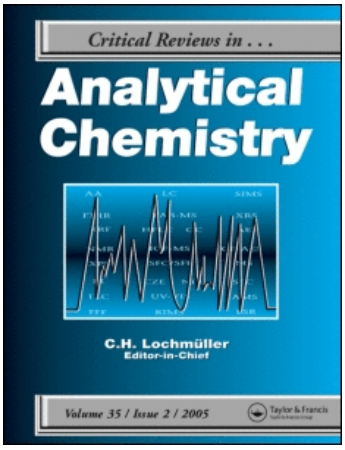

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Biological Fluids as a Source of Information on the Exposure of Man to Environmental Chemical Agents

Żaneta Polkowska[a]; Katarzyna Kozłowska[a]; Jacek Namieśnik[a]; Andrzej Przyjazny[b]
[a] Department of Analytical Chemistry, Chemical Faculty, Technical University of Gdańsk, Gdańsk, Poland [b] Science & Mathematics Department, Kettering University, Flint, USA

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Biological Fluids as a Source of Information on the Exposure of Man to Environmental Chemical Agents

Żaneta Polkowska, Katarzyna Kozłowska, and Jacek Namieśnik
Department of Analytical Chemistry, Chemical Faculty, Technical University of Gdańsk, Gdańsk, Poland

Andrzej Przyjazny
Science & Mathematics Department, Kettering University, Flint, Michigan, USA

The human body is exposed to a variety of environmental pollutants. Many chemical compounds, including volatile organochlorine compounds and metals, have a harmful effect on the tissues and organs of the human body, in many cases causing irreversible damage and illnesses. In order to assess the degree of occupational hazard, it has become more and more common to determine the concentrations of chemical substances or their metabolites in biological fluids (i.e., in urine, blood, or less often, in human milk, bile, saliva, and sperm). However, in order to determine concentrations levels of trace components in biological fluids such as urine, blood, milk, bile, saliva, or sperm, the samples collected have to be prepared for the final analysis because their matrix is so complex as to preclude direct determination of the analytes by any analytical method available. This article contains the literature data on: analyte isolation and/or enrichment techniques from samples of biological fluids prior to the final determination step; and final determination techniques for a variety of compounds, both organic and inorganic, being determined in the above samples. The data include both the primary pollutants that found their way into the human body as a result of environmental and occupational exposure and the products of their conversion (metabolism) in the organism.

Keywords biomarkers, biomonitoring, enrichment, human fluids, isolation

Sources of information about the state of the environment can be the results of analysis of samples of both the abiotic part of the environment (air, water, soil) and the biotic part, including tissues and biological fluids of humans who are constantly exposed to a broad spectrum of xenobiotics.

The analysis of samples of human physiological fluids presents a formidable challenge to the analyst. In order to determine in such samples metals and a variety of organic compounds, the samples have to be subjected to tedious and time-consuming sample preparation operations. Samples of physiological fluids are characterized by a very complex matrix, which essentially precludes direct determination of the analytes using common analytical procedures and techniques.

Recent literature contains increasingly more information on:

- New analytical procedures for the examination of biological fluids, primarily human urine and blood;
- Determined concentration levels of a wide variety of analytes in samples of physiological fluids; and
- Assessment of the effect of environmental and occupational exposure on the concentration level of various types of chemical compounds detected in samples of human biological fluids.

BIOLOGICAL FLUIDS

Directly or following some conversions, xenobiotics absorbed by the human body can circulate throughout the

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Address correspondence to Żaneta Polkowska, Department of Analytical Chemistry, Chemical Faculty, Technical University of Gdańsk, 11/12 Gabriela Narutowicza Str., 80-952 Gdańsk, Poland. E-mail: chemanal@pg.gda.pl

organism with physiological fluids. They can undergo accumulation in various tissues and organs or they can be excreted from the organism unchanged or as polar metabolites. These processes depend on both physiological factor (state of health of the organism) and physicochemical properties of the absorbed substance. Xenobiotics or their metabolites can be present in: cerebrospinal fluid, milk, sweat, tears, saliva, sperm, amniotic fluid, bile, lymph, blood, or urine. The determination of toxic substances or their metabolites is carried out mainly in samples of blood, urine, and exhaled air.

Due to the fact that the sample preparation, analyte isolation, and analysis are difficult tasks, two biological fluids—blood (plasma) and urine—have become of greatest interest of toxicologists, ecotoxicologists, and analysts. A number of sample preparation techniques as well as complete analytical procedures have been developed that enable determination of a variety of components present at trace and ultratrace levels. A brief characteristic of selected biological fluids is presented below.

Urine

As a result of its properties and the method of sample collection, urine is used most often for the assessment of degree of environmental and occupational exposure. Urine is a fluid made in the kidneys in the amount of about 1500 mL per day. In addition to water (95%), it contains hazardous and extraneous products of metabolism (mainly urea). Urine is a material rich in information regarding the functioning of the human body and the effects of its exposure to hazardous substances coming from the environment. The strategy of collection of urine samples depends on the kinetics of excretion of the investigated substances.

