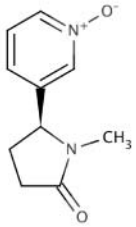
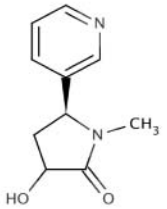
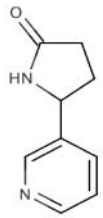
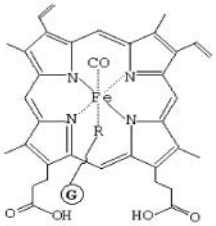
Compound in tobacco smoke	Compounds determined in biological material (compound or metabolite of tobacco smoke compound)	
hydrogen cyanide H—C≡N	hydrogen cyanide H—C≡N	thiocyanate ion $^{\ominus}$S—C≡N half-life: 6 days
nicotine  half-life: 2.5 hours	nicotine  half-time: 2.5 hours	cotinine  half-life: 15-17 hours
	3'-hydroxycotnine-glucuronide 	cotinine N-oxide 
	3-hydroxycotinine 	norcotinine 
carbon monoxide :C≡O:	carboxyhaemoglobin 	exhaled carbon monoxide :C≡O:

TABLE 2
Typical levels of COex and COHb determined in samples of exhaled air and blood taken from subjects exposed to different degrees to the harmful effects of tobacco smoke (Scherer, 2006)

Biomarkers	Non-smokers	Smokers	Heavy smokers
COex	4–7 ppm	20–30 ppm	> 50 ppm
COHb	1–2%	4–7%	> 12%

mucosa is the main pathway in smokers not inhaling smoke or on replacement therapy. But the main route of absorption in smokers is via the pulmonary alveoli. The aqueous fraction of tobacco has a pH of 8.5, whereas the alveolar pH is 7.4. Under these conditions more than 30% of the nicotine is in a neutral state and easily crosses cell membranes (Yildiz, 2004).

The concentration of nicotine and its metabolites in biological fluids may be an indicator of exposure to ETS (Chung et al., 2005). Cotinine is considered to be the most specific and most sensitive biomarker of exposure to ETS constituents (Kaufman et al., 2002). It is a metabolite of nicotine determined in body fluids like saliva, blood, and urine and also in the hair and placenta. As cotinine can be determined in the hair and placenta, it is a good biomarker for fetal exposure to ETS constituents. The half-life of cotinine is 15–17 h, and so it is very much longer than that of nicotine (2.5 h) (Collier and Pritsos, 2003; Polańska et al., 2007; Rogers, 2008). The cotinine level is two to three orders of magnitude greater in smokers than in nonsmokers exposed to ETS (Chung et al., 2005).

Nicotine itself has not been identified as a carcinogen, but several tobacco-specific nitrosamines (TSNAs), which are derived from nicotine and other tobacco alkaloids, may be carcinogenic. Tobacco-specific nitrosamines are formed by N-nitrosation of nicotine during the curing, processing, fermentation, and combustion of tobacco products. TSNAs include such nitrosamines as N′-nitrosonornicotine, 4-(methylnitrosamino-)-1-(3-pyridyl)-1-butanone, N′-nitrosonornicotine, and 4-(methylnitrosamino-)-1-(3-pyridyl)-1-butone. All these compounds are regarded as highly carcinogenic (Chung et al., 2005).

Carbon Monoxide in Blood and Exhaled Air

One of the earliest discovered biomarkers of tobacco smoke was the combination of carbon monoxide and hemoglobin (CoHb) (Scherer, 2005). The affinity of CO for hemoglobin is 200–240 times greater than the affinity of oxygen (Olds, 1997; Topinka et al., 2009). CO is a colorless, odorless toxic gas, slightly lighter than air (Scherer, 2006). Tobacco smoke is one of the sources of exposure to CO (Scherer, 2005). It enters the body through the respiratory tract. Having diffused across the walls of the pulmonary alveoli, CO binds with the iron(II) cations in hemoglobin to form carboxyhemoglobin. This compound is incapable of transporting oxygen. Symptoms of CO poisoning include headache, vertigo, shallow breathing, chest pains, weakness, nausea, loss of consciousness, abdominal pains, and muscle cramps (Scherer, 2006). Exposure to a CO concentration of 2000 ppm for 3–4 h causes 70% of the hemoglobin to bind to it, and death ensues. The half-life of COHb is 1–4 h. The level of carboxyhemoglobin in the blood of a fetus is higher than in the mother, which due to the greater susceptibility of the fetus, increasing risk of exposure to CO effects (Olds, 1997; Topinka et al., 2009). This is because the fetus has a lower capacity for detoxification and DNA repair (Topinka et al., 2009).

The level of CO in exhaled air (COex) is also a biomarker of exposure to ETS constituents. But because of the very short half-life (from 5 to 55 min), the CO concentration should be measured within 30 min of a cigarette having been smoked. CO

TABLE 3
A brief outline of biological materials used in the determination of ETS exposure biomarkers

Biological material	Type of material	Sampling		Sample size		
		Invasive techniques	Noninvasive techniques	Large	Medium	Small
Blood	L	+	−	−	+	−
Urine	L	−	+	+	−	−
Saliva	L	−	+	−	+	−
Tears	L	−	+	−	−	−
Sweat	L	−	+	−	+	−
Bile	L	+	−	−	+	−
Semen	L	−	+	−	+	−
Breast milk	L	−	+	−	−	+
Amniotic fluid	L	+	−	−	−	+
Meconium	S	−	+	−	−	+
Placenta	S	−	+	+	−	−

L: liquid, S: solid, +: yes, −: no.

TABLE 4
Chemical composition of biological materials obtained from humans

Fluid	Constituents	Fluid	Constituents
Sweat	mineral compounds: K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}	Blood	erythrocytes, leukocytes
	0.8% NaCl		protein
	98% water		hemoglobin (HGB)
	ammonia		neutrophils
	fats		blood platelets
	lactic and uric acids		glucose
Bile	mineral compounds: Na^+, K^+, Cl^-, Fe^{2+}, PO_4^{3-}		leukocytes, monocytes, lymphocytes
	bile pigments (bilirubins)		phosphatase: basic and acidic
	cholesterol lipids		creatinine and urea
			urea
	bile acids, responsible for bile's bitter taste		Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Cl^-, HPO_4^-, CO_3^-
	mucus		bilirubin
Semen	steroids and protein hormones	Breast milk	carbohydrate
	prostaglandins		protein
	cytokines		fat
	enzymes (acidic phosphatase, beta-glucuronidase)		Ca^{2+}, Cl^-, Mg^{2+}, P, K^+, Se^{2+}, Zn^{2+}
		Placenta	protein
	citric acid		water
	trace elements (Mg^{2+}, Ca^{2+}, K^+, Zn^{2+})		fat
			ash
Saliva	salivary amylase (ptyalin) and maltase	Urine	Na^+, K^+, NH_3^+, Ca^{2+}, Mg^{2+},Cl^-, PO_4^{3-}, SO_4^{2-},
	mucin—mucilaginous body		water
	mineral salts		urea
	protein		uric acid
	water 99.5%		creatinine
Meconium	residual swallowed amniotic fluid	Amniotic fluid	water and electrolytes 98%, peptides, lipids,
	cholesterol and sterol precursors		proteins, hormones
	blood group substances		peptides
	sugar		lipids
	proteins		proteins
	lipids		hormones
	trace metals	Meconium	bile acids and salts
	desquamated epithelial cells from the		fine neonatal hair
	gastrointestinal tract and skin fatty material		various pancreatic and intestinal secretions
	from the vernix caseosa		water
			Enzymes

in exhaled air is linked to carboxyhemoglobin by the formula $COHb(\%) = 0.6 + 0.3COex$ (ppm). There is a linear dependence between CO and COHb in the 0–10% range.

