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BIOLOGICAL MONITORING FOR EXPOSURE TO VOLATILE ORGANIC COMPOUNDS (VOCs)

(IUPAC Recommendations 2000)

Prepared for publication by
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Biological monitoring for exposure to volatile organic compounds (VOCs)

(IUPAC Recommendations 2000)

Abstract: This paper deals with the appropriate application of biological monitoring (BM) for exposure to volatile organic compounds (VOCs). Sampling guidelines, approved analytical procedures, quality control systems, detailed aspects for the interpretation of biomonitoring data, a compilation of international biological action values for VOC exposure at the workplace (e.g., BAT, BEI®), and state of the art reference values are outlined or referred to in this review for recommendation as guidelines for health professionals in occupational and environmental settings.

VOCs are frequently encountered at the workplace, in daily routines and widely used consumer products. They cover a broad spectrum of chemical classes with different physicochemical and biological properties. Inhalation is a prominent route of exposure due to their volatility but many VOCs can quite readily be absorbed through the skin. BM allows assessment of the integrated exposure by different routes including inhalation and concomitant dermal and oral uptake—a helpful tool for relating exposure to body burden and possible health effects. Because of the different toxicological profiles of VOCs, no uniform approach for BM can be recommended. VOCs in blood and urinary VOC metabolites are most often applied for BM. Limit values for workplace exposure have been established for many VOCs. In this field, profound analytical methodology and extensive experience exist in numerous international scientific laboratories for reliable routine application. Contamination and loss of VOCs during specimen collection, storage and sample treatment, and applied calibration procedure are the most important uncertainties for analytical quantification of VOCs in blood. For interpretation of the analytical results appropriate time of sampling, according to toxicokinetics of the compound, is crucial due to VOC elimination with short but differing biological half-lives. Lifestyle factors (such as smoking habits, alcohol consumption, and dietary habits), workload, personal working habits, exposure to VOC mixtures and endogeous factors (as genetic polymorphism for VOC metabolizing enzymes, body mass) contribute to BM results and have to be considered in detail. Future analytical work should focus on the improvement of analytical methodology of VOC determination in body fluids at low-level environmental exposure and evaluation of corresponding reference intervals.

CONTENTS

1. INTRODUCTION

In recent years the assessment of exposure to hazardous substances has become an integral part of the risk assessment process for an increasing number of inorganic as well as organic chemical substances. Among chemicals of industrial importance the so-called volatile organic compounds (VOCs) belong to a broad scale of chemicals with high production levels all over the world and widespread applications in industry, trade and private households. According to a definition given by the European Communities the expression "volatile organic compound (VOC) means any organic compound having, at 293.15 K, a vapor pressure of 0.01 kPa or more, or having a corresponding volatility under the particular condition of use" [1]).

As VOCs are defined by their vapor pressure, different chemical substance classes with different solubilities and toxicity profiles are covered by this term (Table 1). One of the most important industrial uses of the VOCs is their utility as solvents. Here, the term "organic solvents" will be understood as a "generic name for a group of chemical compounds or mixtures which are liquid in the temperature range of approximately 0–250 °C. They are volatile and relatively chemically inert. Solvents are used industrially to extract, dissolve or suspend materials not soluble in water (e.g., fats, lipids, resins and polymers)" [2]. NOTE

NOTE We have retained in the text of the recommendations and in the tables the names of chemical compounds that have most frequently been encoutered in the literature, which can be described as traditional, semisystematic, or trivial names.

IUPAC, of course, is striving towards a uniform systematic naming of chemical substances and presentation of formulae, which are embodied in IUPAC nomenclature books such as

A Guide to IUPAC Nomenclature of Organic Compounds
R. Panico, W. H. Powell, and J.-C. Richer
Blackwell Scientific Publications, Oxford, 1993

Principles of Chemical Nomenclature – A Guide to IUPAC Recommendations
G. J. Leigh, H. A. Favre, and W. V. Metanomski
Blackwell Science, Oxford, 1998

Therefore, we are providing a listing of the traditional names and their equivalent IUPAC names in Sect. 10.

Table 1 Types of chemical substances among the VOCs.

Type of substance	Example	Formula	lg P[1)]	Application/source
aromatic hydrocarbons	benzene	C_6H_6	2.13	car exhaust fumes, lacquers, printing ink, paint, tobacco smoke
	toluene	C_7H_8	2.69	
	xylenes	C_8H_{10}	2.8–3.2	
aliphatic hydrocarbons	n-hexane	C_6H_{14}		lacquers, adhesives
	white spirit	mixtures (e.g., branched C_6/C_7-isomers)		gasoline, dry cleaning petrol
chlorinated hydrocarbons	dichloromethane	CH_2Cl_2	1.25	paint remover resins, rubbers, dry cleaning
	chlorobenzene	C_6H_5Cl	2.84	
	tetrachloroethylene	C_2Cl_4	2.60	
alcohols	methanol	CH_3OH	–0.82	chemical syntheses; beverages and diet
	ethanol	C_2H_5OH		
esters of carboxylic acids	ethyl acetate	$CH_3COOC_2H_5$	0.66	lacquers, solvents for resins
ketones	2-butanone	$CH_3COC_2H_5$	0.26	lacquers, resins
various:				
glycol ethers	ethylene glycol monoethyl ether	$HOC_2H_4OC_2H_5$		paint, printing ink
ethers	methyl *tert*-butyl ether	$CH_3OC(CH_3)_3$		oxygenated fuels
amides	dimethylformamide	$HCON(CH_3)_2$		lacquers, plastics

[1)] P = c(octanol)/c(blood); octanol/blood partition coefficient [3–5]

Few data are available internationally on the amount of solvents being produced. As one example, about 550 thousand tons of industrial solvents have been produced annually between 1986 and 1993 in Germany [6]. The 10 most often analyzed VOCs in German industrial solvent mixtures are xylenes, toluene, ethyl acetate, butyl acetate, ethylbenzene, butanol, acetone, and ethanol, followed by 2-propanol and 2-butanone [7]. A very similar ranking was observed in Danish companies [8]. In addition, these data indicate that VOC exposure is characterized by the simultaneous presence of several, typically 5 to 10, substances belonging to different chemical classes.

Exposure assessment may be performed by ambient air monitoring and by biological monitoring. Here, the term "exposure" is defined as the concentration or amount of a chemical that reaches the organism [9]. The following terms—given on an international seminar organized by CEC, NIOSH and OSHA [10] and being in line with recent IUPAC recommendations [9] and WHO guidelines on biological monitoring [11]—will be used within this paper:

Monitoring (in preventive health care) is "a systematic continuous or repetitive health-related activity, designed to lead if necessary to corrective action".

Biological monitoring (BM) is "a systematic continuous or repeated measurement and assessment of workplace agents or their metabolites either in tissues, secreta, excreta or any combination of these to evaluate exposure and health risk compared to an appropriate reference". In this definition, BM is related to *monitoring of exposure*. A similar definition has been proposed for biomonitoring of environmental exposure [9].

In 1986 Zielhuis and Henderson [12] proposed the term **biological effect monitoring (BEM)** in order to include early, possibly reversible biological effects (health-related *response)* into the concept of BM: BEM is "the measurement and assessment of early biological effects, of which the relationship to health impairment has not yet been established in exposed workers to evaluate exposure and/or health risk compared to an appropriate reference" as, for example, sister chromatid exchanges, micronuclei analysis, chromosomal aberration, MetHb (methemoglobin).

Recently, instrumental analysis has entered the field of **biochemical effect monitoring** referring preferably to the quantification of *in vivo* formed adducts of the exposure chemical (or its metabolites) to macromolecules, including such proteins as hemoglobin or serum albumin, as well as DNA. The detection of **increased susceptibility** of individuals to chemicals (e.g., due to metabolizing capacity) may be used as biomarkers as well [13].

Another commonly used terminology for different BM types are the terms **biological marker (biomarker) of exposure, of effect, and of susceptibility,** respectively [14].

This paper concerns the area of biological monitoring (BM) of volatile organic compounds (VOCs). Biochemical and biological effect markers will only be described briefly (cf. Sect. 4.3) and are topics of further investigation for their practical approaches in VOC exposure by the Commission on Toxicology of IUPAC.

Biological monitoring provides data that pertain to individual workers. Therefore, their use may involve important ethical problems that have to be solved before starting any biological monitoring program [15].

Biological monitoring (BM) of exposure to organic solvents has been applied as an important tool for worker protection and turned out to be complementary, often even superior, to ambient monitoring of environmental and workplace exposures due to:

- uptake routes additional to inhalation such as skin or oral absorption,
- differences in workload and personal working habits,
- use and efficiency for personal protective devices,

Therefore, the *individual* uptake and its possible impact on the person's health can be estimated.

At present the main problems of evaluating an exposure to VOCs by means of BM include the strategy of sampling, evaluation of exposure to mixtures, influence of inhibition and induction of metabolism on results of BM, and lack of commonly accepted protocols for studies aimed at evaluating relations between the degree of exposure and concentration of markers of exposure and/or early health effects.

While the idea of biomonitoring is generally accepted for exposure to metals, it is still less well established for VOCs although these have widespread applications in industry and private households. As the IUPAC Commission on Toxicology regards the biomonitoring of VOCs as a very helpful tool in health protection of exposed persons, this review is intended to give a concise and up-todate introduction to the field and to recommend the appropriate use of BM of VOCs especially for occupational and environmental health professionals with the main emphasis on sampling, handling, basic principles of applied analytical procedures, as well as on data interpretation.

2. TOXICOKINETICS

The relationship between environmental or occupational exposure, uptake, and the levels in blood, urine, or tissues is determined by the kinetic behavior of the VOCs. The time course of the concentration of a substance in body fluids or the body burden is a result of the dose and the basic kinetic processes of absorption, distribution, and elimination which may be either via biotransformation (metabolism), and/or excretion (including exhalation). These processes are influenced by endogenous (e.g., genetic

phenotype, body mass, age, gender) and exogenous (e.g., workload, dietary habits, smoking) factors. Understanding of these factors is essential for biomonitoring of VOCs, selection of the appropriate biological matrix and sampling time, and interpretation of analytical results.

The basic processes can be described by the extent to which the process occurs and the rate at which it occurs. The toxicokinetic parameters are usually calculated from the time course in body fluids, e.g., plasma or urine concentration time data. At usually occurring low VOC concentrations, the processes are linearly related to exposure and follow first-order kinetics; the time curve plotted on a logarithmic scale becoming a straight line. Following the peak of urinary metabolites at the end of exposure (e.g., phenylglucuronide), or some time after the exposure (e.g., trichloroacetic acid), metabolite excretion usually appears to be monophasic [16]. When the exposure is high and an eliminating process (e.g., metabolism) is saturated, the kinetics are independent of the degree of exposure and follow zero-order kinetics (Figs. 1a and 1b).

The rate at which VOCs enter and leave the body through the lungs is mainly a function of the partitioning of the compound between lipid and aqueous compartments in the body. Components with high lipid solubility will be stored in fatty tissues (lipophilic) and accumulate depending upon extent and duration of exposure. The kinetics of VOCs during the exposure phase show an initial rapid increase and level off afterwards. A corresponding pattern is observed during the elimination phase (rapid initial decrease of VOC concentration that slows down with time) **(**Fig. 2).

In first-order kinetics the concentration decline is described by an exponential function, the rate constant (k_e) indicates the fraction of the determinant in the body removed per unit of time. The biological half-life ($t_{½}$) of a substance in an organ, tissue, or body fluid denotes the time needed to reduce the biological level of the substance by half. Half-life is inversely related to the elimination constant

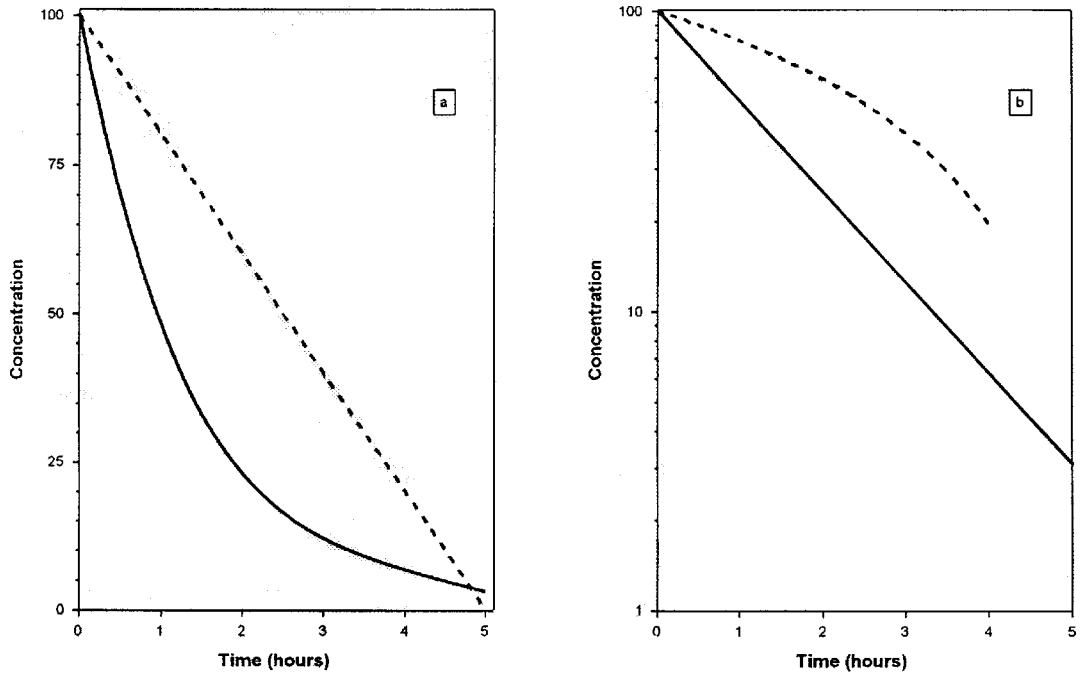

Fig. 1 Elimination curves according to type of elimination kinetics: First-order (——) and zero-order kinetics (-------) presented in normal (**a**) and logarithmic scale (**b**).