The collection and analysis of urine samples carry no associated risk, and the sample volume can be relatively large (up to 800 mL). The optimum method is to collect urine samples over a period of time and to express the results as a rate of excretion, (e.g., in mg/h). During field sampling, only singles urine samples are usually available. Due to the fact that the concentration of many analytes depends on the extent of diuresis, which can undergo substantial fluctuations, the results are generally standardized.

The most commonly used method involves standardizing the results of determination with respect to relative density of urine (Equation 1):

$$C_{st} = C \times \left(\frac{\text{average urine density}}{\text{density of investigated urine}} \right) \qquad [1]$$

where:

C_{st} = analyte concentration in urine after standardization
C = analyte concentration determined experimentally

Another common method of standardization is to express the results per gram of creatinine. The use of a specific method of correction for diuresis aims at obtaining the best correlation between the exposure and the excretion of a substance or its metabolite with urine (1).

In case of medical analysis, the basic examination of urine includes the determination of: specific gravity, color, clarity, pH, and the content of proteins, sugar, ketone bodies, and bile pigments. A more extended examination includes the determination of properties of urine sediment (i.e., the amount of epithelia, red and white blood cells, casts, and mineral components). In recent years, in addition to typical morphological studies of urine, there have been increasing attempts to determine a variety of individual chemical species. Urine can contain specific chemical compounds, xenobiotics, or their metabolites (biomarkers), which normally should be absent. Biomarkers are indicators of changes, which may take place in biological systems as a result of interaction of hazardous agents of a very diverse structure and origin. Biomarkers used to determine the concentration of carcinogens or their metabolites in biological material fall into two categories (2): selective biomarkers (this kind is used in analytical practice) and nonselective biomarkers. This kind of urine analysis may help indicate the source of a problem, such as contaminated air, water, or food eaten by humans.

Significant progress in clinical analysis taken place recently has resulted in much improved determinations of chemicals or their metabolites with high precision and accuracy. Examples of chemical compounds and biomarkers detected in urine are shown in Table 1.

The fact that, for the majority of biomarkers, there is no information regarding their allowed concentrations in biological fluids is a serious problem. Consequently, they can be used only to establish the exposure to a xenobiotic, but the health hazard caused by this ecotoxin cannot be assessed (41).

Blood

Blood along with lymph, intercellular fluid, and cerebrospinal fluid, form the internal medium of the organism. Blood morphology is among the most common tests performed in clinical analyses. It allows assessing the state of health of the examined person, to detect inflammations, poisonings, and other pathological processes taking place in the body. Blood is a universal link between all cells of the organism.

So far, blood has been used less often than urine as a biological material for investigations. This is due to the fact that the collection of blood samples is an invasive process and the blood volume that can be collected does not exceed 20 mL. In addition, sometimes there are problems with obtaining consent to collect blood samples for examination. Blood analysis is usually carried out in cases of exposure of individuals to substances that are excreted very slowly from the organism (e.g., lead or cadmium). The determination of unchanged forms of solvents after cessation of exposure is also performed in order to assess occupational hazard (50). Examples of xenobiotics determined in blood samples are shown in Table 2.

TABLE 1
Examples of Biomarkers Present in Urine and Used for Assessment of Human Exposure to Various Xenobiotics

Hazardous substance	Sources of pollution	Biomarker of exposure (metabolite) detected in urine
Inorganic compounds (1, 3–27)		
Nickel	Potable water, air	Nickel(II)
Mercury (0)	Air (vapor), food	Total mercury
Platinum	Air (vapor), traffic	Platinum
Bismuth	Drugs Cosmetics	Bismuth
Magnesium chromite Chromium	Air (vapor)	Chromium(IV)
Arsenic	Air, food, potable water	As^{+3}, As^{+5} Methyl derivatives of arsenic acids
Cadmium	Air, cigarette smoke	Cadmium
Zinc	Air	Zinc
Lead and its inorganic compounds	Air Atmosphere	Lead 5-Aminolevulinic acid
Fluoride	Air	Fluoride
Organic compounds (1, 28–49)		
Benzene	Air	Phenol, catechol, hydroquinone, muconic acid
Polycyclic aromatic hydrocarbons		1-Hydroxy-benzo(a)pyrene
Ethylbenzene		Mandelic acid
Phenol		Phenol
n-Hexane		2,5-Hexanodione
Toluene		Toluene, benzoic acid, *o*-cresol, hippuric acid, *p*-toluylmercapturic acid
Styrene		Mandelic acid
Xylene		Methylhippuric acid
2-Naphthylamine		2-Naphthylamine
Benzidine		*N*,*N*-Diacetylbenzidine
Phenacetin		*N*-Acetyl-*p*-aminophenol
Acrylonitrile		Acrylonitrile Isothiocyanates
Methyl alcohol		Methyl alcohol Methyl alcohol, formic acid
1,4-Dioxane		β-Hydroxyethoxyacetic acid
2-Ethyltoluene		Sum of *o*-ethylbenzoic and *o*-tolylacetic acid
3-Ethyltoluene		*m*-Ethylbenzoic acid
4-ethyltoluene		*p*-Ethylbenzoic acid
(1,2,4)-trimethylbenzenes (Pseudocumene)	Air	Sum of 2,4-, 2.5-, and 3.4-dimethylbenzoic acids
(1,3,5)-trimethylbenzen (Mesitylene)		3,5-Dimethylbenzoic acid
(1,2,3)-trimethylbenzen (Hemimellitene)		Sum of 2,3- and 2.6-dimethylbenzoic acids
Halohydrocarbons		
Chloroform	Air, potable water, swimming pool water	Chloroform
1,1,1-Trichloroethane	Air, potable water	Trichloroethanol or its glucuronate
Trichloroethene		Trichloroacetic acid, trichloroethanol, or its glucuronate
Tetrachloroethene		Trichloroacetic acid Tetrachloroethene