Table 2 lists typical CO levels in samples of exhaled air and blood taken from subjects exposed to different degrees to the harmful effects of tobacco smoke (Scherer, 2006).

The use of these biomarkers in distinguishing between subjects with no, low, or high levels of ETS exposure is limited because of their lack of sensitivity and specificity (Chung et al., 2005).

Use of Samples of Biological Material in the Analysis of Tobacco Smoke Biomarkers

The term "biological material" includes both physiological fluids and solid tissues. More and more information is being published in the literature about novel analytical methodologies developed to monitor biological solids and liquids, to determine concentration ranges of a wide spectrum of analytes, and to assess the influence of environmental exposure to chemical compounds detected determined quantitatively in samples of biological materials with the aid of the relevant analytical

TABLE 5
Problems appearing during analytical work on samples of biological materials

Type of problem	Problem example
Independent of the analytical procedure	Sample inhomogeneity (placenta)
	Different metabolism of toxic compounds depending on age and sex
	Exposure to biomarkers of the ETS constituents from sources other than ETS (thiocyanate ion, formaldehyde)
	Presence of interferents
	Problems concerning the sampling of biological materials (invasive sampling of blood, amniotic fluid)
	Difficulties with sample representativeness (urine, saliva)
Connected with the analytical procedure	Small volumes of samples (semen, tears, blood)
	Need to select appropriate analytical procedures
	Specific composition of the matrix
	Analyte loss during sample preparation
	Low levels of some analytes
	Lack of reference materials
	Change in chemical composition during storage (decay of biological tissues)
Connected with the interpretation of results	Changes in biomarker concentrations depending on time of exposure (linked to half-life)
	Relationships between contaminants present in samples
	Lack of appropriate norms concerning permissible levels of compounds present in ETS

procedures. The proper selection of biological sample types is a difficult task. Important aspects of a sample, enabling its usefulness for analysis to be assessed, include:

- Metabolic changes of particular elements or compounds,
- Quantity of a sample that can be collected, and

TABLE 6
Operations involved in the preparation of samples of biological materials for analysis

Operation involved in the preparation of samples of biological materials for analysis	Characteristics of each stage	Reference
Sampling	Invasive sampling: specimens of blood, amniotic fluid	Chinaka et al., 1998; Blount and Valentin-Blasini, 2006
	Noninvasive sampling: specimens of urine, saliva, semen, placenta	
	Representative sample	
Sample storage	Samples stored at temperatures from -4°C to -80°C	Chou et al., 2008; Miller et al., 2010
Measurement of mass and/or volume	—	Miller et al., 2010
Dessication	Dessication in a stream of nitrogen after extraction	Page-Sharp et al., 2003
	Lyophilization	
Dilution	Sample diluted with ultrapure water	Chinaka et al., 1998; Glatz et al., 2001
Centrifugation	Removal of suspended matter from fluid samples, blood proteins, urine	Youso et al., 2010
Analyte preconcentration	Extraction techniques	Nakajima et al., 2000

- Analytical quality of the sample, which is affected by the methods of storage and preservation.

Toxic substances that are absorbed by the body can circulate in it together with physiological fluids, accumulate in tissues, or be excreted as polar metabolites or in unchanged form. Biological fluids are materials with a complex matrix and require special attention at the sample preparation stage (Polkowska et al., 2004). Biological fluids such as blood, breast milk, saliva, and urine are commonly used to monitor pollutants. Body-fluid specimens from human subjects that are meaningful as biomonitors fall into two groups: those requiring invasive procedures (whole blood) and those that use noninvasive procedures (saliva, urine) (Iyengar and Rapp, 2001). Table 3 lists basic information on biological materials that are analyzed for ETS exposure biomarkers. Such materials are difficult to analyze because of their complex structure and the possibility of interferents (Polkowska et al., 2004; Sylwanowicz et al., 1980; Heinrich-Ramm et al., 2002; Bochenek and Reicher, 1955; Kratz et al., 2004; Hamosh, 1996; Noguera-Obenza et al., 2003; Iyengar and. Rapp, 2001; Topinka et al, 2009; Gareri et al., 2006; Tong et al., 2009).

Table 4 sets out information on the chemical composition of these materials (Sylwanowicz et al., 1980; Gareri et al., 2006; Bearer, 2003; Tong et al., 2009; Topinka et al., 2009; Hamosh, 1996; Noguera-Obenza, 2003).

Analysis of Biomarkers of Exposure to ETS Constituents on Biological Materials

The analysis of samples of biological materials is problematic because of the variability in target substance concentrations with respect to sampling time. This depends, among other things, on the degree of exposure of the system to a given xenobiotic and on its half-life. Samples of biological materials, with their complex and often variable matrices, present a particular challenge. Another issue to be addressed is the complex and often variable composition of biological materials, which means that they cannot be analyzed directly. Table 5 gives information on the fundamental problems that crop up during analytical work on samples of biological materials.

Techniques of Sample Preparation Prior to Determination of ETS Biomarkers in Biological Materials

The analytical procedure consists of many stages, the most error-prone of which are the sampling and sample preparation steps. Table 6 provides information on the operations involved in the preparation of samples of biological materials for analysis.

Any errors committed during sampling and/or sample preparation may lead to loss of analytes and/or introduce further contaminants to the samples. Another difficulty is the need for invasive sampling in the case of blood or amniotic fluid

TABLE 7
Extraction techniques used for preparing samples of biological materials for analysis of selected contaminants

Extraction	Biological material	Analyte	Reference
LLE	urine	methylmercapturic acid (MMA) 2-hydroxyethylmercapturic acid (HEMA) 2-cyanoethylmercapturic acid (CEMA)	Scherer et al., 2010
		nicotine	Heinrich-Ramm et al., 2002
		nicotine, cotinine	Man et al., 2009
	plasma	nicotine, cotinine	Nakajima et al., 2000
	breast milk	nicotine, cotinine	Page-Sharp et al., 2003
		nicotine (NIC), cotinine (COT), trans-3-hydroxycotinine (TRANS-3-OH-COT), cotinine-N-oxide (COT-N-OX)	Pellegrini et al., 2007
SPE	urine	nicotine, cotinine	Doctor et al., 2004
	plasma, urine	NIC GLUC - nicotine-N-(4-deoxy-4,5-didehydro)-β-D-glucuronide, CNO - (S)-cotinine N-oxiol, 3-HC-3′-hydroxycotinine, NCOT - norcotinine, NNO - trans nicotine-1′-oxide-methyl, COT - cotinine, NNIC - nornicotine, NIC - nicotine, AT - anatabine, AB - anabasine, COT GLUC - cotinine N-β-D-glucuronide	Miller et al., 2010
SPME	urine	benzene, toluene, ethylbenzene, xylenes	Manini et al., 2008
SLE	urine	nicotine, cotinine	Oddoze et al., 1998
PMME	saliva	formaldehyde, acetaldehyde, acrolein, butyraldehyde	Zhang et al., 2006

TABLE 8
Analytical methodologies applied in the determination of different types of biomarkers in samples of biological materials