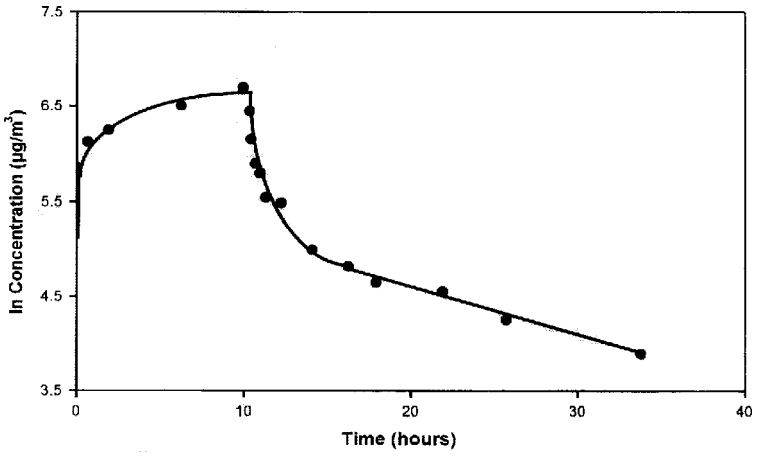

Fig. 2 Uptake and decay of *p*-xylene in a female subject exposed to 11 mg/m³ in a chamber for 10 h. The data for exhaled air were fitted with a four-compartmental model (according to Wallace and Pellizzari [36]).

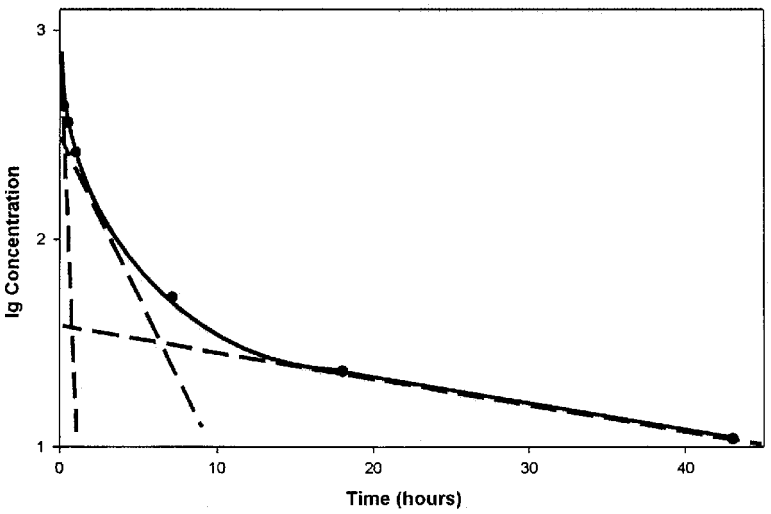

Fig. 3 Phases of kinetic elimination of toluene from capillary blood after termination of exposure. The half-life in capillary blood amounted in the three phases to 4 min; 1.8 h; 24.5 h. Two hours after the experiment the concentration drops down to 15% of its maximum level, and to 2% during the next 16 h (data according to Kostrzewski and Piotrowski [41]).

(k_e = ln2/$t_{½}$). The overall time-course might be the result of several half-lives corresponding to the elimination from different organs or compartments (Fig. 3).

For substances with long half-lives the sampling time is not critical, but the shorter the half-life, the more critical the sampling time becomes (Fig. 4 and Table 2)**.**

2.1 Absorption

Absorption is the process of transfer of a VOC from the site of exposure, most commonly via the airways or across the skin, into the blood circulation.

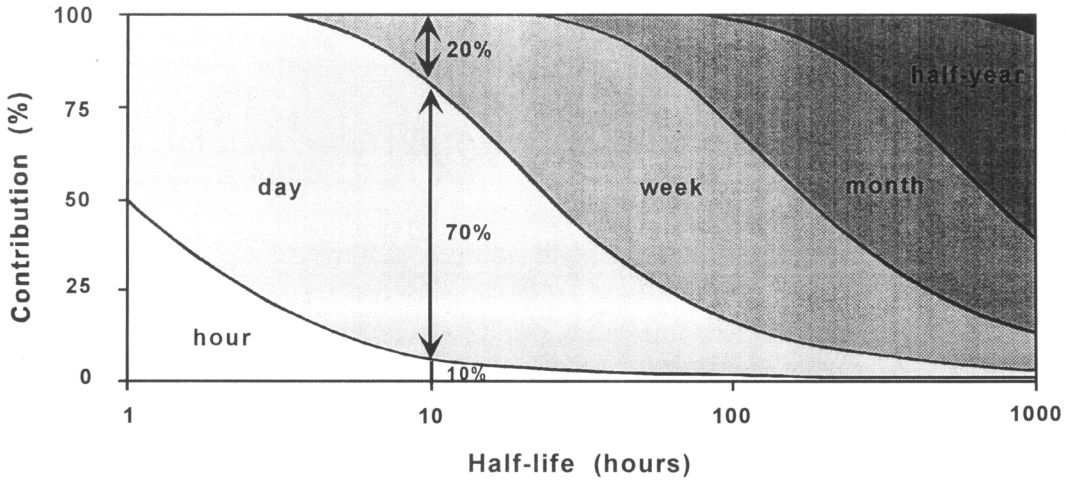

Fig. 4 Effect of half-life (one-compartmental model) on contributions of exposures during the last presampling hour, day, week, month, and half-year to biological levels of determinants. For example, if the determinant is eliminated with a half-life of 10 h, the biological level mainly reflects the exposure on the day prior to sampling (contribution 70%); to a relatively small extent, it reflects the exposure during previous hour and week (contributions 10% and 20%, respectively) (according to Droz et al. [37]).

Table 2 Classification of compounds according to elimination half-life and resulting sampling requirements (adapted from Fiserova [17]).

Half-life	Sampling time reflects
< 2 h	recent rate of absorption
2–10 h	exposure during the day sampling: end of shift/ beginning of next shift
10–100 h	exposure during the week sampling: end of shift/ work week

The rate of absorption of volatile compounds differs in relation to their physicochemical properties. Lipophilicity, water solubility, and protein binding have an important influence considering the body composition of water, lipids and proteins. Lipid-soluble compounds can readily dissolve in membranes and diffuse into the body. Absorption from the lungs tends to be fast because of the large surface area and the thin alveoli membrane. Absorption is usually slow across the skin. The kinetic parameter which describes the absorption rate (k_a) is the absorption half-life; the parameter which describes the extent of absorption is called bioavailability (F).

2.1.1 Pulmonary absorption (absorption through the lungs)

Lungs constitute the main route of absorption of VOCs under occupational and environmental conditions. The introduction of vapor of a given substance into the lungs results in a rise in the alveolar concentration (partial pressure) of that substance. If unimpeded, ventilation would cause the alveolar concentration to rapidly reach the inspired concentration. However, the effect of ventilatory input is opposed by substance

uptake. The alveolar concentration is determined principally by the balance between ventilatory input and the loss of substance through uptake by pulmonary blood.

The uptake of a substance is governed by two types of variables, those characteristic of the compound (blood/air partition coefficient) and those characteristic to the individual (workload, ventilation and circulation, alveolar-venous difference). Highly volatile, inert (stable) compounds are retained only in alveoli. Their uptake accounts for about 57% of the amount in minute ventilation, which is the fraction of minute ventilation that reaches the alveoli. Low volatile compounds, water-soluble compounds, and reactive compounds are retained also in respiratory airways, and therefore, their retention is higher [16].

Gas uptake increases in proportion to the magnitude of the gas partial pressure difference between alveoli and pulmonary venous blood. This difference diminishes when all body tissues achieve equilibrium with the alveolar partial pressure. When the alveolar-venous gas partial pressure difference equals zero, gas uptake from the lungs ceases. Conversely, the difference is greatest at the beginning of exposure.

As results of all these processes, the uptake of VOCs in lungs at the beginning of exposure is high and then decreases when it approaches steady state (equilibrium between partial gas pressure of alveoli and pulmonary venous blood) after 2–3 h. During the early exposure phase blood levels of VOCs increase rapidly and then level off (Fig. 2).

The uptake during steady state is different for particular compounds amounting to 90% for aniline, 80% for nitrobenzene, 50–70 % for aromatic hydrocarbons like benzene, toluene, xylenes, and 35% for carbon disulfide [18].

Increase of physical workload results both in the increase of intake and uptake of VOCs. However, the relative effect of an increase of ventilation and cardiac output varies for substances of different blood/air partition coefficients [19].

2.1.2. Cutaneous absorption (absorption through the skin)

Many chemicals can be absorbed through the skin in toxic amounts, even when airborne concentrations of the chemical are within legal limits.The significance of percutaneous absorption has probably been underestimated in industrial experience and in occupational health practice. VOCs considered to be absorbed through the skin according to the notation of the German MAK and BAT commission [20] and the U.S. ACGIH are summarized in Sect. 7, Table 12.

There are two forms of percutaneous absorption: transepidermal (through the epidermal cells) and appendageal (through the hair follices and sebaceous glands). The appendageal route offers greater permeability and plays an important role in the diffusion of ions and polar nonelectrolytes. However, the transepidermal route is generally more prominent.

Transepidermal transport occurs by passive diffusion. Penetration experiments have demonstrated that the stratum corneum behaves like a passive diffusion barrier. The steady-state penetration flux can be described by the expanded form of Fick's law [21,22].

In the occupational environment, absorption through the skin occurs mainly as the result of contact of **liquid compound** (e.g., solvents) with the skin. The amount of absorbed compound depends on the surface of contaminated skin, properties of compound (K_p, constant of permeability) and vehicle. The rate of absorption of VOC amounts in general from 0.1 to 1.0 mg cm^{-2} h^{-1}, e.g., benzene 0.4 [23]; xylene 0.12 [24]; aniline 0.18– 0.72 [25]; toluene 0.6 [26]; trichloroethylene 0.6 [27].

Vapors may penetrate the skin if they are sufficiently soluble in the horny layer. In the occupational environment such absorption seems of little significance. In the case of xylenes, styrene, toluene, 1,1,1-trichloroethane and tetrachloroethylene the total skin absorption of the vapors contributed respectively 1.3; 1.4; 1.9; 0.9; and 1.1% of concomitant pulmonary absorption [28]. This route of absorption

is significant in the case of compounds that can be adsorbed on the skin. Absorption of phenol, aniline, and nitrobenzene is roughly proportional to the concentration of vapor in the air and can be characterized by the absorption coefficient of about 0.2–0.4 m^3h^{-1}, which means that exposed subjects can absorb the amount of compound contained in 0.2–0.4 m^3 of air through the skin per hour [18]. Absorption of dimethylformamide and 2-butoxyethanol vapors through the skin has also been described [29].

Empirical studies have suggested that the skin penetration (flux, J) rate (in mg cm^{-2} h^{-1}) for skin contact to liquid VOCs can be estimated by the following equation [30]:

$$(J/\text{mg cm}^{-2}\text{h}^{-1}) = \frac{(c_{sat}/\text{mg/ml}^{-1})}{15}(0.038 + 0.153P)\exp(-0.016M_t)$$

where:

c_{sat} - concentration of saturated aqueous solution of the chemical in mg/ml
15 - thickness of stratum corneum epidermidis in µm
P - octanol - water partition coefficient
M_t - molecular weight.
0.038 and 0.153 - denote area fractions and diffusion constants for polar and lipophilic pathways

The penetration rate has been calculated for 132 compounds [30].

2.2 Distribution

Distribution is the process of transfer of the VOCs from the blood into the body tissues (compartments). When the compound is absorbed into the blood stream, it is rapidly distributed throughout the body. The processes involved can be characterized by distribution rate constants and the apparent volume of distribution (V_d). The latter relates the total amount of the substance in the body to the blood concentration. It can be regarded as a dilution parameter and has no anatomical significance. V_d is derived by dividing the dose by the determinant concentration in blood or plasma. In physiologically based models the V_d is defined as the capacity of the compartment to retain the compound.

VOCs pass through biological membranes by the mechanism of passive diffusion. The rates of transfer are determined by the physicochemical properties of the solute, the membrane, and the concentration gradient [31].

Once in blood, the volatile substance is lost primarily by distribution into tissues and metabolism. The rate of gas uptake by tissues (i.e., the speed at which the equilibrum is reached) is determined by the tissue perfusion and partition ratio with blood.

In the 70-kg man 75% of the cardiac output is directed to only 9% of the body tissues (vessel-rich group, VRG). Because of the high blood flow/mass ratio, the body tissues of the VRG rapidly attain equilibrium with the gas partial pressure in arterial blood. As equilibrium is approached, the partial pressure in the venous blood leaving these tissues also rises until at equilibrium it equals the arterial and tissue partial pressure. If no concomitant rise in arterial concentration occurs, this process is complete within 10 to 15 min. The alveolar-venous partial pressure difference therefore, reduces rapidly to 25% of its initial value, and gas uptake is similarly reduced.