TABLE 2
Examples of Hazardous Substances Determined in Blood Samples (to Assess the Degree of Exposure)

		Blood sample donors—kind of exposure	
Hazardous substance	Source of exposure	Endemic	Occupational
Inorganic compounds (1, 5, 6, 8–10, 14, 22, 24, 27)			
Chromium	Air	—	Production of stainless steel boilers, aircraft assembly (binders containing magnesium chromite)
Mercury	Food	Consumers of fish and seafood	Production of dry cells; production of chlorine; extraction of gold from ore concentrates; repair of thermometers, vacuum pumps, transformers, mercury lamps, barometers, and thermostats; dentists (using amalgams for tooth fillings)
Lead	Air	—	Lead smelting from ores or scrap; welding and cutting metal constructions painted with paints containing lead; casting nonferrous metals; production of batteries, pigments, lead glass; lead firing during production of ceramics; repair of automotive radiators; mining lead ores
Cadmium	Air	—	Production of zinc, NiCd batteries, alloys, cadmium pigments; welding metals coated with anticorrosive layer of cadmium
Carbon monoxide	Air	Internal combustion engines (underground parkings, tunnels), Tobacco smoking, house interiors with poor ventilation (kitchens)	—
Organic compounds (1, 45–47, 51, 52)			
Styrene	Air	—	Production of boats made from fiberglass reinforced with resin
Trihalomethanes (THM)	Water, air	Swimmers in indoor swimming pools	—
Tetrachloroethene	Air	—	Dry cleaning
Trimethylbenzene	Air	Painting of construction woodwork, interior and exterior plaster	Car body painting (automobile production)
Polychlorinated biphenyls (PCB)	Air	—	Contaminated indoor air in schools (teachers)
Organophosphorus pesticides	Air		Soil cultivation—farmers

TABLE 3
Examples of Hazardous Substances Determined in Saliva Samples (to Assess the Degree of Occupational Exposure)

Excretion path	Kind of excreted xenobiotics
Saliva	Drugs (penicillin, streptomycin, barbiturates, salicylic acid, quinidine) Ethanol Nicotine Pesticides (carbaryl, kepone) Metals (mercury, cadmium, lead, strontium)

Saliva

Saliva is a fluid secreted by the parotid, submandibular, and sublingual salivary glands as well as other, minor glands located in the lips, inner cheek area, and other linings of mouth and throat. The volume of secreted saliva and its composition depend on the age, sex, and the kind of stimulant. The major component of saliva is water, which constitutes about 99.5%, the rest being solids: 0.2% inorganic and 0.3% organic. Saliva is a material relatively readily available for examination. Prior to its collection, the mouth should be rinsed with warm water, and then about 6–8 mL of the saliva should be allowed to drip freely. The first batch is discarded, and the next ones are collected for examination. A number of organic and inorganic compounds as well as drugs are excreted with saliva. The concentration of a drug in saliva reflects the concentration in serum of the fraction of the drug not bound to protein. Unfortunately, only a few drugs have a constant value of the partition coefficient between saliva and serum. This accounts for the limited use thus far of saliva as a biological material in toxicological analysis (53). Examples of xenobiotics excreted with saliva are shown in Table 3.

Bile

The liver serves as a filter protecting the organism from the action of a number of toxins. Substances absorbed in the gastrointestinal tract are transferred in their entirety through the portal vein to the liver before they enter general circulation. In the liver, they can bind to proteins, undergo biotransformation, or be excreted with bile in an unchanged form or as metabolites (53). During the excretion of xenobiotics with bile a large role is played by the size of a molecule, while their physicochemical properties play only a minor role. Excreted with bile are mainly high-molecular-weight compounds with molar masses over 300–500, primarily in the form of polar conjugation products (53, 54). Polychlorinated insecticides, polynuclear aromatic hydrocarbons, drugs, some alkaloids, and metals such as manganese, silver, organomercury compounds, lead, and zinc are also eliminated through this path.

Substances are transferred along with bile to the intestines, where they can be excreted with feces or reabsorbed to blood.