Analytical technique	Analyte(s)	LOD, LOQ	Mode of sample preparation	Reference
		Urine		
LC-MS/MS	methylmercapturic acid (MMA)	LOD: ~1.4 ng/mL LOQ: 5.0 ng/mL	Urine sample (6 mL) adjusted to pH 1.5 50 μL internal standard (2 ng/μL) added	Scherer et al., 2010
	2-hydroxyethylmercapturic acid (HEMA)	LOD: ~0.3 ng/mL LOQ: 1.0 ng/mL	Extracted twice with 4 mL ethyl acetate by shaking Evaporated to dryness in a SpeedVac evaporator	
	2-cyanoethylmercapturic acid (CEMA)	LOD: ~0.2 ng/mL LOQ: 1.5 ng/mL	Redissolved in 4 mL ammonium hydroxide (5%) Applied to an anion exchange cartridge The eluate evaporated to dryness in a SpeedVac evaporator Redissolved in 100 μL methanol containing 10% N,N-diisopropylethylamine Derivatized with 100 μL pentafluorobenzyl bromide (PFBBr) 10% in methanol at 50°C for 1 h Evaporated to dryness Redissolved in 0.5 mL water Extracted twice with 2 mL ethyl acetate Evaporated Redissolved in 200 μL acetonitrile:water (3:1, v/v) Urine sample (6 mL) adjusted to pH 1.5 50 μL internal standard (2ng/μL) added Extracted twice with 4 mL ethyl acetate by shaking Evaporated to dryness in a SpeedVac evaporator	

GC-MS	nicotine	LOD: 1 μg/L	2 mL of urine with 1.5 mL of hydrochloric acid, 2 mL of sodium hydroxide solution (5 mol/L) and 100 μL deuterated cotinine in 0.1 mol/L hydrochloric acid as internal standard extracted with dichloromethane (3 mL) Centrifuged Upper aqueous phase discarded Cooled to –20°C Organic phase transferred to a glass vial, 100 μL n-decane added Dichloromethane phase evaporated in a gentle stream of nitrogen Residue dissolved in 250 μL of toluene	Heinrich-Ramm et al., 2002
RIA		—	25 μL of urine mixed with 100 μL of rabbit antiserum against nicotine metabolites Incubated for 30 min at room temperature followed by addition of 1000 μL of a precipitating reagent Supernatants removed	
GC-MS	nicotine cotinine	LOD: 0.20 ng/m:	Sample stored at –20°C Extracted	Man et al., 2009
Spectrophotometer	creatinine		—	Tsai et al., 2007
LC-MS/MS	cotinine levels with creatinine correction	LOD: 0.07 ng/mL		

(*Continued on next page*)

TABLE 8
Analytical methodologies applied in the determination of different types of biomarkers in samples of biological materials (*Continued*)

Analytical technique	Analyte(s)	LOD, LOQ	Mode of sample preparation	Reference
Spectrophotometer	thiocyanate ions	—	—	Pre and Vassy, 1991
GC-FPD	nicotine, cotinine	—	Sample storred with carboxylamine melanate in methanol and carbonate buffer Extracted with dichloromethane Organic phase transferred and re-extracted with HCl Liquid phase transferred, buffer added Extracted with dichloromethane Mixed with isoamyl alcohol Dichloromethane evaporated in a stream of nitrogen gas	Moriya and Hashimoto, 2004
GC-MS	cotinine	—	—	Kasperczyk et al., 2008
HPLC-PDA	nicotine	LOD: 5 ng/mL LOQ: 17 ng/mL	Samples stored at –40°C SPE prior to analysis	Doctor et al., 2004
	cotinine	LOD: 2 ng/mL LOQ: 6 ng/mL		
IC	thiocyanate	LOD: 0.02 mg/L	Samples centrifuged and stored at 4°C	Connolly et al., 2002
	nitrate	LOD: 0.02 mg/L 0.04 mg/L	Dilution with deionized water	
AAS	thiocyanate ion	—	[Cu(BPTC)Cl] diluted with deionized water pH adjusted to neutral Sample and isoamyl acetate added Stirring Repetition of above steps to obtain solution Rinsing with buffer solution Dilution of organic layer	Chattaraj and Das, 1992
GC-NPD	cotinine	—	Samples stored at –70°C	Chou et al., 2008

HPLC-UV		—	HNO_3 added to sample (2 mL) and the mixture heated to 60°C Centrifuged Methanol (1 mL), chloroform (4 mL) and sodium hydroxide (1 mL, 5 N) added to the clear liquid (1 mL) Centrifuged Chloroform layer dried with nitrogen Residue dissolved in methanol (0.5 mL) and analyzed	
LC-ESI-MS/MS	NIC GLUC-nicotine-N-(4-deoxy-4,5-didehydro)-β-D-glucuronide	LOQ: 2.5 ng/mL	50 μL deuterated internal standard (1 g/mL) added to 1 mL urine	Miller et al., 2010
	CNO - (S)-cotinine N-oxiol 3-HC-3′-hydroxycotinine	LOD: 1.0 ng/mL	Centrifugation Urine samples acidified with 1.5 mL 5 mM aqueous ammonium formate	
	NCOT - norcotinine NNO - trans nicotine-1′-oxide-methyl	LOQ: 1.0 ng/mL LOD: 1.0 ng/mL	Vortex mixed SPE Extract evaporated to dryness under stream of air at 40°C	
	COT - cotinine NNIC - nornicotine NIC - nicotine AT - anatabine AB - anabasine	LOQ: 2.5 ng/mL LOD: 1.0 ng/mL		
	COT GLUC- cotinine N-β-D-glucuronide	LOD: 25 ng/mL LOQ: 50 ng/mL		

(*Continued on next page*)

TABLE 8
Analytical methodologies applied in the determination of different types of biomarkers in samples of biological materials (*Continued*)

Analytical technique	Analyte(s)	LOD, LOQ	Mode of sample preparation	Reference
		Saliva		
CE	thiocyanate ion	LOD: 0.7–1.5 m*M*	Dilution with deionized water	Glatz et al., 2001
		Plasma		
LC-ESI-MS/MS	NIC GLUC-nicotine-N-(4-deoxy-4,5-didehydro)-β-D-glucuronide	LOQ: 1.0 ng/mL	Blood samples stored at 4°C in 6 mL tubes	Miller et al., 2010
		LOD: 0.25 ng/mL	Centrifuged to separate plasma from the blood	
	CNO - (S)-cotinine N-oxiol		Plasma supernatants transferred to clean silanized glass test tube	
	3-HC-3′-hydroxycotinine		Stored at –20°C	
	NCOT - norcotinine	LOQ: 1.0 ng/mL	50 μL deuterated internal standard (1 g/mL) added to 1 mL plasma	
		LOD: 0.75 ng/mL	1 mL 10% aqueous trichloroacetic acid added to plasma samples to clean up matrix	
	NNO - trans nicotine-1′-oxide-methyl	LOQ: 1.0 ng/mL	Centrifuged	
		LOD: 0.25 ng/mL	Vortex mixed	
	COT - cotinine		SPE	
	NNIC - nornicotine		Extract evaporated to dryness under stream of air at 40°C	
	NIC - nicotine	LOQ: 1.0 ng/mL LOD: 0.75 ng/mL		
	AT - anatabine	LOQ: 1.0 ng/mL LOD: 0.5 ng/mL		
	AB - anabasine	LOQ: 1.0 ng/mL LOD: 0.75 ng/mL		
	COT GLUC - cotinine N-β-D-glucuronide	LOD: 25 ng/mL LOQ: 50 ng/mL		
	NIC GLUC - nicotine-N-(4-deoxy-4,5-didehydro)-β-D-glucuronide	LOQ: 1.0 ng/mL LOD: 0.25 ng/mL		