Other tissue groups continue to remove volatile compounds from arterial blood long after saturation of VRG. Skin and muscle (MG) form 50% of the body volume and at rest receive about 18% of the cardiac output. Saturation of this group proceeds slowly and is not complete for at least 90 minutes. Fat (FG) comprises roughly 20% of the body volume and receives approximately 5% of the cardiac output. Saturation of the FG proceeds more slowly than that of the MG since most of the VOCs are soluble in fat and cumulate in the FG [31].

2.3 Elimination

There are two basic mechanisms of elimination of VOCs from the body—metabolism and excretion. Metabolism eliminates a substance by converting it into a different chemical, the metabolite. Metabolites may undergo further metabolism or are eliminated by an excretory process. Excretion of VOCs and/or their metabolites can occur via body fluids (mainly urine, faeces, and expired air) and can be characterized by the rate of elimination (k_e).

Lipid-soluble compounds are readily reabsorbed in the kidney tubules and are poorly excreted. Only a small fraction, about 1% of the dose of lipophilic aromatic VOCs [32] can be found unchanged in the urine. The main function of metabolism is to transform these compounds into more polar forms that are readily excreted by the kidneys, mainly as conjugates with glycine, sulfuric acid, and glucuronic acid. Some compounds (metabolites) are excreted by glomerular filtration, others by tubular excretion or diffusion. Depending upon the mechanism, the urinary metabolites are related to excretion of solids and might be adjusted to creatinine or to density, or to the urine volume without concentration adjustment.

After termination of exposure the VOCs in unchanged form are excreted mainly in exhaled air. Elimination occurs nearly as a reversal of uptake, the levels drop rapidly at first and then much more slowly.

Half-life is the most useful parameter to describe the elimination process. As shown from decay curves, different half-lives can be observed, reflecting fractions from different body stores: an initial fast release from the blood (< 5 min), an intermediate phase in the muscle (min to h), and a longer phase from the fat (h up to d). The disparities in half-lives are a function of lipid/water partition coefficients and of variations of exposure. Extent and length of exposure affect the elimination kinetics, because a longer and more intense exposure will deposit more compound in the adipose compartment. [33]

2.4 Toxicokinetic modeling

Most VOCs appear and disappear rapidly in biological fluids. Modeling of the kinetics of the selected biomarker in those VOCs might be helpful in the planning and interpretation of biomonitoring studies, e.g., for selecting sampling time. Toxicokinetic models provide tools to establish a relationship between external exposure and internal dose. Physiologically based pharmacokinetic (toxicokinetic) models (PBPK) have been developed to describe the time course of a substance in the body, an organ, or compartment.

Three types of toxicokinetic models, relating available dose and internal dose will be treated in this paper: Correlation models, compartment models based on data for half-lives ($t_{½}$) of substances in the human body, and physiologically based toxicokinetic multicompartment models (PBPK).

It is also possible to simulate the outcome of varying exposure, being either continuous or intermittent. In Fig. 5 the effect of different k (or $t_{½}$) is illustrated [37,38].

2.4.1 Correlation model

Pulmonary uptake (U) has been demonstrated to correlate with the air/blood partition coefficients and time of exposure [34,35]. The model has the form:

$$U = k\ K_{\text{blood/air}}\ t^{0.5}$$

where k is a constant, $K_{\text{blood/air}}$ is the blood/air partition coefficient and t the time.

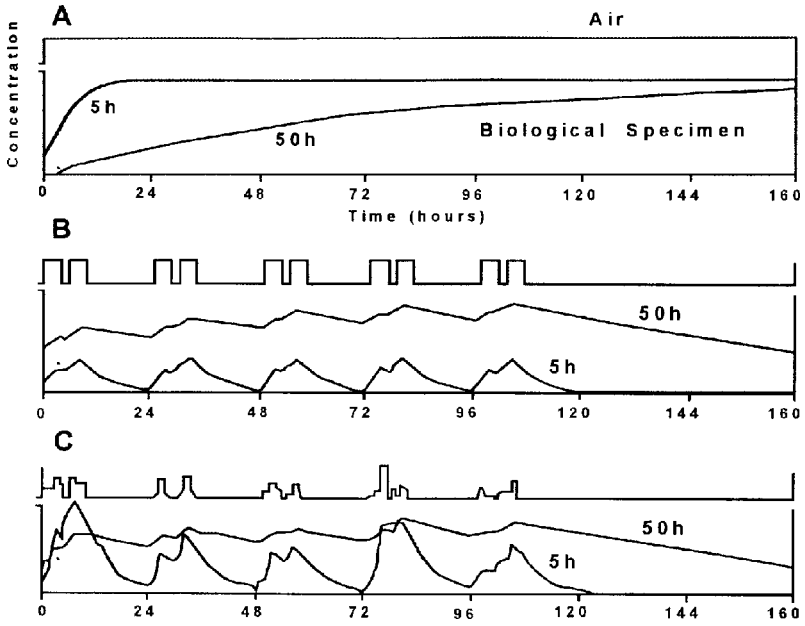

Fig. 5 Effect of half-life (5 h and 50 h, respectively) on changes of biological levels during continuous and intermittent exposure (ordinate is an arbitrary scale for concentration in ambient air and in biological specimens) (according to Droz and Wu [38]).
A: continuous exposure to a constant inspired concentration
B: intermittent occupational exposure to a constant inspired concentration
C: intermittent occupational exposure to a fluctuating, inspired concentration

2.4.2 Compartment model

It is possible to construct kinetic models using input (absorption rate) and output (excretion rate) data, where the whole human body is considered as one or more compartments. The complexities of human physiology make it difficult to model the exact course of a compound in the body. Compartments are mathematical surrogates without physiological meaning, each compartment being defined by a rate constant. The number of compartments is usually derived from the number of decays in the experimental data. For reason of simplicity, dose rate, cardiac output, and alveolar ventilation rate are assumed constant in a one-compartmental linear model.

A mass balance yield for a one-compartment model and for the dose accumulation phase:

$$\int (\mathrm{d}\, d/\mathrm{d}t)\, \mathrm{d}t = V_{\mathrm{body}}\, K_{\mathrm{blood/air}}\, c_{\mathrm{air}}\, (1-\exp[-k_{\mathrm{e}}\, t])$$

where d is the dose, V_{body} is the volume of distribution (m^3), c_{air} the concentration in the air inhaled (mole m^{-3}), t is the time (h) and k_{e} is the elimination rate constant.

The elimination curve for a multicompartmental model (i compartments) is described as the sum of the exponential functions:

$$c(t) = c_{01}\ \mathrm{e}^{-k_1 t} + c_{02}\ \mathrm{e}^{-k_2 t} + \ldots + c_{0i}\ \mathrm{e}^{-k_i t}$$

In most cases one- or two-compartment models are sufficient to describe the general kinetics of VOCs and estimate appropriate sampling time points. It is advisable to take samples during the period of slow decay rather than fast decay. More elaborate models have applied more compartments, e.g., to

describe the decay following *p*-xylene exposure (Fig. 2) [36].

Data on half-lives are available from human volunteer studies and from monitoring people, who have experienced excessive exposure, e.g., due to accidental spillage. When $t_{½}$ for a substance is known the value of *k* can be calculated, because *k* is related to $t_{½}$ (assuming first-order kinetics) by the equation:

$$k = \frac{\ln 2}{t_{1/2}}$$

where ln 2 is the logarithm of 2 (base e).

2.4.3 Physiologically based toxicokinetic model (PBPK)

Physiologically based toxicokinetic models provide a detailed description of a substance in the human body. In principal these models include the major organ systems, plus any site of interest, each representing a physiological compartment with its own blood flow, volume, uptake, and elimination process. The disadvantages are that they are more complex, need more input data, and cannot be solved analytically [39]. More compartments will give a more detailed description of the kinetics and more accurate result, provided that the large amount of input data for the equation are all of sufficient quality. If data for an additional compartment are of low quality, it might be wiser not to increase the number of compartments. Consequently, the physiologically based toxicokinetic models found in the literature are of different complexity. Commercial computer programs are available to solve the equations. The value of these models, however, is limited for biomonitoring purposes. They are especially useful for risk assessment in extrapolating data from animal experiments and estimation of concentrations at target sites. These models have been used for analysis of dichloromethane inhalation kinetics, carcinogenic risk assessment of tetrachloroethylene, and regulatory risk assessement and standard setting of other VOCs [40].

2.5 Toxicokinetics and implication for exposure estimation

The application and interpretation of biological monitoring depend upon the sampling strategy, which must be based on the toxicokinetic properties of the substance. Generally, the following basic parameters can be distinguished.

2.5.1 Evaluation of the rate of absorption

The half-life of the chemical in the given compartment of the body is so short that the concentration in biological media reflects the actual exposure. An example can be the concentration of volatile solvents in blood and exhaled air in samples collected during exposure.

2.5.2 Evaluation of daily exposure

The half-life of the chemical in the body is sufficiently short (e.g., < 5 h) and biological level reflects the dose absorbed on a given day. Toluene, xylenes, and phenol belong to this group. In this case, biological monitoring is useful in industrial toxicology even if the measured levels fluctuate significantly from day to day. Time of sample collection at the end of exposure is recommended; under practical circumstances this will be 15–20 min after termination of exposure [41]. The use of biological monitoring in environments other than industry, where the source of exposure has not been identified, could give misleading results.

2.5.3 Evaluation of cumulative exposure

The half-life of the VOC and its metabolites is very long (e.g., < 50 h), allowing substantial accumulation of the substances. Here, the fluctuations of levels from day to day and within one day are small and, therefore, the exact timing related to the source of exposure is not necessary. Under continuous exposure a steady state is reached after 6 half-lives.

Most VOCs do not belong to either of these classes, having neither very short nor very long half-lives. For substances such as nitrobenzene, trichloroethylene or tetrachloroethylene, biological levels depend both on the actual exposure during the day and on the past exposure over the last week.

This situation is common in the case of elimination of organic solvents from the fat tissue. None of the situations is usually clear enough to allow proper interpretation of the data without adequate knowledge of the toxicokinetic properties of the substances.

2.5.4 Kinetics in exhaled air

Analysis of exhaled breath/alveolar air can be used for the estimation of exposure to VOCs and might be put forward as biological equivalents to TLVs [42]. End-exhaled-air represents the alveolar air concentration and mixed-exhaled-air represents the gas mixture coming from the dead space of the bronchial tree and the alveolar gas-exchange space. The mass-balance assumption for alveolar breath concentrations can be described by:

$$c_{alv} = f \; c_{air} \; \Sigma \, a_i \, [1 - \exp (-t / t_i)]$$

where c_{alv} alveolar breath concentration, f = fraction of the parent compound exhaled at equilibrium, c_{air} ambient air concentration, t_i = residence time in the compartment i , a_i = fraction of breath concentration contributed by the compartment i at equilibrium, t = time of exposure and $\Sigma \, a_i = 1$. The residence time is the time it takes for a chemical to decline to 1/ e of its initial concentration in the compartment. Values for estimates of t_i and f for some VOCs have been provided from inhalation chamber studies [42,43]. The exchange in alveoli is between air and blood. At equilibrium, there is no uptake, and the ratio of concentrations in blood and alveolar air is given by the blood/gas partition coefficient. After exposure, the exchange occurs between pulmonary venous blood and alveolar air. The contribution of compartments to the venous blood levels and hence alveolar air depends upon the time following exposure, the compound's solubility in tissues [16]. The estimated contribution of the main compartments to alveolar air concentration at equilibrium amounted to 30% for blood and fat respectively and 20% for the vessel-rich (VRG) and the vessel-poor groups (VPG) each [36].

The method of breath analysis by collection of exhaled air is noninvasive but bears some limitations (cf. Sect. 5.1.3): The VOC concentrations may fluctuate very rapidly and are very sensitive to minor changes in sampling time and preceding breath-holding time. Specifically, concentration differences in mixed-exhaled air, end-exhaled air (alveolar air), and forced-exhaled air have to be taken into account [16]. The risk of contamination reflecting the external air concentrations is always present. Individual variation has to be considered, such as gender, age, body mass index, and the effect of exercise, since alveolar toluene concentrations tended to be higher in females and under exercise [46]. For persons with altered pulmonary function this biomarker may not be suitable.

Recently, attempts have been made to improve sampling and measurement methods for breath analysis [e.g., 44]. Portable systems have quite recently been applied for population based studies [36] in environmental settings [45].

3. SAMPLING AND ANALYTICAL PROCEDURES, FACTORS AFFECTING UNCERTAINTY COMPONENTS, AND QUALITY ASSURANCE

3.1 Specimen collection

Due to the ubiquitous presence of VOCs in ambient and indoor air, special precautions must be taken to protect sampling equipment and the specimen from contamination. On the other hand, losses during specimen collection, shipping, and storage due to the volatility of the VOC have to be minimized by appropriate procedures.