TABLE 4
Types of Xenobiotics Excreted with Saliva, Sweat and Milk (55–62)

Excretion path	Kind of excreted xenobiotics
Sperm	Polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), styrene
Sweat	Ethanol, salicylic acid, benzoic acid, phenazone, lead, arsenic, mercury, iron, iodine, bromine, phenol
Milk	Drugs (inhalational general anesthetics, barbiturates, tetracyclines, thiazides, lithium salts), ethanol, nicotine, insecticides, chlorinated pesticides, polychlorinated biphenyls, ethers of polybrominated biphenyls, methylmercury, lead, cadmium, copper, radioactive elements (^{90}Sr, ^{131}I)

The substances most often reabsorbed are lipophilic compounds, which reenter the liver (53).

Excretion of Hazardous Substances from the Organism via Other Paths

Small amounts of xenobiotics are excreted from the organism with sperm, sweat, and milk. Xenobiotics excreted with saliva are usually swallowed and enter the gastrointestinal tract. The types of xenobiotics excreted with saliva, sweat, and milk are listed in Table 4. Xenobiotics penetrating into milk can cause poisonings or allergies in infants (53).

PREPARATION OF SAMPLES OF BIOLOGICAL FLUIDS FOR ANALYSIS

The concentrations of toxic substances or their metabolites in biological materials are usually variable and the variability depends on the rhythm of exposure and the biological half-life of a xenobiotic in the body. Consequently, it is vital to strictly adhere to the schedule of sample collection, particularly in case of substances with short biological half-lives. In addition urine, blood, saliva, bile, and sperm are biological materials that have a very complex organic matrix, which precludes their direct analysis. Preparation of biological samples for analysis is a complicated task and the operations and processes included in this step can result both in analyte losses and the source of contamination and errors (63). The analytical process includes the following steps:

1. sample collection;
2. sample transport, preservation, and storage;
3. physical and chemical treatment;
4. analyte isolation and enrichment;
5. extract clean-up;

6. separation of analytes;
7. identification of analytes;
8. quantitative determination; and
9. validation of analytical procedures used.

The steps that are of vital importance for the quality of analytical results will be discussed below.

Sample Collection

Urine samples should be collected in time described in the analytical protocol. Samples overly dilute or concentrated should be rejected and an appropriate procedure correcting for the degree of diuresis selected. A number of physiological factors can affect the concentration of toxic substances in urine. For example, a fivefold increase in the concentration of chromium was observed following a 2-h run. Kidney damage can result in retention in the organism of compounds excreted primarily with urine.

The concentration of toxic substances in blood is also affected by the condition of a donor. In a standing position the volume of plasma decreases, which can result in a 10% increase in concentration of nondiffusing substances. Meal consumption influences the blood content of triglycerides, which can cause changes in distribution of fat-soluble organic compounds. Physical exercise results in changes in blood plasma, most likely due to leakage of intracellular components. Intensive exercise brings about dilution of blood and a decrease in concentration of substances bound to erythrocytes. During pregnancy the volume of plasma increases by one-third, which results in changes in concentration of many blood components. Accordingly, standardization of conditions of collection of blood samples is recommended.

The results of biomonitoring also can be distorted by disorders of some organs. Liver diseases can influence metabolic clearance of substances undergoing transformations in this organ or being excreted with bile (1).

Transport, Preservation, and Storage of Samples

A number of factors, such as evaporation, precipitation, adsorption, lability, and redistribution can affect the stability of concentration of analytes in samples of biological fluids.

On storage, urine samples tend to undergo precipitation. Trace elements can undergo coprecipitation or adsorption on the surface of the precipitate. The extent of this phenomenon varies and depends on the kind of element and pH of urine (losses of nickel amounted to 1% at pH 1 and 6% at pH 6) (1). During the storage of samples of acidified urine for 2 days, an increase in concentration of arsenic, copper, antimony, chromium, mercury, selenium, and zinc in the precipitate was observed. On the other hand, manganese, cobalt, cesium, and rubidium remained in the supernatant. No losses of chromium and nickel were observed in acidified urine samples stored in polyethylene containers at 6°C or −20°C. The results of a number of investigations demonstrate lack of losses of lead in blood samples stored in plastic or glass containers. Precipitation in urine samples does not affect the concentration of organic compounds. However, insufficient data are available on the stability of volatile organic compounds in samples of biological material. Numerous organic compounds are unstable in biological matrices due to the action of enzymes or bacteria present in urine. Phenylglyoxylic, hippuric, and methylhippuric acids were found to be unstable in urine refrigerated or stored at room temperature, while benzoic, methylbenzoic, and mandelic acids remained stable for several weeks. The concentration of components such as phenol and tri-, tetra-, and pentachlorophenol did not change in urine samples stored in a refrigerator (1).

In cases of blood and serum, the concentration of chromium was found to remain constant in samples stored for 4 days, 3 weeks, and over 18 months at room temperature, −4°C, and −10°C, respectively, in plastic test tubes (1). On the other hand, a rapid decrease in concentration of toluene and ethylbenzene from blood samples stored in open test tubes was observed. In contrast, there were no changes in concentration of toluene in blood stored in glass test tubes closed with stoppers having aluminum or Teflon liners (1).