GC-MS	protein-bound thiocyanate	LOD: 2.5 ng/mL	Samples mixed with an EDTA anticoagulant Centrifuged Stored at −80°C Plasma proteins isolated by precipitation using acidified acetone and diethyl ether three times Proteins dessicated Proteins dissolved in an aqueous 0.1 M carbonate buffer Initiation of thiocyanate adducts Shaking at room temperature for 1 h to extract the protein-bound thiocyanate 200 μL of the extracted protein mixed with PFBBr in ethyl acetate and 20 mM of TBAS in saturated sodium borate Heated sample at 80°C for 1 h, shaken, and centrifuged to separate organic layers Organic layer collected	Youso et al., 2010
RIA	cotinine	—	Frozen at −80°C until analysis	Seccarecia et al., 2003
CE	thiocyanate ion	—	Dilution with deionized water Addition of acetonitrile to remove proteins	Glatz et al., 2001
HPLC-UV	nicotine	LOQ: 0.2 ng/mL	Alkalized with NaOH Internal standard added Extracted with dichloromethane Conc. HCl added Organic layer evaporated Residue dissolved in mobile phase	Nakajima et al., 2000
	cotinine	LOQ: 1.0 ng/mL		

(*Continued on next page*)

TABLE 8
Analytical methodologies applied in the determination of different types of biomarkers in samples of biological materials (*Continued*)

Analytical technique	Analyte(s)	LOD, LOQ	Mode of sample preparation	Reference
		Blood		
GC-NPD	cotinine	—	Samples stored at −70°C	Chou et al., 2008
HPLC-UV		—	HNO_3 added to the sample (2 mL) and the mixture heated to 60°C Centrifuged Methanol (1 mL), chloroform (4 mL), and sodium hydroxide (1 mL, 5 N) was added to the clear liquid (1 mL) Centrifuged Chloroform layer dried with nitrogen Residue dissolved in methanol (0.5 mL) and analyzed	
IC-UV, IC-FD	cyanide ion	LOD: 3.8 pmol/mL	Water and methanol added Centrifuged	Chinaka et al., 1998
	thiocyanate ion	LOD: 86 pmol/mL	Derivatized	
		Breast milk		
LC-ESI-MS/MS	nicotine (NIC)	LOD: 1.6 μg/L	Storage at −20°C	Pellegrini et al., 2007
		LOQ: 5.0 μg/L	Internal standard (10 mL) and phosphate buffer (to pH 6.8) was added to milk sample (1 mL)	
	cotinine (COT)	LOD: 1.6 μg/L	Extraction with a solution of chloroform/isopropanol (2.5 mL, 95:5 v/v)	
		LOQ: 5.0 μg/L	Ammonium chloride (2 mL) was added to the aqueous phase containing milk, re-extracted	
	trans-3-hydroxycotinine (TRANS-3-OH-COT)	LOD: 1.6 μg/L	Organic phase evaporated under a stream of nitrogen	
		LOQ: 5.0 μg/L	Residue dissolved in ammonium formate (100 μL, 50 mM)	
	cotinine-N-oxide (COT-N-OX)	LOD: 1.6 μg/L LOQ: 5.0 μg/L	20 μL injected to the column	

HPLC-UV	nicotine, cotinine	—	Alkalized with aq. NaOH Extracted with dichloromethane, centrifuged Extracted with HCl Dichloromethane and NaOH added, sample shaken and centrifuged Dichloromethane and conc. HCl added Evaporated in a stream of nitrogen	Page-Sharp et al., 2003
		Amniotic fluid		
IC-ESI-MS/MS	perchlorate	LOD: 0.020 μg/L	Fluid samples collected at 15–20 weeks of gestation	Blount and Valentin-Blasini, 2006
	thiocyanate	LOD: 7.4 μg/L	Centrifuged	
	nitrate	LOD: 50 μg/L	Stored at -80°C	
	iodide	LOD: 0.57 μg/L	After 30 days stored at room temperature Sample stirred to remove suspended matter 0.5 mL sample diluted with 0.5 mL internal standard Solution transferred to microcentrifuge vials and centrifuged Clear sample analyzed	
Tears				
Quantiplate lysozyme test kit	lysozyme	—	Tear sample (100 μL from one eye) collected with a micropipette Stored at 4°C for up to 4 days	Satici et al., 2003
		Semen		
RIA	nicotine	detectable range	Centrifuged	Pellegrini et al., 2007
		0.5–50 ng/mL	Stored at -20°C without preservatives until analysis	
	cotinine	detectable range 0.2–20 ng/mL		
RIA	nicotine	—	Centrifuged and stored at -20°C	Wong et al., 2000
HPLC	cotinine	—	Centrifuged	Shen et al., 1997

TABLE 9
Literature data on biomarkers detected and determined in samples of biological materials

Biological material	Analyte(s)	Concentration range	Reference
Urine	methylmercapturic acid (MMA)	19.1–27.2 μg/24 h	Scherer et al., 2010
	2-hydroxyethylmercapturic acid (HEMA)	25.7–272.8 μg/24 h	
	2-cyanoethylmercapturic acid (CEMA)	6.79–30.4 μg/24 h	
	nicotine	smokers: 1002.2 μg/g creatinine	Heinrich-Ramm et al., 2002
		non-smokers: 42.0–45.8 μg/g creatinine	
		smokers: 21.2–2684 μg/g creatinine	
		non-smokers: 1.7–148 μg/g creatinine	
	nicotine	nicotine: 1.3–417.3 ng/mL	Man et al., 2009
	cotinine	cotinine: 21.4–1277.8 ng/mL	
	cotinine levels with creatinine correction	non-smokers: 0.0032 mg/g	Tsai et al., 2007
		secondhand smokers: 0.0073 mg/g	
		smokers: 1.93 mg/g	
	nicotine	non-smokers: 1.60 μg/mL	Doctor et al., 2004
		smokers: 5.40 μg/mL	
		chewers: 2.22 μg/mL	
		snuff users: 2.12 μg/mL	
	cotinine	non-smokers: 3.36 μg/mL	
		smokers: 10.28 μg/mL	
		chewers: 5.87 μg/mL	
		snuff users: 3.87 μg/mL	
	thiocyanate	non-smokers: 6.48 mg/L	Connolly et al., 2002
		smokers:	
		female: 11.62 mg/L	
		male: 17.10 mg/L	
	nitrate	non-smokers: 224 mg/L	
		smokers: female: 259 mg/L	
		male: 141 mg/L, 152 mg/L	
	cotinine	urine: 0.53–8.9 μg/L	Willers et al., 2000
	thiocyanate ion	urine: non-smokers: 84.0 μM	Glatz et al., 2001
		smokers: 216.5 μM	
	NIC GLUC - nicotine-N-(4-deoxy-4,5-didehydro)-β-D-glucuronide	5.2–46 ng/mL	Miller et al., 2010
	CNO - (S)-cotinine N-oxiol	4.1–37 ng/mL	
	3-HC-3′-hydroxycotinine	10–103 ng/mL	
	NCOT - norcotinine	4.3–38 ng/mL	
	NNO - trans nicotine-1′-oxide-methyl	0.90–99 ng/mL	
	COT - cotinine	9.6–88 ng/mL	
	NNIC - nornicotine	4.4–37 ng/mL	
	NIC - nicotine	5.4–46 ng/mL	
	AT - anatabine	8.7–83 ng/mL	
	AB-anabasine	4.0–37 ng/mL	
	COT GLUC - cotinine N-β-D-glucuronide	82–327 ng/mL	