For the determination of internal exposure to volatile compounds in blood, the following measures have been proven to be suitable:

- Disinfection of the skin with a hydrogen peroxide (H_2O_2) solution (3%) or simply soap. Alcoholic, phenolic, or other disinfectants are unsuitable. Similar precautions have been introduced for forensic purposes in blood alcohol sampling.
- Blood is taken by venipuncture with stainless steel needle and 10 ml K-EDTA syringe (e.g., Sarstedt Monovette).
- The blood is transferred by septum puncture, injected into a PTFE (polytetrafluoroethylene) lined rubber-stopped (Bördelcapped) head-space glass vial containing ammonium oxalate or liquid K-EDTA solution gently swirled until the anticoagulant is dissolved.
- The head-space glass vial must be thoroughly cleaned before use, and the vial and the rubber diaphragm septum heated at 80 °C for at least several days (limited to 80 °C to prevent ammonium oxalate decomposition and septum deformation leading to reduced tightness) before capping in an uncontaminated atmosphere (best choice: clean bench with activated carbon-filtered air, automated crimper system).

Applying this procedure, VOC losses or contamination are very unlikely to occur because of the immediate separation of the blood specimen from the ambient atmosphere. A second advantage is the fact that the analytical procedure of gas chromatographic head-space analysis can be carried out in the identical vial without further clean-up steps. The samples should be analyzed as soon as possible but experience has shown that in many cases storage in a refridgerator at +4 °C for one week maximum or up to several months in the freezer at –18 °C is possible without VOC losses. Spiked blood standards for aromatic hydrocarbons (e.g., benzene, toluene, xylenes), chlorinated aliphatic compounds (e.g., dichloromethane, trichloro-, tetrachloroethylene) as well as for alcohols and ketones in blood (e.g., methanol, acetone, butanone) can be used for calibration at the µg/l level (mg/l for alcohols and ketones) up to several months if stored deep-frozen. The refrigerator or freezer must be uncontaminated by solvents, standard solutions, etc. which has to be tested by parallel storage of control or blank specimen. At freezer temperatures, long-time storage with perforated septum, however, should be avoided because of reduced tightness [47]. Shipment of the samples in a cooler to the laboratory should be performed as soon as possible, preferentially by express mail or courier.

For very low concentrations (ng/l level) concerns for contamination control during all steps of specimen handling increase dramatically. Here, anticoagulant, blood collecting apparatus and storage container, blank water, laboratory air and analytical reagents have to be cleaned by special treatments before being free of VOC traces [48,49].

For urine collection, pre-cleaned (by rinsing with diluted nitric acid followed by ultrapure water) polyethylene screw cap vessels (volume 250 ml or up) are recommended for most VOC metabolites. If unchanged VOCs are to be determined in urine (e.g., for alcohols, ketones) an aliquote of the urine has to be transferred into a head-space vial (without anticoagulant) after voiding.

For controlling contamination during specimen collection and shipping, the following procedure is recommended (*field blanks*):

A head-space vial filled with uncontaminated 5 ml ultrapure water (or blood with low but known VOC content) is shipped and analyzed with each batch of the specimen samples. As blank for sampling, head-space vials filled with 15 ml ultrapure water or blood are prepared and for each sampling around 10 ml are withdrawn and handled exactly like the blood specimen (see above), including storage and shipment.

3.2 Principles of analytical procedures for VOCs in body fluids and interference factors

The main analytical principle for the analysis of unchanged VOCs in blood and urine is the so-called **head-space technique** (static or dynamic) **followed by capillary gas chromatography** applying mass selective, electron capture, photoionizaton or flame ionization, nitrogen-phosporous selective, or other detection. This technique is based on the physical laws by Henry/Dalton that state a proportionality between the VOC blood concentration and the corresponding vapor pressure in a closed compartment above the blood specimen (*head-space*). Injection of an aliquot of this head-space gas from the blood-containing gas-tight vial by an automatic head-space sampler into a gas chromatograph enables the analyst to estimate the VOC blood content, using an appropriate calibration with VOC-spiked blood standards. Due to the high dependence of the vapor pressure on temperature, the blood is incubated at a constant temperature (approx. 40 to 60 °C) to obtain an increase in the gas-phase VOC concentration. Head-space analysis may also be applied to VOC determinations in urine.

Lower detection limits in the ng/l range for biomonitoring of environmental VOC exposure or for the evaluation of reference values (cf. Sect. 6) can be achieved as follows: Techniques, such as dynamic mode, with purge-and-trap-technique or possibly by solid-phase microextraction [50,51,52] will increase the fraction of VOCs in the specimen that is subjected to gas chromatographic analysis, thus decreasing the detection limit. These methods have, however, a higher degree of analytical problems and uncertainty and should be restricted to well-equipped and specially trained scientific laboratories.

Analytical results for VOCs in blood seem to be to some extent dependent on the type of head-space technique applied (purge-and-trap vs. static). Especially for small VOC levels and without using specific techniques as shaking, heating of the blood vial, the static head-space results were distinctly lower (approx. 60–80 %) than those with the purge-and-trap technique. There was apparently a fraction of VOCs in blood that was not in immediate equilibrium with the head-space. This was not corrected by spiked blood standards unless they were allowed to equilibrate for a long time period (possibly several weeks) [53].

Table 3 summarizes important interference factors during a head-space gas chromatographic procedure of VOCs in body fluids. Among the numerous listed interference factors calibration conditions seem to be one of the most important. In order to prepare blood standards of well-defined µg/l- or even ng/l-concentrations of an organic solvent with high volatility, several dilution/transferring steps have to be carried out that are prone to systematic errors. The development of a reliable and standardized procedure to prepare matrix calibration standards is highly recommended in this context.

Due to the low VOC levels in blood, direct spiking into blood cannot be performed reliably, and diluted solutions of VOCs have to be prepared before spiking. Ethanol is an effective solvent for most VOCs and is used successfully in many laboratories for preparing a VOC stock solution (adding, e.g., 100 µl VOCs to several ml ethanol in a volumetric flask, weighing the exact mass of VOCs and adding ethanol to the mark). Ethanolic solutions of VOCs may be directly added to blood in small quantities (e.g., 100 µl to 10 ml blood) with stirring to prevent the blood from deproteinization. Ethanolic VOC solutions can also be further diluted with physiological saline (aqueous sodium chloride solution, 0.9%). This solution is isotonic to blood serum and can be added to blood in any volume without

Table 3 Head-space gas chromatographic analysis of VOCs in blood and corresponding interference factors[1)].

Procedure	Interference factors
Blood drawing	Contamination by ambient air or disinfection chemicals
Transfer to a gas-tight vial	Analyte loss due to high volatility
Shipping, storage	Analyte loss, contamination
Incubation (e.g., 40 min, 50 °C)	Fluctuations in duration and temperature of incubation
Injection of a head-space aliquot into the gas chromatograph	Analyte loss, memory effects, thermal discrimination of mixtures
Separation of the gas mixture	Insufficient separation, coelution
Analyte detection	Selectivity, linearity, sensitivity of the detector
Calibration	Matrix effects, preparation of standards, analyte loss during standard preparation, selection of an emulsifier, primary VOC content of matrix
Calculation of the analytical result (electron	Peak area quantification, extrapolation/range of linearity capture detection)

[1)]**interference factors** include all factors that alter the result *in vitro* (accord. to [54])

coagulation or deproteinization. Finally, VOC blood standards should be transferred and stored in those crimped head-space vials that are used for the analytical measurement. All handling during VOC blood standard preparation should be organized in a way to prevent losses due to evaporation, from contamination, and from coagulation of the native blood. Baseline VOC levels of the blood used for standard preparation have to be analyzed and taken into account for the calibration line. These base levels of VOCs should be kept as low as possible.

Urinary VOC metabolites that are to be quantified for biomonitoring purposes will in general have to be separated from the aqueous urinary matrix by clean-up steps such as liquid/liquid extraction, solid-phase extraction, steam distillation, etc. before instrumental analysis. In many cases the metabolites have to be released from conjugation with e.g., glucuronic or sulfuric acids, amino acids by hydrolysis. Especially for gas chromatography, derivatization may be required for the polar metabolites (e.g., carboxylic acids, alcohols, phenols). Thus, the analytical demands differ due to the individual properties of the corresponding group of metabolites. Table 4 gives an overview of VOC metabolites suitable for biomonitoring together with their principle of analytical determination.

General interference factors for urinary metabolites are inhomogeneity of matrix due to urine sedimentation and—for conjugated metabolites such as phenols, amines—incompleteness of hydrolysis to the free analyte. The latter item is of major importance as the conjugated forms are rarely available as calibrators. Numerous other factors depend on the applied analytical procedure and cannot be discussed in detail here. For VOCs and their metabolites in biological fluids the combined analytical standard uncertainties in general are *at best* 15 to 30%, depending on the actual concentration. Within the German external quality assessement scheme, the tolerance range accepted for the participating laboratories was ± 39–51% of the assigned value for VOCs in blood and ± 18–30% for VOC metabolites in urine [55]. The assigned value is obtained by analyses in highly qualified reference laboratories, and the tolerance range is the threefold standard deviation of their results.

3.3 Quality assurance

To meet the demands for reliable VOC biomonitoring determinations is not an easy task. The low analyte levels require in part complex sample treatment procedures that have to be carried out with a high degree of reliability in order to allow an exposure evaluation. An approach widely applied today in

Table 4 Examples of important VOC urinary metabolites and their analytical determination.

VOC	*Metabolite*/example	Principle of clean-up	Analytical determination
Aromatic hydrocarbons	*Phenols*/ *o*-cresol, dimethylphenols, phenol	Hydrolysis, steam distillation, SPE-RP[1], extractive derivatization	RP-HPLC/UV[2] GC/ECD[3]
	Carboxylic acids/ mandelic; *t,t*-muconic acid	Extraction SPE-anion exchange	RP-HPLC/UV
Aliphatic hydrocarbons	*Ketones*/ 2,5-hexanedione[4]	Hydrolysis, SPE-cation exchange, SPE-XAD	GC/FID[5]
Chlorinated hydrocarbons	*Alcohols, phenols*/ 4-chlorocatechol; chlorophenols	Hydrolysis, extraction steam distillation, SPE-RP, extraction, derivatization	RP-HPLC/UV GC/ECD GC/MSD[6]
Glycol ethers	*Alkoxyacetic acids*/ Ethoxyacetic acid	Pre-extraction of matrix, derivatization, SPE-adsorption[7]	GC/ECD
Dimethylformamide	Methylformamide[8]	Extraction	GC/NPD[9]
Carbon disulfide	2-Thioxothiazolidine-4-carboxylic acid	Extraction	RP-HPLC/UV

[1] SPE-RP solid-phase extraction-reversed phase
[2] RP-HPLC/UV reversed phase high-performance liquid chromatography with UV detection
[3] GC/ECD gas chromatography/electron capture detection
[4] Sum of 2,5-hexanedione and 4,5-dihydroxy-2-hexanone
[5] GC/FID gas chromatography/flame ionization detection
[6] GC/MSD gas chromatography/mass selective detection
[7] SPE-adsorption solid-phase extraction-adsorption chromatography
[8] Sum of *N*-methylformamide (NMF) and *N*-hydroxymethyl-*N*-methylformamide (DMF-OH).
[9] GC/NPD gas chromatography/nitrogen selective detection

order to achieve, maintain, and document the quality of the work of a biological monitoring laboratory is the adoption of a quality management program [56–58]. Traditional internal quality control and external quality assurance are important parts of the quality management. The following measures should be applied for quality assurance:

- Give an exact protocol about the person's working time and the individual exposure situation (e.g., workload, skin contact to VOCs, time of specimen collection with respect to the exposure)
- standardize all procedures during the pre-analytical phase that cannot be controlled by classical quality control (see below) including conditions of specimen collection, shipping, and storage,
- work out and follow strictly a validated method, e.g., a *Standard Operation Procedure (SOP)* for the complete analytical procedure of the VOC determination in body fluids. The U.S. BEI committee refers to appropriate analytical procedures described in the literature in their BEI documents [74]. Analytical procedures for selected VOCs are mentioned by the WHO [11]. Numerous

Table 5 International external quality assessment schemes for VOCs and their metabolites in body fluids according to [60–64].

VOC/metabolite	Body fluid	Organization/Nation
Benzene	Blood	DGAUM[1)]/Germany
Dichloromethane	Blood	DGAUM/Germany
Ethylbenzene	Blood	DGAUM/Germany
Tetrachloroethylene	Blood	DGAUM/Germany
Toluene	Blood	DGAUM/Germany
Trichloromethane	Blood	DGAUM/Germany
Trichloroethylene	Blood	DGAUM/Germany
Xylenes	Blood	DGAUM/Germany
Butoxyacetic acid	Urine	DGAUM/Germany
o-Cresol	Urine	DGAUM/Germany
Ethoxyacetic acid	Urine	DGAUM/Germany
2,5-Hexanedione	Urine	FIOH[2)]/Finland JFOHO[3)]/Japan
Hippuric acid	Urine	DGAUM/Germany JFOHO/Japan KISCO[4)]/Korea
Mandelic acid	Urine	DGAUM/Germany JFOHO/Japan FIOH/Finland
N-Methylformamide	Urine	DGAUM/Germany JFOHO/Japan
Methylhippuric (toluric) acids	Urine	DGAUM/Germany JFOHO/Japan FIOH/Finland
trans, trans-Muconic acid	Urine	DGAUM/Germany
Phenol	Urine	DGAUM/Germany FIOH/Finland
Phenylglyoxylic acid	Urine	DGAUM/Germany
2-Thioxo-4-thiazolidine-carboxylic acid (TTCA)	Urine	DGAUM/Germany
Trichloroacetic acid	Urine	DGAUM/Germany JFOHO/Japan FIOH/Finland

[1)]Deutsche Gesellschaft für Arbeitsmedizin und Umweltmedizin/German Society of Occupational and Environmental Medicine; Institute of Occupational, Social and Environmental Medicine at University Erlangen/Nuremberg, Germany
[2)]Finnish Institute of Occupational Health; [3)]Japan Federation of Occupational Health Organizations; [4)] Korea Industrial Health Corporation

examples of SOPs for the quantification of VOCs and their metabolites in body fluids can be found in the collection of analytical methods by the MAK and BAT committee of the German Science Foundation [59]. These publications contain a set of reliable experimentally validated analytical procedures with sufficient specificity and sensitivity as well as practicability for the investigation of large groups of exposed persons.