Physical and Chemical Treatment

One of the methods of preparation of urine samples for the determination of heavy metals (e.g., chromium, cadmium) involves dilution with nitric acid. Other reagents, such as potassium dichromate or sulfuric acid and hydrogen peroxide, are also added to urine samples. Samples with added reagents are heated to 120–140°C (e.g., arsenic). On the other hand, blood is deproteinated with a nitric acid solution (1).

Urine containing metabolites of toluene, xylene, and styrene is subjected to alkaline hydrolysis, and the analytes are converted to nonpolar derivatives by silylation using *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). In order to determine phenol in urine samples, the urine is subjected to acid hydrolysis, followed by extraction with diethyl ether (1).

Isolation and Enrichment of Analytes

One of the major steps in preparation of a biological sample for analysis is analyte isolation and/or enrichment involving the transfer of analytes from the primary matrix (original sample) to the secondary matrix with the simultaneous removal of interferences (isolation) and the increase in analyte concentration to a level above the detection limit of the instrument (enrichment). Among common techniques for the isolation and enrichment of volatile organohalogen compounds (trihalomethanes (THMs), tetrachloroethylene) is headspace analysis, static (HS) or dynamic (purge and trap (PT), thin-layer headspace (TLHS) (49–50, 63–75).

DETERMINATION OF XENOBIOTICS IN SAMPLES OF BIOLOGICAL FLUIDS

Determination of Inorganic Xenobiotics

The determination of metals and other elements in biological materials is important not only for the assessment of environmental and occupational exposure and diagnostics of acute poisonings, but also in the diagnostics of diseases resulting from a deficiency of essential metals. Depending on the need, various biological materials are analyzed. However, most often these are physiological fluids—blood, serum, and urine; sometimes hair and nails are also used.

Methods of Isolation of Metals from Physiological Fluids

The determination of trace amounts of metals in urine, blood, or milk often requires the destruction of organic compounds via oxidation (mineralization) (76). This process results in a conversion of metallic xenobiotics into their ionic form. There are two methods of mineralization: dry and wet. The selection of one depends on the element being determined and the method of final determination. In addition, the following mineralization techniques find use in the analytical practice:

- microwave-assisted sample decomposition (in an open or closed system),
- ultraviolet-assisted mineralization,
- ultrasound-assisted sample decomposition in oxidizing mixtures.

A sample prepared in this way can then be analyzed using appropriate measuring devices. Often, samples of urine or blood are diluted using only, for example, dilute nitric acid.

Final Determination of Metals

Metals are determined by means of a variety of analytical techniques. Among the most important are the following methods:

- atomic absorption spectroscopy (flame or flameless),
- atomic emission spectroscopy,
- inductively coupled plasma-mass spectrometry,
- inductively coupled plasma-atomic emission spectrometry,
- stripping voltammetry, and
- neutron activation analysis.

The selection of one techniques depends on the kind of analyzed sample (matrix interference), the amount of analyte in the sample, and the desired accuracy of the determination. Examples of techniques used for the determination of selected elements in samples of human biological fluids are shown in Table 5.

Validation of the analytical procedures and techniques used is an important part of the analytical process. In the case of determination of inorganic analytes suitable reference materials are available, which facilitates validation (Table 6).

Determination of Organic Xenobiotics

The determination of organic contaminants in samples of biological fluids, such as urine, blood, milk, or sperm, is a difficult and complex task due to: large variety of these compounds; very diversified concentration levels; complex matrix of biological fluids; and problems with validation of procedures used (as a result of lack of suitable reference materials), particularly for organic components. The only reference material available contains metabolites of styrene: mandelic acid and phenylglyoxylic acid in human urine (83). Interlaboratory (even international) comparative studies on the quality of results obtained in individual laboratories are gaining more and more importance (84).

The majority of analytical procedures are based on chromatography at the stage of separation of mixtures into individual chemical species. Prior to introduction of a sample into the chromatographic column, analytes have to be isolated and/or enriched from a very complex matrix.

Methods of Isolation and/or Enrichment of Organic Compounds from Physiological Fluids

A search of the literature reveals information about an ever-increasing spectrum of organic compounds present in various elements of the environment and, hence, in the human body. These compounds are often toxic; however, not all of them have been included in standards pertaining to the quality of the environment.

Samples of urine, blood, milk, or sperm require special pretreatment due to very complex composition of the matrix, low concentration levels of the analytes, and incompatibility of the sample matrix with the chromatographic technique used. Consequently, the analytes present in a biological sample undergo the process of isolation and/or enrichment prior to the final determination step. Single-step isolation/enrichment processes are preferred to minimize errors.

The sample preparation step must take into consideration the following factors:

- nature of matrix of a sample of biological fluid,
- method of sample introduction into the chromatographic column,
- conditions of chromatographic process, and
- characteristics of detectors used (e.g., detection limit).

Often the selection of a particular analytical method is limited by the quantity of sample available (blood, sperm, bile). However, this inconvenience is eliminated when analyzing samples of urine or milk.