(*Continued on next page*)

TABLE 9
Literature data on biomarkers detected and determined in samples of biological materials (*Continued*)

Biological material	Analyte(s)	Concentration range	Reference
Saliva	thiocyanate ion	non-smokers: 1.05 mM smokers: 2.05 mM	Glatz et al., 2001
	cotinine	0.3–2.6 μg/L	Willers et al., 2000
Plasma	NIC GLUC - nicotine-N-(4-deoxy-4,5-didehydro)-β-D-glucuronide	5.2–41 ng/mL	Miller et al., 2010
	CNO - (S)-cotinine N-oxiol	4.4–39 ng/mL	
	3-HC-3′-hydroxycotinine	1.0–92 ng/mL	
	NCOT - norcotinine	4.6–42 ng/mL	
	NNO - trans nicotine-1′-oxide-methyl	0.83–106 ng/mL	
	COT - cotinine	1.1–96 ng/mL	
	NNIC - nornicotine	4.8–46 ng/mL	
	NIC - nicotine	5.3–42 ng/mL	
	AT - anatabine	0.97–107 ng/mL	
	AB -anabasine	4.8–40 ng/mL	
	COT GLUC - cotinine N-β-D-glucoronide	74–434 ng/mL	
	protein-bound thiocyanate	non-smokers: 35.4 ng/mL smokers: 89.1 ng/mL	Youso et al., 2010
	nicotine	0.18–24.92 ng/mL	Nakajima et al., 2000
	cotinine	1.02–79.42 ng/mL	
Blood	cyanide ion	smokers: 509–686 pmol/mL non-smokers: 434–550 pmol/mL	Chinaka et al., 1998
	thiocyanate ion	smokers: 28.6–96.1 pmol/mL non-smokers: 5.2–16.6 pmol/mL	
Milk	nicotine (NIC)	40.0–240.0 μg/L	Pellegrini et al., 2007
	cotinine (COT)	20.5–173.9 μg/L	
	trans-3-hydroxycotinine (TRANS-3-OH-COT)	14.4–17.3 μg/L	
	cotinine-N-oxide (COT-N-OX)	5.0–18.4 μg/L	
Amniotic fluid	perchlorate	0.057–0.71 μg/L	Blount and Valentin-Blasini, 2006
	thiocyanate	1.7–170 μg/L	
	nitrate	$<$ 10–5860 μg/L	
	iodide	650–8900 μg/L	
Tears	lysozyme	smokers: 1217 μL/mL non-smokers: 1472 μL/mL	Satici et al., 2003
Semen	nicotine	12.4 ng/mL	Pacifici et al., 1995
	cotinine	5.2 ng/mL	

specimens. Figure 2 shows a general scheme of the procedures used for analyzing samples of biological materials with a complex matrix composition (Polkowska et al., 2004).

Analytical procedures that are useful generally require extraction and preconcentration steps. During extraction, the compounds are isolated from the matrix and concentrated to enable their identification or quantification. Environmental, biomedical, and other types of analyses use extraction techniques like liquid-liquid extraction (LLE) (Kintz et al., 1998; James et al., 1998; Man et al., 2006; Song et al., 2005), solid-phase extraction (SPE) (Connolly et al., 2002; Zuccaro et al., 1995; Wang and Lu, 2009; Bentley et al., 1999; Kim et al., 2000; Bazylak

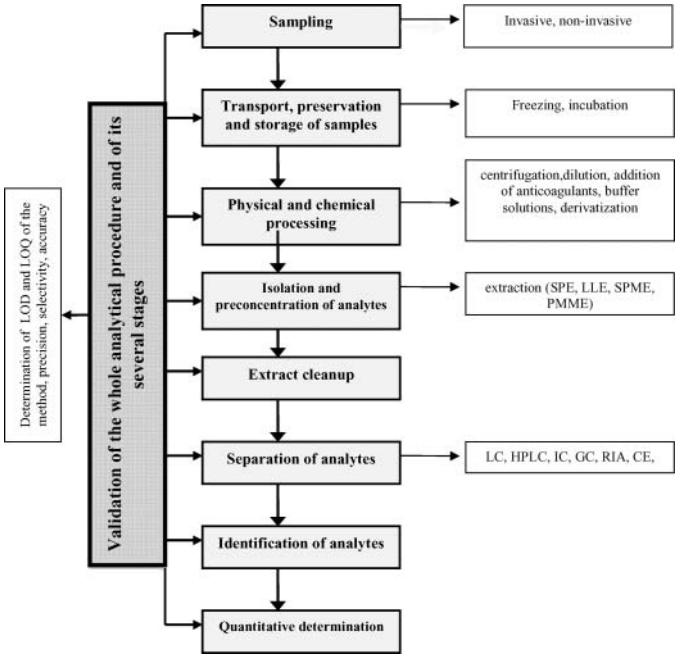


FIG. 2. General scheme of the procedures used for analyzing samples of biological materials with a complex matrix composition.

et al., 2000), solid-phase microextraction (SPME) (Manini et al., 2008), solid-liquid extraction (SLE) (Oddoze et al., 1998), and polymer monolith microextraction (PMME) (Zhang et al., 2006) (Table 7).

One of the most popular trends in analytical chemistry is the miniaturization of the sample, which enables solvent consumption in analytical laboratories to be reduced, and is expected to contribute significantly to the reduction of analytical costs (Kawaguchi et al., 2006). Table 8 lists basic information on the analytical methodologies applied in the determination of different types of biomarkers in samples of biological materials.

Modern analytical techniques enable substances that are contaminants and biomarkers specific to ETS to be detected at very low concentrations (ng/mL). Most literature reports provide data on biomarkers of exposure to ETS constituents, but research is also taking place on other contaminants, the occurrence of which is the result of being exposed to ETS constituents. Most literature data refer to biological materials and fluids that can be obtained noninvasively. This is because of the problems involved in obtaining permission from bioethics commissions to take samples invasively. In any case, samples that can be obtained in larger volumes are of greater interest, as this considerably facilitates their preparation and analysis. The literature data on concentrations of biomarkers and other ETS contaminants in samples of biological materials are summarized in Table 9.

CONCLUSION

Information about the state of the environment can be obtained not only from its abiotic part (air, water, soil), but also from its biotic part, and this includes biological materials obtained from humans (excreta, secretions, tissues, and physiological fluids), which are constantly exposed to a whole range of xenobiotics. These contaminants include the compounds formed during the smoking of tobacco. Tobacco smoke is a complex mixture of more than 4000 compounds, many of which are harmful to human health. A large proportion of these latter are proven toxicants or carcinogens.

Exposure to the constituents of environmental tobacco smoke (ETS) is ever more frequently being assessed on the basis of the measured concentrations of toxic substances or their metabolites in tissues, secretions, or excreta. In order to assess the extent to which the human body is exposed to the toxic substances present in ETS, substances specific to ETS—biomarkers—are determined, among them carbon monoxide, thiocyanate ion, and nicotine and its metabolites. One of the most troublesome aspects regarding the use of biological material for assessments of xenobiotic exposure is the choice of a representative sample for analysis.