- Establish a well-performing system of internal and external quality control. Unfortunately, there are no commercially available control materials for VOCs in body fluids up to now so that each laboratory engaged in this field has to prepare its own. For selected urinary metabolites, spiked matrix standards with assigned values of metabolite content can be purchased (e.g., *Lyphochek* by BioRad, *ClinRep* by Recipe, Munich/Germany). For the most important external quality control there exist several international schemes for VOCs and their metabolites in body fluids (Table 5) in concentrations relevant to occupational medicine.

4. UNCERTAINTY OF VOC ANALYSIS IN BODY FLUIDS

The result of a measurement is always an approximation of the true values. Therefore, when reporting results of unchanged volatile organic compounds and/or their metabolites in biological fluids, it is now accepted that it is essential to give some quantitative indication of the uncertainty. The uncertainty of a

Table 6 Type of uncertainties associated with biological VOC measurement results.

Sampling	Calibration	Analytical procedure
Nonrepresentative sampling	Calibrator concentration and purity	Definition of the measured entity
Contamination	Analytical variability	Volumetric equipment
Degradation/synthesis	Nonrepresentative matrix	Matrix effects, e.g., anticoagulants, hemolysis
Loss by adsorption	Nonlinearity of calibration function	Specificity
	Statistical estimation of the calibration function	Environmental factors

measurement result is an estimate of the limits between which the results are expected to vary with a stated confidence level. It may be expressed as a standard deviation, the half-width of a confidence interval. Without such an indication it is not possible to compare biological measurement results, nor to assess the reliability of the result and the confidence that can be placed in any decision based on its use [65]. Furthermore, the EN 45000 [58] and ISO/IEC Guide 25 [56] require that the laboratory has estimated the uncertainty of the method included in the accreditation [66].

A basic premise of the uncertainty concept is that it is composed of contributions from many different sources, so-called *uncertainty components* [67,68]. Uncertainty components are classified according to the method of evaluation: Type A describes a standard uncertainty evaluated by statistical analysis of series of observations, and Type B describes those uncertainty components that are evaluated by other means, i.e., evaluation from the literature data, from data obtained by other means or from data judged by an expert. The estimation procedure gives valuable information to the laboratory of the uncertainty components, which influence the total uncertainty of a measurement: The laboratory may be able to improve the performance by reducing or eliminating the largest uncertainty component [69].

When estimating the total uncertainty of a measurement, all uncertainty components should be evaluated and assigned an uncertainty. They include preanalytical factors; sampling and transportation, preservation, matrix effects, dilution of the sample, calibration, etc. However, the preanalytical factors are often complicated or impossible to estimate. In many cases, it is possible to reduce their contributions by appropriate instruction and training. Those preanalytical factors that contribute with more than 1/5 of the largest uncertainty component must be included in the uncertainty. The factors before sampling may often influence the result much more than the uncertainty of measurement, but they are not included in the estimation. Table 6 lists uncertainty components related to biological VOC-measurement results.

The uncertainty components are quantified using data from internal control charts, experimental data from method validation, reference materials or external quality assessment schemes, information from the literature, etc. Measurement results should be corrected for known systematic effects. The total standard uncertainty of a VOC measurement result is then obtained by combining the standard uncertainties of the components according to the rules of combining variances. An expanded uncertainty is obtained by multiplying the combined standard uncertainty by a coverage-factor k. The choice of the factor k is usually 2 or 3, based on the level of significance. In the case of a normal distribution, the factor k = 2 means that limits of the overall uncertainty have a confidence level of approximately 95%.

5 BIOMONITORING OF EXPOSURE

In principle, two different types of exposure can be found for VOCs:

In environmental settings, the exposure profile is often unknown. Therefore, a qualitative over-

view of the VOCs present in the body fluids of the study group might be of interest before selecting VOCs for a quantitative analysis. Environmentally caused VOC levels are at least one order of magnitude lower compared to occupational limit values in biological material (cf. Section 6, 7). Kinetic influences are often not as distinct as in occupational settings where comparatively higher and fluctuating exposure levels are to be expected.

At the workplace a typical VOC profile in body fluids starts with an initial increase in VOC level often followed by a steady state with nearly constant VOC level. After the end of exposure at work VOC level decreases due to elimination by excretion and metabolism. In industry, VOC exposure is in general well characterized. Quantitative analysis of VOC levels in body fluids, the evaluation of these biomonitoring results with established occupational limit values are the main points of interest in the industrially applied biomonitoring.

In the following section different approaches for biomonitoring of VOC exposure will be presented and discussed along with their advantages and limitations in practical application.

5.1 Volatile organic compounds (VOCs)

5.1.1 VOCs in blood

The determination of the unchanged volatile organic compounds in blood is specific. For the majority of the VOCs, they are not physiologically present in body fluids. Environmentally caused base blood levels lie in the ng/l range for most of the important industrial solvents in the aromatic hydrocarbons, and chlorinated aliphatic hydrocarbons classes. One of the VOC's target organs is the central nervous system, because of its high perfusion and the usually high lipophility of VOCs, resulting in prenarcotic symptoms during chronic exposure. VOCs in blood reflects the individual internal load and potential CNS toxicity. This response is caused by the unchanged VOC and in general not by its metabolites.

The VOC level in blood is appropriate for biomonitoring nearly every VOC except for esters of carboxylic acids (e.g., ethyl acetate). These esters undergo rapid enzymatic hydrolysis by ester cleavage into carboxylic acid and alcohol with half-lives of a few minutes [70]. Because of the high stability of the esterases this reaction might even continue *in vitro* after blood collection. A better choice for ester biomonitoring would be to estimate the released alcohol moiety.

Another advantage of determining unchanged VOCs lies in the fact that due to the volatility of the substances the same analytical approach (head-space technique in combination with capillary gas chromatography, cf. Sect. 3) allows the simultaneous quantification of all VOCs of interest in one blood specimen (at least if applying an universal detection system such as mass spectrometry). This is of special interest for biomonitoring as occupational and environmental VOC exposure is characterized by complex mixtures of sometimes unknown composition.

A main drawback is its high dependence on the usually fast elimination kinetics of the VOC (cf. Sect. 2). With elimination half-lives of minutes to a few hours it is obvious that a specimen collection strategy and protocol has to be strictly followed for interpretation of a VOC quantification in blood. This is of utmost importance for fluctuating exposure profiles at work sites. Environmental exposure (e.g., indoor atmosphere) might be characterized by a more steady ambient VOC content.

Three principle approaches in specimen collection points can be found in the literature:

- during exposure,
- at the end of exposure,
- a defined time period after end of exposure.

The first approach might be used in experimental studies as well as for environmental exposure but is by far less acceptable for a systematic monitoring program at work sites because of hygienic, work processing, and other practical reasons. For occupational exposure, specimen collection at the end

of the shift (second possibility) and—to a lesser degree—after a given time after exposure (third) is frequently used and is recommended in many biomonitoring guidelines (e.g., BAT and BEI values, cf. Sect. 7 [71–74]).

As pointed out in Section 2, elimination of VOCs from blood might be the result of several half-lives (cf. Fig. 3). It should be clearly stated here that the very short initial elimination phase with half-lives of several minutes, as found in experimental settings, is not covered in biomonitoring practice. After a lag of some 15 min the second phase of VOC elimination starts with half-lives of several hours. Blood collection should be done during that time period.

For half-lives of a few hours (second phase of elimination), the BAT refers to the blood level at the end of the shift (e.g., dichloromethane, styrene, toluene, xylenes), whereas for accumulating substances (e.g., tetrachloroethylene, 1,1,1-trichloroethane) sampling should be performed prior to the following shift. In the latter case, the elapsed time from end of exposure to specimen collection is related to VOC levels in different body compartments. In the case of tetrachloroethylene, the increasing half-lives ($t_{½}$ of the I, II and III phase of elimination amounting to about 1.5 h, 9 h and 56 h respectively) are reflecting concentrations in blood, blood-rich organs such as brain, heart, liver, kidney, muscles, and fatty tissue, and less drained tissues such as cartilage and bones [75–77].

From the analytical point of view it might be advisible to choose the time of specimen collection with the highest VOC content—usually at the end of exposure especially for low VOC levels such as benzene in blood. Today, benzene air concentrations at the workplace are about 1 ppm (at least in the developed countries) causing benzene blood levels range at or below 5 µg/l. This is already of the same order of magnitude as the limit of determination for static head-space technique capillary gas chromatography. A change to the more sensitive *dynamic* head-space technique (cf. Sect. 3), however, would lead to a decrease in analytical accuracy due to the still high rate of interference for this analytical procedure.

Specimen collection at a defined period of time after the end of exposure might be less affected by small variation in timing, but there are very few VOC guidelines reported in the scientific literature that could be adopted for the evaluation of quantitative VOC body fluid levels. VOC concentrations in specimens collected after a definded period of exposure are influenced more by factors such as body fat, exercise, and smoking, since these specimens represent release (washout) from the fat compartment. In addition, exact kinetic parameters for the VOC elimination are generally unknown. Any extrapolation to other times after exposure is therefore impossible (with only few exceptions as, e.g., ethanol, tetrachloroethylene). Reconsideration is recommended if in future agreements a defined period of time after end of exposure (e.g., 15–20 minutes) should replace the present approach of end of exposure sampling because of the rapid decrease within the first few minutes after termination of exposure.

5.1.2 VOCs in urine

VOCs in urine is especially suitable for short-chain alcohols and ketones as they possess a high-to-medium water solubility. Thus, they are easily excreted in the urine without metabolism by a simple diffusion process (examples: acetone, 2-butanone, methyl isobutyl ketone, methanol). In many cases, determination of alcohols and ketones in urine is specific for exposure (no metabolic transformation), but in some cases (as for acetone) a high urinary base level exists, and the substance may also be formed by biotransformation of other VOCs (acetone is a metabolite of 2-propanol). Because the urinary specimen reflects a sampling period of several hours, it is correlated with the absorbed dose for specimens collected at the end of exposure. Compared to VOCs in blood, kinetic influences are distinctly lower for VOC excretion in urine. Other positive analytical aspects of biomonitoring are noninvasive urine collection, in contrast to blood venipuncture, and a higher concentration for the water-soluble VOCs in urine than in blood (mg/l for the stated examples).

While excretion of unchanged VOCs is related to the urine/blood partition coefficient and to urinary *volume*, urinary concentrations VOC metabolites are significantly influenced by *kidney functions*, drinking habits, sweating, etc. To overcome this problem adjustment to either urinary density or creatinine content in urine has been recommended in the literature [78–81]. Generally, before applying any adjustment for urine concentration, the user should establish that a closer relationship exists between the adjusted metabolite concentration in urine and the air concentration for the individual VOC. This is best accomplished through experimental or field studies. The German BAT group, and the U.S. BEI committee have established certain biological action levels with creatinine adjustments (cf. Table 12, Sect. 7). Examples are BEIs for carbon disulfide, chlorobenzene, ethylene glycol ethers, n-hexane; BAT values for 4-chlorocatechol excretion after chlorobenzene exposure. It is suggested that spontaneous urinary specimens with extreme relative densities (higher than 1.024 or less than 1.010) or creatinine concentration less than 0.5 g/l or higher than 2.5 g/l should be excluded from analysis in order to prevent the analyst and physician from misinterpretation (e.g., in case of altered renal function).

VOC excretion in urine has been recommended for biomonitoring purposes by few scientific groups [82–86] for other chemical classes of VOCs (e.g., aromatic and chlorinated hydrocarbons). Arguments *for* this are the noninvasive specimen collection, the minor kinetic influences in comparison with the VOC levels in blood, and the simultaneous quantification of mixture compounds in a single urine sample. Arguments *against* the use of VOCs in urine include (1) the small percentage of lipophilic VOCs excreted in the urine, (2) increased analytical requirements necessary to detect these low levels, (3) high probablity of VOC loss during the pre-analyical phase, and (4) more complex sample handling [87]. Table 7 shows blood and urinary concentrations for some VOCs at ambient air concentrations of the actual German MAK levels and demonstrates that distinctly higher blood concentrations are observed at the same time of specimen collection.

Table 7 Comparison of blood and urinary VOC concentrations corresponding to actual German MAK values [71] at end of shift. Blood concentrations refer to German BAT values [71] while urinary concentrations were calculated with quantitative relationships between urinary and environmental concentrations for occupational exposure by Pezzagno et al. [83].