In cases of analysis of volatile and semivolatile organic compounds, a single-step isolation and/or enrichment process is the best solution. This requirement is met by the techniques enabling both analyte isolation and enrichment in a single stage. In analytical practice, gas-phase extraction, liquid-phase extraction, and solid-phase extraction, which are suitable for volatile, semivolatile, and nonvolatile compounds, are used

TABLE 5
Selected Information on the Techniques of Determination of Metals and their Metabolites in Samples of Human Blood and Urine

Separation and/or final determination technique	Preparation of samples for analysis	Analyte	Ref.
AAS*	Urine samples were transferred to reaction tubes containing 6 M HCl, a tablet of antifoaming agent and water	As—its metabolites: (methyl derivatives of arsenic(V) acid)	(4)
	Possibly, dilution of a sample with nitric acid prior to final determination	Cr, Cd,Fe, Co, Cu, Mn, Ni, Pb	(5, 14, 15, 77)
	Coprecipitation using samarium hydroxide as a carrier	Cd, Fe, Ni, Co, Pb, Cr, Cu, Mn,	(77)
	Wet mineralization	Bi	(26)
		Hg	(8, 9, 78, 79)
		Zn, Cd, Pb	(10)
		Ni, Cd, Cu, Co, Pb	(80)
		Pb	(81)
IC-AAS	Urine samples were diluted with the mobile phase used for ion chromatography	As—its metabolites: (methyl derivatives of arsenic(V) acid)	(12)
ETAAS	No preparation required	Cd, Ni	(7, 8)
	Microwave-assisted wet mineralization	Bi, Cd, Pb	(27)
IC-ETAAS	Wet mineralization	Pb	(82)
		Bi	(16)
		Ni	(13)
ICP-MS	Urine samples were diluted with water in a 1:9 solution	Sc, V, Mn, Fe, Co, Ni, Cu, Zn	(10)
ICP-MS HPLC-MS	No sample preparation required	As—its metabolites: (methyl derivatives of arsenic(V) acid)	(3)
Stripping voltammetry	UV mineralization	Pt	(11)

*Atomic absorption spectroscopy; used in a number of modes, including flame, flameless, cold vapor, hydride generation.

most commonly (65). None of these techniques is universal. By selecting a particular enrichment technique, the range and number of compounds that can be determined is narrowed. The most often used techniques of analyte isolation and enrichment from urine samples are compiled in Table 7. Each of the listed techniques has shortcomings and a limited use. The information on individual techniques is also provided in Table 7.

Determination of Organic Compounds in Samples of Biological Fluids

The analysis of obtained extracts/eluates includes identification and quantitative determination of individual components and is generally carried out by a suitable chromatographic technique. Of a variety of chromatographic techniques available, gas chromatography with the detector selective for a specific group

TABLE 6
Reference Materials Which Can Be Used in Urine Sample Analysis (1)

Material	Certified metal content	Manufacturer
Urine	Cu, Se—normal level	NIST SRM 2670-72
	As, Cd, Cr, Cu, Pb, Hg, Se—elevated level	
	Hg—normal level	
	F—normal and elevated level	
	As, Cu, Se, Be, Cd, Cr, Mn, Ni, Pb	LGC 91-02-03
	As, Cu, Se, Be, Cd, Cr, Cu, Mn, Ni, Pb	NRCCRM; GBW 09102-103
Serum	Cd, Cr, Cu, Pb, V	NIST 909

TABLE 7
Information on Selected Isolation and/or Enrichment Techniques for Organic Compounds (63–72, 85–86)

Extraction technique	Description
Gas-phase extraction	
Headspace (HS) techniques	HS is based on partition of analytes between the liquid and gaseous phase. The concentration of analyte in the condensed phase is determined by analyzing the headspace—the gaseous phase in contact with the sample. The most effective release of analytes is achieved for volatile and semivolatile nonpolar or moderately polar organic compounds
Static headspace	Both phases being in contact—aqueous (sample) and gaseous (receiving matrix) are stationary. The analysis proceeds in two steps: the examined sample is placed in a closed container at a constant temperature, the system is brought to thermodynamic equilibrium, and a sample of headspace is collected manually or automatically
Dynamic headspace (TLHS)	The gaseous phase is passed continually through or over a sample (concurrently or countercurrently) and the analytes carried with it are retained in a trap with a sorbent (e.g., water). The technique is often combined with direct aqueous injection (DAI) onto the GC column of a chromatograph equipped with an electron capture detector (ECD)
Purge and trap (PT)	A stream of gas is bubbled through the analyzed liquid sample. The purged analytes are then retained in a trap, from which they are subsequently released, most often thermally, into a gas chromatographic column. The technique is widely used for the determination of volatile and semivolatile organic compounds in a variety of aqueous matrices
Distillation techniques	These techniques are used for the isolation of volatile, more polar compounds from liquid matrices. They enable the determination of analytes in samples with a high content of inorganic or high-molecular-weight organic compounds, which would otherwise require an extensive pretreatment procedure prior to chromatographic analysis. The basis for the separation of a mixture into components is diverse partition of individual components between the liquid phase and the gaseous phase equilibrated with it. The gaseous phase is enriched in more volatile components, which following condensation becomes a concentrate of these components—distillate
Liquid-phase extraction	
Liquid-liquid extraction (LLE)	The principle of isolation is based on partition of the analytes between the liquid phase (sample) and an organic solvent. Solvents immiscible with water are used for extraction. The analytes dissolve better in the solvent than in water. The technique is used for semivolatile and nonvolatile compounds
Solid-phase extraction	
Solid-phase extraction (SPE)	Solid phase extraction involves the transfer of analytes from a liquid sample to a solid sorbent, followed by their release using extraction with a solvent of high elution strength or, less frequently, thermal desorption. A large selection of solid sorbents ensures obtaining proper selectivity and optimal enrichment factor of the analytes. Typical sorbents used for analyte retention include: porous polymers, such as styrene-divinylbenzene copolymers; carbon sorbents; and silica gels with chemically bonded stationary phases containing various functional groups. SPE enables isolation and enrichment of analytes with a wide range of volatility and polarity
Solid-phase microextraction (SPME)	In SPME, the sorption medium constitutes a layer of liquid or solid coating on a fused silica fiber. This ensures rapid transport of the analyte from the sample to the sorbent and simplifies introduction of analytes into a chromatographic column