The examination of samples of human physiological fluids and materials poses a great challenge to analysts. Many scientific institutions are working on the development of new analytical methodologies that have metrological parameters enabling them to be used on a routine basis. Samples of physiological fluids and biological materials have a very complex matrix, and in the vast majority of cases the direct determination of analytes using known analytical methodologies and techniques is not possible. Which technique is to be applied depends on the group of compounds to which the target biomarker belongs and on its half-life. In accordance with the principles of green chemistry, environmentally friendly extraction techniques are increasingly being used, which means that the consumption of toxic substances like organic solvents can be much reduced.

REFERENCES

Avşar, A.; Darka, Ö.; Bodrumlu, E. H.; Bek, Y. Evaluation of the Relationship between Passive Smoking and Salivary Electrolytes, Protein, Secretory IgA, Sialic Acid and Amylase in Young Children. *Arch. Oral Biol.* **2009**, *54*, 457–463.

Baker, R. R. Smoke Generation Inside a Burning Cigarette: Modyfing Combustion to Develop Cigarettes That May Be Less Hazardous to Health. *Energy Comb. Sci.* **2006**, *32*, 373–385.

Bazylak, G.; Brózik, H.; Sabanty, W. HPTLC Screening Assay for Urinary Cotinine as Biomarker of Environmental Tobacco Smoke Exposure among Male Adolescents. *J. Pharm. Biomed. Anal.*, **2000**, *24*, 113–123.

Bearer, C. F. Meconium as a Biological Marker of Prenatal Exposure. *Ambul. Pediatr.*, **2003**, *3*, 40–43.

Bentley, M. C.; Abrar, M.; Kelk, M.; Cook, J.; Phillips, K. Validation of an Assay for the Determination of Cotinine and 3-Hydroxycotinine in Human Saliva Using Automated Solid-Phase Extraction and

Liquid Chromatography with Tandem Mass Spectrometric Detection. *J. Chromatogr. B* **1999**, *723*, 185–194.

Blount, B. C.; Valentin-Blasini, L. Analysis of Perchlorate, Thiocyanate, Nitrate and Iodide in Human Amniotic Fluid Using Ion Chromatography and Electrospray Tandem Mass Spectrometry. *Anal. Chim. Acta* **2006**, *567*, 87–93.

Bochenek, A.; Reicher, M. *Anatomia człowieka, układ trawienny*; National Institute of Medical Publications: Warsaw, 1955, vol. III (in Polish).

Borgerding, M.; Klus, H. Analysis of Complex Mixtures—Cigarette Smoke. *Exp. Toxicol. Pathol.* **2005**, *57*, 43–73.

Chattaraj, S.; Das, A .K. Indirect Determination of Thiocyanate in Biological Fluids Using Atomic Absorption Spectrometry. *Spectrochim. Acta* **1992**, *5*, 675–680.

Chinaka, S.; Takayama, N.; Michigami, Y.; Ueda, K. Simultaneous Determination of Cyanide and Thiocyanide in Blood by Ion Chromatography with Fluorescence And Ultraviolet Detection. *J. Chromatogr. B* **1998**, *713*, 353–359.

Chou, S. Y.; Hsu, H. H.; Kuo, H. H.; Kuo, H. W. Association between Exposure to Environmental Tobacco Smoke (ETS) and Breastfeeding Behaviour. *Acta Pædiatr.* **2008**, *97*, 76–80.

Chung, S.; Baker, L.; Jenkins, P.; Julian, D.; Lum, S.; Miyasato, L.; Polakoff, J.; Shimer, D.; Takemoto, B.; Winder, B. *Proposed Identification of Environmental Tobacco Smoke as A Toxic Air Contaminant Part A: Exposure Assessment*, As approved by the Scientific Review Panel: June 24, 2005. California Environmental Protection Agency: Sacramento.

Collier, A. C.; Pritsos, C. A. Environmental Tobacco Smoke in the Workplace: Markers of Exposure, Polymorphic Enzymes and Implications for Disease State. *Chem.-Biol. Interact.* **2003**, *146*, 211–224.

Connolly, D.; Barron, L.; Paull, B. Determination of Urinary Thiocyanate and Nitrate Using Fast Ion-Interaction Chromatography. *J. Chromatogr. B* **2002**, *767*, 175–180.

Counts, M. E.; Hsu, F. S.; Laffoon, S. W.; Dwyer, R. W.; Cox, R. H. Mainstream Smoke Constituent Yields and Predicting Relationships from a Worldwide Market Sample of Cigarette Brands: ISO Smoking Conditions. *Regul. Toxicol. Pharmacol.* **2004**, *39*, 111–134.

DeMarini, D. M. Genotoxicity of Tobacco Smoke and Tobacco Smoke Condensate: A Review. *Mutat. Res.* **2004**, *567*, 447–474.

Doctor, P. B.; Gokani, V. N.; Kulkarni, P. K.; Parikh, J. R.; Saiyed, H. N. Determination of Nicotine and Cotynine in Tobacco Harvester's Urine by Solid-Phase Extraction and Liquid Chromatography. *J. Chromatogr. B* **2004**, *802*, 323–328.

Frost-Pineda, K.; Zedler, B. K.; Liang, Q.; Roething, H. J. Environmental Tobacco Smoke (ETS) Evaluation of a Third-Generation Electrically Heated Cigarette Smoking System (EHCSS). *Regul. Toxicol. Pharmacol.* **2008**, *52*, 118–121.

Gareri, J.; Klein, J.; Koren, G. Drugs of Abuse Testing in Meconium. *Clin. Chim. Acta* **2006**, *366*, 101–111.

Glatz, Z.; Novakova, S.; Sterbova, H. Analysis of Tiocyanate in Biological Fluids by Capillary Zone Electrophoresis. *J. Chromatogr. A* **2001**, *916*, 273–277.

Hamosh, M. Breastfeeding: Unraveling the Mysteries of Mother's Milk. *MedGenMed* [online] **1996**, http://www.medscape.com/viewarticle/718175 (accessed 5/26/2011).

Heinrich-Ramm, R.; Wegner, R.; Garde, A. H.; Baura, X. Cotinine Excretion (Tobacco Smoke Biomarker) of Smokers and Non-Smokers: Comparison of GC/MS and RIA Result. *J. Hyg. Environ. Health* **2002**, *205*, 493–499.

Henderson, A. J. The Effects of Tobacco Smoke Exposure on Respiratory Health in School-Aged Children. *Pediatr. Resp. Rev.* **2009**, *9*, 21–28.

Howard, S.; Sanderow, D. L. *The Invisible Environment Fact Sheet Series Environmental Tobacco Smoke*; The Ohio State University, 2008.

Iyengar, G. V.; Rapp, A. Human Placenta as a 'Dual' Biomarker for Monitoring Fetal and Maternal Environment with Special Reference to Potentially Toxic Trace Elements. Part 1: Physiology, Function and Sampling of Placenta for Elemental Characterization. *Science. Total Environ.* **2001**, *280*, 195–206.

James, H.; Tizabi, Y.; Taylor, R. Rapid Method for the Simultaneous Measurement of Nicotine and Cotinine in Urine and Serum by Gas Chromatography–Mass Spectrometry. *J. Chromatogr. B* **1998**, *708*, 87–93.