VOC	MAK value 1998	VOC in blood (BAT 1998)	VOC in urine acc. to [83]
	ml/m^3	µg/l	µg/l
Toluene	50	1000	110
Xylenes	100	1500	170
Tetrachloroethylene	50 [1)]	1000 [1) 2)]	125
1,1,1-Trichloroethane	200	550 [2)]	550

[1)] MAK /BAT values valid until 1996; tetrachloroethylene will then be classified as carcinogen.
[2)] Time of specimen collection: 16 h after shift (prior to next shift)

5.1.3 VOCs in exhaled air

The main advantage of VOCs in exhaled air is the noninvasive specimen collection. In addition, specificity can be assumed as the unchanged VOCs are measured. Drawbacks, however, are as follows: (1) practical and analytical deficiencies with sampling, shipping, and storage of alveolar air. Recently considerable attempts have been undertaken to overcome these preanalytical difficulties [43–45] for environmental population studies. (2) For persons with altered pulmonary function, systematic errors in the specimen collection process might occur that are caused by the individual and cannot be eliminated by improved analytics. (3) Elimination in alveolar air is as highly affected by toxicokinetic influences as blood, so sampling time recommendations have to be followed very strictly. (4) In general, measurement of VOCs

in exhaled air is not as sensitive to exposure as measurement of VOCs in blood. Applying the currently available methods to similar environmental exposure scenarios, breath measurements tend to report more non-detects than blood mesurements. This is especially true with very low level exposures [53]. Summarizing, this biomarker can be recommended only to a limited degree.

5.2 VOC metabolites

5.2.1 VOC metabolites in urine

Metabolite excretion may be a superior parameter for biological monitoring if the relevant toxicity of the exposure chemical is caused by the metabolite or its precursor and not by the unaltered chemical itself. Examples for this situation are: 2,5-hexanedione for n-hexane exposure, ethoxyacetic acid for ethylene glycol ethers. Other advantages, due to the urine sampling period and to longer half-lives of many VOC metabolites, are a more integrative measure for exposure compared to the VOC concentration in blood. Blood reflects the actual exposure level shortly before specimen collection (e.g., toluric acids in urine/xylenes in blood). Other examples for VOC metabolites suitable for biomonitoring may be found in Table 12 in Sect. 7. For practical biomonitoring purposes, the amount of VOC metabolite excreted must be in sufficient quantity to detect and must exceed background levels in a non-exposed population. VOC metabolite levels are, in general, not affected by determinant loss or contamination during preanalytical phase and clean-up. The analytical requirements for the metabolites' quantification differ to a large degree on the excreted amount, chemical properties of the metabolite (*in vitro* stability, polarity, etc.) (cf. Sect. 3).

The following disadvantages have to be stated: Unspecificity in terms of either an observed "base" level for unexposed persons or because the metabolite may be formed by several exposure chemicals independently. In the first case (example: hippuric acid) details of the quantity of "normal" physiological excretion (reference values, upper reference limit as defined by IUPAC ComTox and IFCC [88,89]) have to be known for an appropriate interpretation of biological data in relation to the exposure situation. In the second case (example: mandelic acid that occurs as a metabolite after styrene as well as ethylbenzene exposure) and for complex exposure situations an additional, specific parameter (the VOC level in blood) may be required. Table 8 gives a short overview about specificity of urinary VOC metabolites commonly applied in biomonitoring.

5.2.2 VOC metabolites in blood

In some cases metabolite levels in blood may be more relevant for biological monitoring than those in urine. Carbon monoxide is a metabolite of dichloromethane and the portion of COHb (carboxyhemoglobin) reflects the internal dose of dichloromethane as well as its health impact. 2,2,2-Trichloroethanol seems to be responsible for the central nervous symptoms caused by trichloroethylene as the parent substance. With the brain as the target organ, blood levels of 2,2,2-trichloroethanol reflect its brain concentration and are thus more relevant to neurotoxicity than urinary metabolite levels [90].

5.3 Other

For some VOCs, biological effect parameters can be applied for exposure effects in addition to dose monitoring (example among the VOCs: induced elevated Met-Hb levels for aromatic amines or nitro compounds). BEI values for aniline and nitrobenzene exposure refer to Met-Hb levels of 1.5%.

Another approach of biological monitoring is the use of VOC adducts to macromolecules, namely protein adducts—especially those of hemoglobin or albumin. Several examples for corresponding BAT/

Table 8 Specificity of urinary metabolites of selected VOC applied for biomonitoring.

VOC	Urinary metabolite	Metabolite specificity	Remark
benzene	phenol	low*	diet, metabolite of aromatic amino acids
	trans, *trans*-muconic acid	medium	metabolite of sorbic acid (preservative)
	S-phenylmercapturic acid	high	
toluene	hippuric acid	low*	diet, sodium benzoate (preservative)
	o-cresol	high	
xylenes	methylhippuric acids	high	
	dimethylphenols	high	
ethylbenzene	mandelic acid	medium	
	phenylglyoxylic acid	medium	metabolite formation by
styrene	mandelic acid	medium	styrene and ethylbenzene[1)]
	phenylglyoxylic acid	medium	
1,1,1-trichloroethane	trichloroacetic acid	medium	metabolite formation by several chlorinated hydrocarbons
tetrachloroethylene	trichloroacetic acid	medium	
trichloroethylene	trichloroacetic acid	medium	
vinyl chloride	thiodiglycolic acid	low-medium	metabolite formation by several VOCs (e.g., 1,2-dichloroethane) and drugs (carbocysteine)
ethylene glycol monoethyl ether	ethoxyacetic acid	high	
n-hexane	2,5-hexanedione (total)	medium	metabolite formation *in vitro* by physiologically excreted precursors
isopropyl alcohol	acetone	medium	physiological acetone excretion

[1)]This fact has no practical implication for occupational settings as coexposure of styrene and ethylbenzene seems not to occur.
* This VOC metabolite is not recommended to be used in practical biomonitoring mainly because of its low specificity and sensitivity.

EKA values after VOC exposure may be found in Table 12 (cf. Sect. 7). For aromatic amines (e.g., aniline) and nitro compounds (e.g., nitrobenzene) nitroso-metabolites bind to thiol groups of hemoglobin. The resulting sulfinic acid amide hemoglobin conjugate remains stable *in vivo* for erythrocyte life time (approx. 120 days) [91]. *In vitro*, the conjugate may be cleaved after erythrocyte isolation by alkaline hydrolysis to release the parent compound (e.g., aniline) that can be determined by gas chromatography/mass spectrometry analysis after clean-up.

For alkylating compounds (e.g., acrylonitrile), a specific terminal hemoglobin adduct of the parent compound is released by derivatization *in vitro* (so-called Edman-degradation). The resulting substituted amino acid (cyanoethylvaline) can be quantified by instrumental analysis.

These protein adduct levels might be related closer to the relevant "target dose" than the VOCs in blood, for instance. The link between protein adduct level and risk assessment which would be very helpful especially for carcinogenic compounds has, however, not yet been established.

5.4 Evaluation of exposure to mixtures

In many industrial settings as well as for environmental exposure situations persons are exposed to complex VOC mixtures. They are of different and changing compositions. Two common practical approaches for applying biological monitoring for VOC mixture exposure exist:

- Single VOC measurement in human biological material and interpretation as a so-called "leading compound",
- "fingerprint" measurements of VOC mixtures in body fluids as a qualitative assessment.

Both approaches are inadequate from the toxicological point of view. For most solvent combinations that are present at work sites we either do not know their combinated toxicology, or whether synergistic toxicological mechanisms exist that would exclude this approach (e.g., butanone/ n-hexane or 2-hexanone) [92,93].

In industrial hygiene it is common practice to add up the fractions of mixture compounds in relation to airborne limit values and conclude occupational safety if the sum of these fractions is below 1. A similiar approach has been suggested by Ogata [94] for biological monitoring assuming no interaction at all. This proposal cannot be recommended because mixture components may possess diverse toxicological profiles including different target organs, kinetics, and possible synergistic effects (see above for examples).

5.5 Influence factors on biomonitoring results after VOC exposure

Influence factors are defined according to the International Federation of Clinical Chemistry (IFCC) and affect the biomonitoring results *in vivo* [54]. Their impact on VOC biomonitoring data is considered to be quite high. Some of the most important ones, such as kinetics, lack of specificity of urinary metabolites, nutritional influences on base levels, lifestyle factors (smoking, ethanol consumption, drugs), and different urinary dilution have already been mentioned above (cf. Sect. 2 to 5.3). Other important influence factors might be induction or inhibition of VOC metabolizing enzymes and genetic polymorphism of specific enzymes.

Studies of experimental animals exposed to very high VOC concentrations point to a possible influence of enzymatic induction or competitive inhibition on the toxicity and metabolism of VOCs [95, 96]. It was suggested that the induction or inhibition of microsomal enzymes may also affect the results of biological monitoring of exposure.

In vivo, the extent of hepatic metabolism of chemical substances is determined by the relation between the metabolic clearance and the hepatic blood flow [97]. At current exposures occuring in occupational environments, hepatic blood flow rate limits the metabolism. This might lead to the impression that enzyme induction might not be of major importance for the current industrial exposure situation. This conclusion, however, does not take into account the relevant contributions of extrahepatic metabolism/phase II enzymes to the overall metabolic clearance.

Metabolic interactions—although very common in practice—have not been studied in a systematic and comprehensive manner according to literature data, possibly because the combinations of the

Table 9 Experimental exposure of volunteers to solvent mixtures: effects on metabolism [98] (either the maximum non-effective dose or the minimum effective dose is given when two or more concentrations were tested).

Combination	Effect	References
25 ppm[1)] benzene + 100 ppm toluene	No change in clearance	Sato and Nakajima (1979) [99]
66 ppm toluene + 21 ppm *p*-xylene	No effect on both	Wallen et al. (1985) [100]
50 ppm toluene + 40 ppm *m*-xylene	No effect on both	Tardif et al. (1993) [101]
95 ppm toluene + 80 ppm *m*-xylene	Mutual metabolic suppression	Tardif et al. (1993) [101]
200 ppm *m*-xylene +400 ppm 1,1,1-trichloroethane	No effect on both	Savolainen et al. (1981) [102]
150 ppm *m*-xylene + 150 ppm ethylbenzene	Mutual metabolic suppression	Engström et al. (1984) [103]
100 ppm *m*-xylene + 200 ppm 2-butanone	Decrease in *m*-xylene metabolism, but no effect on that of 2-butanone	Liira et al. (1988) [104]
71 ppm *m*-xylene + 69 ppm toluene	No effect on xylene metabolism	Jakubowski and Kostrzewski (1989) [105]
69 ppm *m*-xylene + 72 ppm n-butyl acetate	Increase in xylene metabolism	Jakubowski and Kostrzewski (1989) [105]
49 ppm *m*-xylene + 46 ppm n-butyl acetate	Increase in xylene metabolism	Jakubowski and Kostrzewski (1989) [105]
69 ppm *m*-xylene + 68 ppm n-hexane	No effect on xylene metabolism	Jakubowski and Kostrzewski (1989) [105]
69 ppm *m*-xylene + 69 ppm n-butanol	No effects on xylene metabolism	Jakubowski and Kostrzewski (1989) [105]
46 ppm *m*-xylene + 45 ppm toluene	No effects on xylene metabolism	Jakubowski and Kostrzewski (1989) [105]
72 ppm styrene + 517 ppm acetone	No effect on styrene metabolism	Wigaeus et al. (1984) [106]

[1)] ppm: ml/m^3

chemicals are large and the exposure intensities vary greatly. There is, however, a set of experimental studies with human volunteers as well as a limited number of field studies available. Table 9 summarizes data on experimental studies of combined VOC exposure and effects on metabolism.

Field studies for effects on VOC metabolism after combined exposure mostly for aromatic hydrocarbons [107–114] are summarized in Table 10.

Table 10 Occupational exposure of workers to solvent mixtures: effects on metabolism.

Combination	Effect	References
15.2 ppm[1)] toluene +13.9 ppm xylenes +16.7 ppm methyl isobutyl ketone	no statistically significant differences in regression lines for toluene in air vs. o-cresol/hippuric acid in urine and for xylenes in air vs. methylhippuric acid in urine compared to single exposure	Ogata et al. 1995 [107]
2.7 ppm toluene +3.1 ppm xylenes	absence of metabolic interaction for hippuric and methylhippuric acid excretion	Huang et al. 1994 [110]
6.2 ppm benzene +11.9 ppm toluene	biotransformation of benzene to phenol and quinol but not catechol suppressed as was metabolism of toluene to hippuric acid and o-cresol	Inoue et al. 1998 [109]
11 ppm toluene +7 ppm styrene	urinary metabolites of toluene and styrene as expected	Kawai et al. 1992 [112]
72.2 mg/m^3 styrene +225.7 mg/m^3 acetone	slower urinary kinetics of mandelic and phenylglyoxylic acid in presence of acetone; tendency to accumulate these metabolites	Marhuenda et al. 1997 [108]
63 ppm styrene +160 ppm acetone	urinary mandelic acid concentration increased	Ferioli et al. 1990 [113]
12.4 ppm styrene +23.5 ppm methanol +229 ppm methyl acetate	no evidence for suppression of styrene metabolism by methanol/methyl acetate coexposure	Kawai et al. 1995 [111]
7 ppm n-hexane +40 ppm 2-butanone	2,5-hexanedione vs. 2-butanone exposure positive correlated	Perbellini et al. 1985 [114]

[1)] ppm: ml/m^3

Ikeda [98] reviewed both sets of data from experimental as well as from industrial studies and concluded that the different effects depend on the amount of exposure so that caution should be exercised where the intensity of the combined exposure is high but not necessarily when it is low (although the threshold cannot be set so far). Alessio [115] proposed lists of effects of combination VOC exposures as a useful guide to interpret results of biological monitoring for combined VOC exposure. These compiled data, however, are incomplete and only indicative of the conditions studied. This fact should stimulate scientific groups to extend their knowledge of combined exposure to solvents.