TABLE 8
Selected Information on the Techniques of Determination of Organic Xenobiotics and Their Metabolites in Samples of Human Urine, Blood, Milk, and Sperm

Final determination technique	Sample preparation for analysis	Analyte	Ref.
Gas chromatography			
GC-MS	Addition of a drop of antifoaming agent High-temperature (90°C) purge and trap (PT)	Methyl-*tert*-butyl ether (MTBE) Its metabolite: *tert*-butyl alcohol	(87)
	Headspace analysis (HS)	Trichloroethene Its metabolites: trichloroacetic acid, chloroform, trihalomethanes (THMs)	(45–50, 68–69, 73)
		Acrylaldehyde	(88)
	Headspace analysis (HS) Solid-phase microextraction (SPME)	Benzene, toluene, ethylbenzene, xylenes	(89)
	Enzymatic hydrolysis Solid-phase extraction (SPE) Acylation of aromatic amines*	Nitroarenes aromatic amines*	(90)
	Solid-phase extraction (SPE)	Phenols	(91)
		Polychlorinated dibenzodioxins (PCDD), dibenzofurans (PCDF), polychlorinated biphenyls (PCB)	(92)
	—	Polychlorinated biphenyls (PCB)	(93)
		Hippuric acid	(94)
	Acid hydrolysis	Thiodiglycolic acid	(95)
	Enzymatic hydrolysis Solid-phase extraction (SPE)	Monohydroxy derivatives of polynuclear aromatic hydrocarbons	(96)
	Solid-phase microextraction (SPME)	Toluene (and its metabolites)	(35)
	Sample dilution Liquid-liquid extraction (LLE)	Phenol	(32)
	Liquid-liquid extraction (LLE)	Mercapturic acids	(97)
	Liquid-liquid extraction (LLE)	S-*p*-toluylmercapturic acid *o*-Cresol Hippuric acid	(39)
	Hydrolysis Liquid-liquid extraction (LLE) Solid-phase extraction (SPE)	*o*-Cresol 1-Naphthol 2-Naphthol	(98)
	Liquid-liquid extraction (LLE)	Solid organic contaminants (PCB, pesticides)	(99)
	Liquid-liquid extraction (LLE) Esterification	2-Methoxyethoxyacetic acid (MEAA)	(100)
	Acid hydrolysis Basic hydrolysis Headspace analysis (HS)	4-Heptanone Trimethylamine	(101)
	Cryogenic trap	Toluene, trichloroethene, *n*-hexane	(102)
GC-ECD	Solid-phase extraction (SPE)	Polychlorinated biphenyls (PCB)	(52, 103)
	—	Chlorinated pesticides	(62)

(Continued on next page)

TABLE 8
Selected Information on the Techniques of Determination of Organic Xenobiotics and Their Metabolites in Samples of Human Urine, Blood, Milk, and Sperm *(Continued)*