Kasperczyk, A.; Ostałowska, A.; Grucka-Mamczar, E.; Birkner, E. Porównanie stężenia kadmu, cynku i selenu we krwi i w nasieniu ludzkim. *Bromat. Chem. Toksykol.* **2008**, *1*, 81–87.

Kaufman, F. L.; Kharrazi, M.; Delorenze, G. N.; Eskenazi, B.; Bernert, J. T. Estimation of Environmental Tobacco Smoke Exposure during Pregnancy Using a Single Question on Household Smokers versus Serum Cotinine. *J. Expo. Anal. Environ. Epidemiol.* **2002**, *12*, 286–295.

Kawaguchi, M.; Ito, R.; Saito, K.; Nakazawa, H. Novel Stir Bar Sorptive Extraction Methods for Environmental and Biomedical Analysis. *J. Pharm. Biomed. Anal.* **2006**, *40*, 500–508.

Kim, I.; Darwin, W. D.; Huestis, M. A. Simultaneous Determination of Nicotine, Cotinine, Norcotinine, and *Trans*-3′-Hydroxycotinine in Human Oral Fluid Using Solid Phase Extraction and Gas Chromatography–Mass Spectrometry. *J. Pharm. Biomed. Anal*. **2000**, *24*, 113–123.

Kintz, P.; Henrich, A.; Cirimele, V.; Ludes, B. Nicotine Monitoring in Sweat with a Sweat Patch. *J. Chromatogr. B* **1998**, *705*, 357–361.

Kośmicki, M. Palenie tytoniu przez pacjentów z chorobą niedokrwienną serca - możliwości terapii. *Przew. Lek.* **2001**, *4*, 38–47.

Kratz, E. M.; Pupek, M.; Chełmowska-Soyta, A.; Kątnik-Prastowska, I. The Molecular Forms of Immunoglobulin A in Human Seminal Plasma. *Adv. Clin. Exp. Med.* **2004**, *13*, 541–547.

Krzemieniecki, J. Szkodliwość biernego palenia tytoniu, *Służby życiu*, [Online] **2001**, http://www.pro-life.pl/_ow/read.php?y = Sluzba_Zyciu&n = Nikotynizm&a = 04_szkodliwosc (accessed 6/13/2011).

Man, C. N.; Gam, L.-H., Ismail, S.; Lajis, R.; Awang, R. Simple, Rapid and Sensitive Assay Method for Simultaneous Quantification of Urinary Nicotine and Cotinine Using Gas Chromatography–Mass Spectrometry. *J. Chromatogr. B* **2006**, *844*, 322–327.

Man, C. N.; Fathelrahman, A. I.; Harn, G. L.; Lajis, R.; Shalihin, A.; Samin, M.; Omar, M.; Awang, R.; Bayanuddin, N. A. Correlation between Urinary Nicotine, Cotinine and Self-Reported Smoking Status among Educated Young Adults. *Environ. Toxicol. Pharmacol.* **2009**, *28*, 92–96.

Manini, P.; De Palma, G.; Andreoli, R.; Poli, D.; Petyx, M.; Corradi, M.; Mutti, A.; Apostoli, P. Biological Monitoring of Low Benzene Exposure in Italian Traffic Policemen. *Toxicol. Lett.* **2008**, *181*, 25–30.

Miller, M. D.; Broadwin, R.; Green, S.; Marty, M. A.; Polakoff, J.; Salmon, A. G.; Winder, B. *Proposed identification of Environmental Tobacco Smoke as a Toxic Air Contaminant Part B: Health Effects*,

As approved by the Scientific Review Panel: June 24, 2005. California Environmental Protection Agency: Sacramento.

Miller, E. I.; Norris, H. R. K.; Rollins, D. E.; Tiffany, S. T.; Wilkins, D. G. A Novel Validated Procedure for the Determination of Nicotine, Eight Nicotine Metabolites and Two Minor Tobacco Alkaloids in Human Plasma or Urine by Solid-Phase Extraction Coupled with Liquid Chromatography–Electrospray Ionization–Tandem Mass Spectrometry. *J. Chromatogr. B* **2010**, *878*, 725–737.

Moriya, F.; Hashimoto, Y. Nicotine and Cotinine Levels in Blood and Urine from Forensic Autopsy Casus. *Leg. Med.* **2004**, *6*, 164–169.

Nakajima, M.; Yamamoto, T.; Kurowa, Y.; Yokoi, T. Improved Highly Sensitive Method for Determination of Nicotine and Cotinine in Human Plasma by High-Performance Liquid Chromatography. *J. Chromatogr. B* **2000**, *742*, 211–215.

Noguera-Obenza, M. J.; Ochoa, T.; Gomez, H. F.; Lourdes Guerrero, M.; Herrera-Insua, I.; Morrow, A. L.; Ruiz-Palacios, G.; Pickering, L. K.; Guzman, C. A.; Celary, T. G. Human Milk Secretory Antibodies against Attaching and Effacing Escherichia coli. *Emerg. Infect. Dis.*, [Online] **2003**, http://wwwnc.cdc.gov/eid/article/9/5/02-0441_article.htm (accessed January 12, 2011).

Nusbaum, M. L.; Gordon, M.; Nusbaum, D.; McCarthy, M. A.; Vasilakis, D. Smoke Alarm: A Review of the Clinical Impact of Smoking on Women. *Prim. Care Update Ob. Gyns.* **2000**, *7*, 207–214.

Oddoze, C.; Pauli, A. M.; Pastor, J. Rapid and Sensitive High-Performance Liquid Chromatographic Determination of Nicotine and Cotinine in Nonsmoker Human and Rat Urines. *J. Chromatogr. B* **1998**, *708*, 95–101.

Olds, D. Tobacco Exposure and Impaired Development: A Review of the Evidence. *Ment. Retard. Dev. Disabil. Res. Rev*. **1997**, *3*, 257–269.

Page-Sharp, M.; Hale, T. W.; Hackett, L. P.; Kristensen, J. H.; Ilett, K. F. Measurement of Nicotine and Cotynine in Human Milk by High-Performance Liquid Chromatography with Utlraviolet Absorbance Detection. *J. Chromatogr. B* **2003**, *796*, 173–180.

Pellegrini, M.; Marchei, E.; Rossi, S.; Vagnarelli, F.; Durgbanshi, A.; Garcia-Algar, O.; Vall, O.; Pichini, S. Liquid Chromatography/Electrospray Ionization Tandem Mass Spectrometry Assay for Determination of Nicotine and Metabolites, Caffeine and Arecoline in Breast Milk. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 2693–2703.

Polańska, K.; Hanke, W.; Laudański, T.; Kalinka, J. Stężenie kotoniny w osoczu jako biomarker czynnej i biernej ekspozycji kobiet ciężarnych na dym tytoniowy. *Ginekol Pol*. **2007**, *78*, 796–801.

Polkowska, Ż.; Kozłowska, K.; Witkowska, E.; Namieśnik, J. *Bioanalityka w ocenie zanieczyszczeń środowiska*; Wardencki, W.: Gdańsk, 2004, pp 75–101.

Pré, J.; Vassy, R. Urine Thiocyanate: Creatinine Ratio as a Reliable Indicator of Cigarette Smoking. *Clin. Chim. Acta* **1991**, *204*, 87–94.

Pritchard, J. D. *Hydrogen Cyanide Toxicological Overview*, Version 2; Health Protecion Agency, 2002.