Consumption of ethanol during exposure may cause an increase in concentration of unchanged compounds in blood, reduction of metabolism, and delay of peak excretion of metabolites and change of ratios between metabolites for some VOCs [116]. For example, p.o. ethanol consumption simultaneously with inhalative toluene exposure of volunteers increased toluene blood levels and decreased toluene clearance [117]. For styrene, efficiency of metabolism to mandelic and phenylglyoxylic acids was inhibited, and peak excretion of metabolites was delayed [118]. Excretion of hippuric and methylhippuric acids was reduced for smokers and drinkers after toluene and xylene exposure [110].

Genetic polymorphism of xenobiotically active enzymes (e.g., *N*-acetyl transferase NAT2, glutathione transferases GST) may cause different individual susceptibility to VOC exposure. This topic is

currently under extensive scientific investigation, and knowledge in this field will probably increase significantly within the next decade. Several recent studies suggest a relationship between VOC exposure, polymorphism-related differences in susceptibility, health effects, and results of biomonitoring.

Persons occupationally exposed to trichloroethylene and carrying at least one functional GST T1 or GST M1 gene had a higher risk for renal cell cancer than those with negative genotypes [119] due to a more pronounced glutathione transferase dependent formation of the reactive metabolite *S*-(1,2-dichlorovinyl)-L-cysteine [120]. Another example for a polymorphism effect might be found for NAT 2 positive persons ("fast acetylators"). These individuals have a higher risk for colon cancer probably caused by dietary heterocyclic aryl amines, but a lower risk for bladder cancer after occupational exposure to aromatic amines than slow acetylators [121]. Acute methyl bromide exposure [122] led to severe neurotoxic symptoms and comparatively low levels of *S*-methylcysteine adducts in albumin and globin for one person with normal red cell GST T1 activity ("conjugator") whereas another patient without detectable enzyme activity ("non-conjugator") showed only mild and reversible effects and distinctly higher adducts levels. This was interpreted as a possible relationship between methyl bromide neurotoxicity and GST-dependent formation of toxic metabolites such as methanethiol.

6. REFERENCE VALUES AND INTERVALS

For the interpretation of biological monitoring data of VOC exposure, an exact knowledge of the baseline concentration of these analytes and their metabolites in the relevant biological material is required. These so-called *reference values* have been defined by the IFCC as measured values in samples of a reference sample group, statistically adequate numerically to represent the reference population [89]. A *reference interval* is the interval between, and including, two *reference limits* that are derived from the statistical distribution of reference values. However, frequently it is not possible to produce the required number of reference values. In this case, the calculation of indicative reference intervals and reference limits from a lower number of reference values may be based on the statistically well-defined confidence or tolerance intervals as has been recommended by IUPAC [88].

For VOCs in blood the current data on reference values are quite limited and represent an analytical borderline situation because, for most laboratories, these levels are below the corresponding analytical limits of detection. Despite the lack of detailed data for many VOC reference values, the present knowledge seems to be sufficient for the interpretation of exposure levels found in occupational biological monitoring. For environmental exposures, e.g., of persons living near dry-cleaning shops, reference values are often not available. For these cases it is recommended to include a well-defined control reference group with minimal exposure in each biological monitoring study (applying an identical analytical design).

Table 11 gives an overview of literature data for the important aromatic hydrocarbons in whole blood for occupationally unexposed persons. This class of VOCs is comparatively well investigated concerning baseline values. For benzene in blood, an upper normal level (according to the 95 percentile of the reference values) of about 500 ng/l for nonsmokers has been found by three independent scientific groups in the United States, Italy, and Germany. For toluene, the corresponding concentration reaches several µg/l. These two compounds are of special interest for environmental toxicology due to a widespread use for many indoor applications (toluene) or due to a high environmental exposure caused mainly by automobile exhaust for a proven human carcinogen (benzene). Corresponding data for urinary metabolites can be found in Table 13, Sect. 7.3.

Table 11 Human blood concentration (ng/l) for aromatic hydrocarbons in whole blood of occupationally unexposed persons.

Analyte	Year of Publication	Number of persons	Mean	Median	Range	95th Percentile	Reference
benzene	1988	8 nonsmokers	127	127	49–191		Perbellini et al. [123]
		11 smokers	584	578	109–1136		
	1989	58[1)]	332	235	10–1657		Brugnone et al. [124]
	1989	13 nonsmokers	218	190	112–455		Hajimiragha et al.
		14 smokers	547	493	287–947		[129])
	1991	8 nonsmokers	176	165	80–300		Angerer et al. [130]
		2 smokers	211	180	130–430		
	1991	15 nonsmokers	262		202–345		Chriske et al. [132]
	1991	21 nonsmokers	239	216	83–571		Goergens et al. [133]
		9 nonsmokers	163	144	52–278		
	1992	67 nonsmokers	247	220	<60–780	540	Angerer et al. [131]
		26 smokers	335	280	<60–920	550	
	1992	293 nonsmokers	205	163	7–924	514	Brugnone et al. [126]
		138 smokers	381	291	7–2241	901	
	1994	883[2)]	130	61		480	Ashley et al. [135]
	1994	25 nonsmokers	111	99	50–219		Kok and Ong [82]
		50 smokers	329	316	81–629		
	1996	nonsmoker		241			Fustinoni et al. [136]
		smokers		365			
	1998	171 nonsmokers	123	110	15–462		Brugnone et al. [137]
		72 smokers	264	210	28–940		
toluene	1989	37[1)]	830	573	23–5013		Brugnone et al. [124]
	1989	13 nonsmokers	1630	1141	495–4614		Hajimiragha et al.
		14 smokers	2133	2001	1316–3804		[129]
	1991	15 nonsmokers	809		514–1058		Chriske et al. [132]
	1991	21 nonsmokers	576	460	210–1383		Goergens et al. [133]
		9 nonsmokers	384	425	79–558		
	1993	179 nonsmokers	809	424	–8241	3393	Wang et al. [134]
		53 smokers	897	606	–4153	2722	
	1994	604[2)]	520	280		1500	Ashley et al. [135]
	1995	269[2)]	1100	500		7100 (98perc.)	Brugnone et al. [128]
	1996	nonsmokers		759			Fustinoni et al. [136]
		smokers		1327			
ethyl benzene	1989	13 nonsmokers	651	431	175–2284		Hajimiragha et al.
		14 smokers	837	533	378–2697		[129,123]
	1991	15 nonsmokers	226		128–355		Chriske et al. [132]
	1991	21 nonsmokers	252	108	30–918		Goergens et al. [133]
		9 nonsmokers	148	115	46–340		
	1994	631[2)]	110	60		250	Ashley et al. [135]
	1996	nonsmokers		140			Fustinoni et al. [136]
		smokers		233			
m/p-xylene	1989	13 nonsmokers	1580	1094	548–5602		Hajimiragha et al.
		14 smokers	1705	1490	916–3008		[129]

Table continues on next page.

Table 11 *Continued*.

Analyte	Year of Publication	Number of persons	Mean	Median	Range	95th Percentile	Reference
m/p	1991	15 nonsmokers	590		339–940		Chriske et. al. [132]
xylene	1991	21 nonsmokers	687	378	170–2287		Goergens et. al. [133]
(cont'd.)		9 nonsmokers	479	405	234–924		
	1994	649[2)]	370	190		780	Ashley et al. [135]
o-xylene	1989	13 nonsmokers	409	324	129–1472		Hajimiragha et. al.
		14 smokers	463	352	236–1130		[129]
	1991	15 nonsmokers	191		106–291		Chriske et. al. [132]
	1991	21 nonsmoker	223	134	46–872		Goergens et al. [133]
		9 nonsmokers	153	118	50–340		
	1994	711[2)]	140	110		300	Ashley et al. [135]
total	1996	nonsmoker		604			Fustinoni et al. [136]
xylenes		smokers		794			
styrene	1989	33	177	155	0–854		Brugnone et al. [124]
	1993	81	221	172	7–963	512	Brugnone et al. [127]
	1994	657[2)]	74	41		180	Ashley et al. [135]
cumene	1989	33	176	147	31–929		Brugnone et al. [124]

1) Significant differences between smokers and nonsmokers (benzene: mean 485 vs. 130 ng/l; toluene: mean 1135 vs. 472 ng/l)
2) Smoking status not mentioned

7 BIOMONITORING ACTION LEVELS

Biological monitoring is extensively applied to field studies and other experimental designs of toxicological and medical research. In addition, it is used on a broad scale in practical occupational medicine in many developed countries. In some of them biological threshold values have been developed to control the workers' exposure. On an international level several Council Directives of the European Communities deal with health protection of industrially exposed workers, some of them bringing forward the idea of biological monitoring for selected chemicals, e.g., lead (80/1107/EEC; 82/605/EEC) [138, 139]. A currently adopted "Council Directive on the protection of the health and safety of workers from the risks related to chemical agents at work" (98/24/EC) [140] suggests establishing biological limit values (in addition to airborne occupational exposure limits) on a European level.

7.1 German BAT values

In Germany, the first set of "Biologische Arbeitsstoff-Toleranz-Werte, BAT" (biological tolerance values) has been recommended in 1980 by a scientific expert group of the "Deutsche Forschungsgemeinschaft" (German Research Foundation) [141]. BAT values are published as a list being updated annually combined with airborne threshold limit values for occupational exposure, the so-called "Maximale Arbeitsplatzkonzentrationen MAK" (maximum workplace concentrations) [71]. Presently, BAT values have been developed for 43 substances. Among them more than two third are VOCs [72].

The legal implementations of the BAT values are given by the fact that their annual list is adopted by the Federal Ministry for Employment and Social Affairs as Technical Guidelines [142,143]. There-

fore, employers are advised to apply biological monitoring. Performance of biomonitoring in occupational settings is regulated in a recently passed Technical Guideline [144]. For substances with proven dermal absorption under usual working conditions biological monitoring is mandatory [145].

BAT values are defined as "the maximum permissible quantity of a chemical substance or its metabolites or the maximum permissible deviation from the norm of biological parameters induced by these substances in exposed humans. ... BAT values are conceived as **ceiling values for healthy individuals**. ...BAT values are intended to protect employees from impairment of health at work."

For carcinogenic compounds "exposure equivalents for carcinogenic substance, Expositionsäquivalente für krebserzeugende Arbeitsstoffe, EKA "have been investigated by the Commission. An EKA value is based on the relationship of the air concentration of a carcinogen and that of the substance or its metabolites in biological material. From this relationship, the internal exposure which results from uptake of the substance *exclusively by inhalation* may be determined.

Two main approaches are applied so far in order to establish BAT values:

- correlation between air and biological concentrations derived by results of several independent field studies,
- health-based values as for dichloromethane exposure (CO-Hb) or aromatic amines or nitro compounds (MetHb).

In addition, experience from long-term medical supervision of occupationally exposed workers, data of experimental exposure studies as well as pharmacokinetic models provide further sources of knowledge to substantiate the BAT concept.

7.2 ACGIH BEI values

The U.S. Biological Exposure Indices (BEI) are developed by the American Conference of Governmental Industrial Hygienists (ACGIH), with the first values published in the early 1980s. Although BEIs are not considered to represent a legal standard, the ACGIH as an important professional society of industrial hygienists offers a highly accepted guidance for the interpretation of biological exposure data. The current booklet of BEIs contains 22 volatile organic compounds for which BEIs have been developed out of a total of 35 [73].

BEI values represent the levels of analytes that are **most likely to be observed** in specimens collected from a healthy worker who has been exposed to chemicals to the same extent as a worker with **inhalation exposure to the TLV** (Threshold Limit Value). BEIs are understood as "advisory levels" that may be exceeded by individuals of an observed group. Industrial hygiensts or occupational physicians are expected to reduce the exposure if BEIs are exceeded for a longer time period or if the BEI is exceeded for a substantial group within the exposed population.

Table 12 lists these BAT/BEI values for volatile organic substances.

As can be seen from Table 12, the quantitative values of BAT and BEI differ in some cases (e.g., butanone) although the scientific documentations cited for both categories [72,74] clearly show that each value is derived from the same scientific data pool, applying the same recommended sampling time and based on the same principle of evaluation (field studies/correlation between air and biological concentrations). The main reason for the difference lies in the different definitions (BAT/ "ceiling values for healthy individuals"; BEI/ " levels that are most likely to be observed"—see above). In several other cases deviations between BAT and BEI values are caused by different levels of their airborne standards (MAK and TLV-TWA, respectively). The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area as well as the U.S. working group of the American Conference of Governmental Industrial Hygienist are currently engaged to harmonize their occupational limit values for air and biological concentrations.

7.3. Finnish biomonitoring action levels

For several VOCs, the Finnish Institute of Occcupational Health recommends so-called *biomonitoring action levels* as listed in Table 13 together with the corresponding upper reference limit values. Some of them are directly health-based, other have been extrapolated from the relationship between exposure at the Finnish occupational exposure limit and the biological measurement [146, 147]. These values are not binding to the employer.

For phenol in urine and TTCA in urine *indicative limit values* are recommended by the Ministry for Social Affairs and Health with the objective that "the employer has to consider these limit values when assessing the exposure to the chemicals in question" [148]. In 1998, two further indicative values for urinary mandelic acid (as a biomarker of ethylbenzene exposure) and for blood toluene were given by the Ministry of Social Affairs and Health [149,150].