Final determination technique	Sample preparation for analysis	Analyte	Ref.
		Cresols	(94)
	Enzymatic hydrolysis	Ethanol, aldehyde, acetone, 2,3-butanediol	(104)
	Liquid-liquid extraction (LLE), Solid-phase extraction (SPE)	Chloro- and methylthiotriazine derivatives	(105)
	Derivatization Acid hydrolysis Liquid-liquid extraction (LLE)	2,5-Hexanedione	(102)
	Headspace analysis (HS)	Trichloroethene, trichloroethanol, trichloroacetic acid	(106)
		Tetrachloroethene, trichloroacetic acid	(107)
		Tetrachloroethene, trichloroethene	(108)
GC-FID	Headspace analysis (HS)	Methanol Formic acid	(37)
		Dichloromethane	(44)
		Ethanol	(109)
		Toluene, xylenes	(94, 110, 111)
		Toluene, hexane, xylenes Ethylbenzene, styrene Methanol	(112)
		Trimethylbenzene Dimethylbenzoic acid	(113)
		Benzene, ethylbenzene, styrene, toluene, *m*-xylene, *n*-hexane, hemimellitene, mesitylene, pseudocumene	(108)
	Purge and trap (PT)	Styrene (and its metabolites)	(52)
	Solid-phase microextraction (SPME)	Toluene	(35)
GC-FMD	Acid hydrolysis Liquid-liquid extraction (LLE)	Hydroquinone	(114)
GC	Headspace analysis (HS)	1-Butanol 1,1,1-Trichloroethane Trichloroacetic acid Trichloroethanol Methanol Formic acid Toluene	(115)
		Toluene, hexane Ethyl acetate	(116)
		Toluene, styrene Methanol	(117–119, 127)
		Xylene	(120, 121)
	—	(*o*-, *m*-, *p*-) Xylenes	(122)
	—	*o*-Cresol	(123)
		Trichloroethanol, trichloroacetic acid	(102)
	Acid hydrolysis Silylation	*m*-Methylhippuric acid	(121)

(Continued on next page)

TABLE 8
Selected Information on the Techniques of Determination of Organic Xenobiotics and Their Metabolites in Samples of Human Urine, Blood, Milk, and Sperm *(Continued)*

Final determination technique	Sample preparation for analysis	Analyte	Ref.
Liquid chromatography			
LC-MS	Liquid-liquid extraction (LLE)	Diuretics	(124)
	Acid hydrolysis	1-Hydroxypyrene	(125)
LC-MS/MS	—	Hydroquinone Catechol	(30)
LC-ES-MS/MS	—	*S*-Methylmercapturic acid *trans*, *trans*-muconic acid	
HPLC-UV	Sample acidification Liquid-liquid extraction (LLE)	*S*-Phenylmercapturic acid	(38)
	Solid-phase extraction (SPE) Ion exchange	*trans*, *trans*-Muconic acid	(38)
	Acidification, Liquid-liquid extraction (LLE)	Hippuric acids (*ortho*-, *meta*-, *para*-)	(111)
	—	Methylhippuric acids (*ortho*-, *meta*-, *para*-)	
	Liquid-liquid extraction (LLE)	Mandelic acid	(97)
	—	Methylhippuric acids (*ortho*-, *meta*-, *para*-)	(126)
	Solid-phase extraction (SPE)	*trans*, *trans*-Muconic acid	(114)
	—	Mandelic acid, phenylglyoxylic acid	(83)
HPLC-UV-VIS	Acidification, Liquid-liquid extraction (LLE)	Benzylmercapturic acid	[127]
HPLC-FSD	—	1-Hydroxypyrene	(128–130)
	Enzymatic hydrolysis Solid-phase extraction (SPE)		
	Enzymatic hydrolysis	Sum of polynuclear aromatic hydrocarbons	(40)
	Enzymatic hydrolysis Solid-phase extraction (SPE)		(96)
	Liquid-liquid extraction (LLE)	17 Diuretic drugs	(131)
HPLC-FID	—	1-Hydroxypyrene	(132)
HPLC		Mandelic acid	(133)
		Hippuric acid, *o*-cresol	(102, 119, 134)
		Hippuric acids (*ortho*-, *meta*-, *para*-)	(122, 123)

of analytes (e.g., mass spectrometry (MS), flame ionization detector (FID), and electron capture detector (ECD)) has found the widest use for the determination of organic components of biological fluids. High-performance liquid chromatography with fluorimetric detection is used less often. The information on application of these techniques for the determination of organic xenobiotics in urine and blood samples is compiled in Table 8.

INTERPRETATION OF RESULTS

Biological monitoring is defined as a "systematic measurement of concentrations of toxic substances or their metabolites in the tissues, secretions or excretions, separately or jointly, aiming at the assessment of exposure and health risk based on appropriate interpretative data" (1). In this case, the data enabling interpretation of the results of determination of analytes are the values of biological exposure indices (BEI).

In most countries, the use of biomonitoring is optional. Biomonitoring can be used for the risk assessment for both communal and occupational exposure. Consequently there exist appropriate reference data, which can exhibit significant differences for the general population, depending on the degree of environmental pollution, diet, cigarette smoking, and so forth. However, systematic determinations of, for example, lead in blood, allow revealing trends and drawing of conclusions regarding potential health hazards for the population in a given area and the factors causing these hazards. The data regarding allowed concentrations of toxic substances or the products of their conversion under conditions of industrial exposure as well

as the early, reversible effects of their action are published in the form of recommendations by various international organizations and the institutions responsible for occupational safety and hygiene in individual countries. The two widely recognized organizations publishing the lists of biological exposure indices are the American Conference of Governmental Industrial Hygienists and the German Deutsche Forschungsgemeinschaft. These lists are updated and published annually. In general, the values of BEI can be based on health criteria and allow a direct risk assessment for toxic materials or serve as equivalents of maximum allowable concentrations (MAC) of toxic substances in air (1).

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