Rogers, J. M. Tobacco and Pregnancy: Overview of Exposures and Effects. *Birth Defects Res*. **2008**, *84*, 1–15.

Rothberg, M.; Heloma, A.; Svinhufvud, J.; Kähkönen, E.; Reij, K. Measurement and Analysis of Nicotine and Other VOCs in Indoor Air as an Indicator of Passive Smoking. *Ann. Occup. Hyg*. **1998**, *42*, 129–134.

Rummenie, V. T.; Matsumoto, Y.; Dogru, M.; Wang, Y.; Hu, Y.; Ward, S. K.; Igarashi, A.; Wakamatsu, T.; Ibrahim, O.; Goto, E.; Luyten, G.; Inoue, H.; Saito, I.; Shimazaki, J.; Tsubota, K. Tear Cytokine and Ocular Surface Alterations Following Brief Passive Cigarette Smoke Exposure. *Cytokine* **2008**, *43*, 200–208.

Satici, A.; Bitiren, M.; Ozardali, I.; Vural, H.; Kilic, A.; Guzey, M. The Effects of Chronic Smoking on the Ocular Surface and Tear Characteristics: A Clinical, Histological and Biochemical Study. *Acta Ophthalmol. Scand.* **2003**, *86*, 583–587.

Scherer, G. Biomonitoring of Inhaled Complex Mixtures—Ambient Air, Diesel Exhaust and Cigarette Smoke. *Exp. Toxicol. Pathol*. **2005**, *57*, 75–110.

Scherer, G. Carboxyhemoglobine and Thiocyanate as Biomarkers of Exposure to Carbon Monoxide and Hydrogen Cyanide in Tobacco Smoke. *Exp. Toxicol. Pathol*. **2006**, *58*, 101–124.

Scherer, G.; Urban, M.; Hagedorn, H. W.; Serafin, R.; Shixia, F.; Kapur, S.; Muhammad, R.; Yan Jin; Sarkar, M.; Roethig, H. J. Determination of Methyl-, 2-Hydroxyethyl- and 2-Cyanoethylmercapturic Acids as Biomarkers of Exposure to Alkylating Agents in Cigarette Smoke. *J. Chromatogr. B* **2010**, *878*, 2520–2528.

Seccareccia, F.; Zuccaro, P.; Pacifici, R.; Meli, P.; Mannozo, F.; Frejman, K. M.; Santaquilani, A.; Giampaoli, S.; the Research Group of the MATISS Project. Serum Cotinine as a Marker of Environmental Tobacco Smoke Exposure in Epidemiologic Studies: The Experience of the MATISS project. *Eur. J. Epidem.* **2003**, *18*, 487–492.

Shen, H.; Chia, S.; Ni, Z.; New, A.; Lee, B.; Ong, C. Detection of Oxidative DNA Damage in Human Sperm and the Association with Cigarette Smoking. *Reprod. Toxicol.* **1997**, *11*, 675–680.

Song, L.; Davis, W.; Abrams, S. M.; Hemiup, J.; Kazim, A. L.; Cummings, K. M.; Mahoney, M. C. Sensitive and Rapid Method for the Determination of Urinary Cotinine in Non-Smokers: An Application for Studies Assessing Exposures to Second Hand Smoke (SHS). *Anal. Chim. Acta* **2005**, *545*, 200–208.

Stedman, R. L. The Chemical Composition of Tobacco and Tobacco Smoke. *Chem. Rev.* **1968**, *68*, 153–207.

Sylwanowicz, W.; Michalik, A.; Ramotowski, W. *Anatomia i fizjologia człowieka*; National Institute of Medical Publications: Warsaw, 1980 (in Polish).

Tong, X. L.; Wang, L.; Gao, T. B.; Qin, Y.G.; Qi, Y.Q.; Xu, Y. P. Potential Function of Amniotic Fluid in Fetal Development—Novel Insights by Comparing the Composition of Human Amniotic Fluid with Umbilical Cord and Maternal Serum at Mid and Late Gestation. *J. Chin. Med. Assoc.* **2009**, *72*, 368–373.

Topinka, J.; Milcova, A.; Libalova, H.; Novakova, Z.; Rossner, Jr., P.; Balascak, J.; Srama, R. J. Biomarkers of Exposure to Tobacco Smoke and Environmental Pollutants in Mothers and Their Transplacental Transfer to the Foetus. Part I: Bulky DNA Adducts. *Mutat. Res.* **2009**, *669*, 13–19.

Torikaiu, K.; Uwano, Y.; Nakamori, T.; Tarora, W.; Takahashi, H. Study on Tobacco Components Involved in the Pyrolytic Generation of Selected Smoke Constituents. *Food Chem. Toxicol.* **2005**, *43*, 559–568.

Tsai, H. T.; Tsai, Y. M.; Yang, S. F.; Wu, K. Y.; Chuang, H. Y.; Wu, T. N.; Ho, C. K.; Lin, C. C.; Kuo, Y. S.; Wu, M. T. Lifetime Cigarette Smoke and Second-Hand Smoke and Cervical Intraepithelial Neoplasm—A Community-Based Case-Control Study. *Gyn. Oncol.* **2007**, *105*, 181–188.

Wang, V.-S.; Lu, M.-Y. Application of Solid-Phase Microextraction and Gas Chromatography–Mass Spectrometry for Measuring Chemicals in Saliva of Synthetic Leather Workers. *J. Chromatogr. B* **2009**, *877*, 24–32.

Willers, S.; Amon, A.; Feyerabend, C.; Nielsen, J.; Skarping, G.; Skerfving, S. Assesment of environmental tobacco smoke exposure in children with asthmatic symptoms by questionnaire and cotinine concentrations in plasma, saliva, and urine. *J. Clin. Epidemiol.* **2000**, *53*, 715–721.

Wong, W. Y.; Thomas, C. M. G.; Markus, H. M. W. M.; Gielhuis, G. A.; Doesburg, W. H.; Steegers-Theunissen, R. P. M. Cigarette Smoking and the Risk of Male Factor Subfertility: Minor Association between Cotinine, Seminal Plasma and Semen Morphology. *Fertil. Steril.* **2000**, *74*, 930–935.

World Health Organization. (2008). *The MPOWER package. Fresh and alive*, World Health Organization Report on the Global Tobacco Epidemic; World Health Organization, 2008.

Yildiz, D. Nicotine, Its Metabolism and an Overview of Its Biological Effects. *Toxicon* **2004**, *43*, 619–632.

Youso, S. L.; Rockwood, G. A.; Lee, L. P.; Logue, B. A. Determination of Cyanide Exposure by Gas Chromatography–Mass Spectrometry Analysis of Cyanide-Exposed Plasma Proteins. *Anal. Chim. Acta* **2010**, *677*, 24–28.

Zhang, H.-J.; Huang, J.-F.; Wang, H.; Feng, Y.-Q. Determination of Low-Aliphatic Aldehyde Derivatizatives in Human Saliva Using Polymer Monolith Microextraction Coupled to High-Performance Liquid Chromatography. *Anal. Chim. Acta* **2006**, *565*, 129–135.

Zuccaro, P.; Altieri, I.; Rosa, M.; Passa, A. R.; Pichini, S.; Pacifici; R. Solid-Phase Extraction of Nicotine and Its Metabolites for High-Performance Liquid Chromatographic Determination in Urine. *J. Chromatogr. B* **1995**, *668*, 187–188.