7.4. Other biomonitoring action levels (Japan, France, Italy, United Kingdom, Poland)

The Japan Society for Occupational Health (JSOH) recommends Occupational Exposure Limits Based on Biological Monitoring (OEL-B) for several substances, among them the VOC n-hexane for which an OEL-B of 3 mg/g creat. 2,5-hexanedione in urine (after acid hydrolysis) and 0.3 mg/g creat. 2,5-hexanedione in urine (without acid hydrolysis) has been established [151] for a sampling time at end of shift at end of work week. For toluene, OEL-B of 0.6 mg/l (toluene in blood) and 0.06 mg/l (toluene in urine) were set in 1999 for sampling within 2h before shift end in the second half of work week [152]. OEL-Bs are defined as values at or below which adverse health effects do not appear in most workers who are exposed to the chemical.

In France, a Scientific Group for the Monitoring of Workplace Environments publishes Biological Exposure Indices IBE (*Indificateurs Biologiques d'Exposition*) based on the BEI by ACGIH after consideration of the French situation. For aniline, benzene, chlorobenzene, *N*,*N*-dimethylformamide, ethylbenzene, carbon disulfide, furfural, n-hexane, methanol, 2-butanone, nitrobenzene, phenol, styrene, toluene, 1,1,1-trichloroethane, trichloroethylene, and xylenes the values are identical to the BEI listed in Table 12 while for tetrachloroethylene levels of 1 mg/l in blood (prior to last shift of the work week) and 7 mg/l for trichloroacetic acid in urine (end of work week) are recommended [153].

The Italians, through the "Fondazione Clinica del Lavoro", have established biological equivalent concentrations (*LBE, Limiti Biologici Equivalenti*) for a variety of VOCs. These values are principally VOCs in urine and are guidelines (Table 14, [154]).

In the United Kingdom, the framework of legislation for the use of biomonitoring is set out by the Control of Substances Hazardous to Health Regulation 1994. However, there is no requirement in those regulations for compliance with biological monitoring guidance values [155]. Two types of biological monitoring guidance values may be set in the United Kingdom—the health-based *health guidance values* and the practicable *benchmark guidance values* (set at the 90th percentile of available biomonitoring results collected from a representative sample of workplaces with good occupational hygiene practice). For several VOCs, *health guidance values* have been established by the Health and Safety Executive [156] which are listed in Table 15.

Biological exposure indices for VOCs recommended in Poland are summarized in Table 16 [157].

Text continues on page 427.

Table 12 Biological monitoring of volatile organic compounds—international biological limit values for occupational exposure.

Substance	CAS #	Boiling point °C	Vapor pressure hPa	Skin absorption	Biological monitoring analyte	Biological limit value	Sampling time
Acetone	67-64-1	56	240	- -	Acetone in urine	BAT[1)] 80 mg/l BEI[2)] (100 mg/l)[4)]	end of shift
Acrylonitrile	107-13-1	77	116	H[3)] S[3)]	Cyanoethylvaline in erythrocytes	no BAT values are established as acrylonitrile is a carcinogen; quantitative correlations for the listed parameters are given in relation to ambient air concentrations in the range of 0.14 to 3 ml/m^3	at any time
Aniline	62-53-3	184	64	H	Aniline, free in urine	BAT: 1 mg/l	after several shifts
					Aniline, released from aniline hemoglobin conjugate (whole blood)	BAT: 100 µg/l	end of shift
				S	Total *p*-aminophenol in urine	BEI: 50 mg/g creatinine	
					Methemoglobin in blood	BEI: 1.5% of hemoglobin	during or end of shift
Benzene	71-43-2	80	101	H	Benzene in blood *S*-Phenylmercapturic acid in urine *trans, trans*-Muconic acid in urine	no BAT values are established as benzene is a human carcinogen; quantitative correlations for the listed parameters are given in relation to ambient air concentration of benzene in the range of 0.3–6 ml/m^3	end of shift
				S	*S*-Phenylmercapturic acid in urine	BEI: 25 µg/g creatinine	end of shift
2-Butanone	78-93-3	79.6	105	H -	2-Butanone in urine	BAT: 5 mg/l BEI: 2 mg/l	end of shift
Carbon disulfide	75-15-0	46.3	400	H S	2-Thioxothiazolidine-4-carboxylic acid in urine	BAT: 4 mg/g creatinine BEI: 5 mg/g creatinine	end of shift

Table continues on next page

Table 12 *Continued.*

Substance	CAS #	Boiling point °C	Vapor pressure hPa	Skin absorption	Biological monitoring analyte	Biological limit value	Sampling time
Carbon tetrachloride	56-23-5	76.5	120	H S	Carbon tetrachloride in blood	BAT: 70 µg/l	end of exposure after several shifts
Chlorobenzene	108-90-7	131.7	12	-	Total 4-chlorocatechol in urine	BAT: 70/300 mg/g creatinine BEI: 150 mg/g creatinine	prior/end of shift end of shift
				-	Total *p*-chlorophenol in urine	BEI: 25 mg/g creatinine	end of shift
Dichloro-methane	75-09-2	40.1	475	- -	CO-Hb in blood Dichloromethane in blood	BAT: 5% BAT: 1 mg/l	end of shift end of shift
N,*N*-Dimethy-acetamide	127-19-5	166	3	H S	*N*-Methacetamide in urine	BEI: 30 mg/g creatinine	end of shift at end of work week
N,*N*-Dimethy-formamide	68-12-2	153	3.5	H S	*N*-Methyformamide in urine	BAT: 15 mg/l BEI: (40 mg/g creatinine)[4)]	end of shift
Ethylbenzene	100-41-4	136.2	9	H -	Ethylbenzene in blood Mandelic acid in urine	BAT: 1.5 mg/l BEI: 1.5 g/g creatinine	end of shift end of shift at end of work week
					Mandelic and phenyl glyoxylic acid in urine	BAT: 2000 mg/g creatinine	
Ethylene glycol dinitrate	628-96-6	198	0.07	H S	Ethylene glycol dinitrate in blood	BAT: 0.3 µg/l	end of shift
Ethylene glycol monobutyl ether	111-76-2	171.2	1	H S	Butoxyacetic acid in urine	BAT: 100 mg/l	after several shifts
Ethylene glycol monobutyl ether acetate	112-07-2	192	0.4	H	Butoxyacetic acid in urine	BAT: 100 mg/l	after several shifts

Table 12 *Continued.*

Substance	CAS #	Boiling point °C	Vapor pressure hPa	Skin absorp-tion	Biological monitoring analyte	Biological limit value	Sampling time
Ethylene glycol monoethyl ether	110-80-5	136.2	5	H S	Ethoxyacetic acid in urine	BAT: 50 mg/1 BEI: 100 mg/g creatinine	end of shift after several shifts
Ethylene glycol monoethyl ether acetate	111-15-9	156.4	2.7	H S	Ethoxyacetic acid in urine	BAT: 50 mg/l BEI: 100 mg/g creatinine	end of shift after several shifts
Furfural	98-01-1	162	1	H S	Total furoic acid in urine	BEI: 200 mg/g creatinine	end of shift
Halothane (2-Bromo-2-chlo-ro-1,1,1-trifluoro-ethane)	151-67-7	50.2	242	- -	Trifluoroacetic acid in blood	BAT: 2.5 mg/l	end of exposure after several shifts
n-Hexane	110-54-3	69	160	H -	2,5-Hexanedione in urine (BAT: plus 4,5-dihydroxy- 2-hexanone)	BAT: 5 mg/l BEI: 5 mg/g creatinine	end of shift
2-Hexanone (Methyl butyl ketone)	591-78-6	128	13.3	- S	Hexane-2,5-dione plus 4,5-dihydroxy- 2-hexanone in urine	BAT: 5 mg/l	end of shift
Hexone (Methyl isobutyl ketone)	108-10-1	115.8	8	H -	Hexone in urine	BAT: 3.5 mg/l BEI: 2 mg/l	end of shift
Hydrazine	302-01-2	114	1.3	H S	Hydrazine in plasma Hydrazine in urine	no BATvalues are established as hydrazine is a carcinogen; quantitative correlations for the listed parameters are given in relation to ambient air concentra-tions of hydrazine in the range of 0.01 to 0.10 ml/m³	end of shift

Table continues on next page

Table 12 *Continued*.

Substance	CAS #	Boiling point °C	Vapor pressure hPa	Skin absorp-tion	Biological monitoring analyte	Biological limit value	Sampling time
Isopropyl alcohol	67-63-0	82.4	40	-	Acetone in blood	BAT: 50 mg/l	end of shift
				-	Acetone in urine	50 mg/l	
Methanol	67-56-1	64.7	128	H S	Methanol in urine	BAT: 30 mg/l BEI: 15 mg/l	end of exposure after several shifts
Nitrobenzene	98-95-3	211	0.13	H S	Aniline, released from aniline-hemoglobin conjugate	BAT: 100 µg/l	end of shift after several shifts
					Total p-nitrophenol in urine	BEI: 5 mg/g creatinine	end of shift at end of workweek
					Methemoglobin in blood	BEI: 1.5% of hemoglobin	end of shift
Phenol	108-95-2	182	47	H S	Phenol in urine	BAT: 300 mg/l BEI: 250 mg/g creatinine	end of shift
Styrene	100-42-5	146	6	-	Mandelic acid in urine	BEI: 800/300 mg/g creatinine	end of/prior to next shift
				-	Phenylglyoxylic acid in urine	BEI: 240/100 mg/g creatinine	end of/prior to next shift
					Mandelic plus phenylglyoxylic acid in urine	BAT: 600 mg/g creatinine	end of shift after several shifts
					Styrene in blood	BEI: 0.55/0.02 mg/l	end of/prior to next shift
Tetrachloro-ethylene	1027-18-4	121	19	H	Tetrachloroethylene in blood	no BAT-values are establishied as tetrachloroethylene is suspected to have a carcinogen potential; a BAT value valid until 1997 of 1 mg/l tetrachlorethylene in blood can be used as guidelines in biomonitoring. BEI: 0.5 mg/l	prior to next shift
				-	Tetrachloroethylene in alveolar air	BEI: 5 ppm	prior to last shift of the work week
					Trichloroacetic in urine	BEI: 3.5 mg/l	end of shift at end of work week

Table 12 *Continued*.

Substance	CAS #	Boiling point °C	Vapor pressure hPa	Skin absorption	Biological monitoring analyte	Biological limit value	Sampling time
Tetrahydrofuran	109-99-9	66	200	- -	Tetrahydrofuran in urine	BAT: 8 mg/l	end of shift
Toluene	108-88-3	111	29	-	Toluene in blood	BAT: 1.0 mg/l BEI: (1 mg/l)[4)]	end of shift
				S	Hippuric acid in urine	BEI: (2.5 g/g creatinine)[4)]	end of shift
					o-Cresol in urine	BAT: 3.0 mg/l	end of shift
1,1,1-Trichloroethane	71-55-6		74	-	1,1,1-Trichloroethane in blood	BAT: 550 µg/l	prior to shift after several shifts
				-	1,1,1-Trichloroethane in alveolar air	BEI: 40 ppm	prior to shift after several shifts
					Total 2,2,2-trichloroethanol in blood	BEI: 1 mg/l	end of shift at end of work week
					Total 2,2,2-trichloroethanol in urine	BEI: 30 mg/l	end of shift at end of work week
					Trichloroacetic acid in urine	BEI: 10 mg/l	end of work week
Trichloroethylene	79-01-6	87	77	-	2,2,2-Trichloroethanol in blood	no BAT values are established as trichloroethylene is a carcinogen;	end of shift after several shifts
					Trichloroacetic acid in urine	BAT values valid until 1996 of 5 mg/l 2,2,2-trichloroethanol in blood and 100 mg/l trichloroacetic acid can be used as guidelines in biomonitoring	end of shift after several shifts
				-	2,2,2-Trichloroethanol in blood	BEI: 4 mg/l	end of shift at end of work week
					Trichloroacetic acid in urine	BEI: 100 mg/g creatinine	end of work week
					2,2,2-Trichloroethanol and trichloroacetic acid in urine	BEI: 300 mg/g creatinine	end of shift at end of work week

Table continues on next page

Table 12 *Continued*.

Substance	CAS #	Boiling point °C	Vapor pressure hPa	Skin absorption	Biological monitoring analyte	Biological limit value	Sampling time
Xylenes	1330-20-7	138-144	7-9		Xylenes in blood	BAT: 1.5 mg/l	end of shift
				--	Methylhippuric (toluric) acids in urine	BAT: 2000 mg/l BEI: 1500 mg/g creatinine	end of shift

[1] BAT: German Biological Tolerance Values for Occupational Exposure according to report no. 35 (1999) by Deutsche Forschungsgemeinschaft.
[2] BEI: U.S. Biological Exposure Indices according to booklet 1997 by American Conference of Governmental Industrial Hygienists.
[3] H, S: Substance is considered to be absorbed through the skin, i.e., systemic exposure may be increased by percutaneous absorption (H: according to classification in the German list of MAK and BAT values 1999; S: according to booklet 1997 by American Conference of Governmental Industrial Hygienists.)
[4] BEIs are under discussion to be lowered/changed: Acetone in urine: 60 mg/l; *N*-Methylformamide in urine: 20 mg/g creat.; toluene in blood: 0.05 mg/l (prior to next shift); hippuric acid in urine: 1.6 g/g creat.

Table 13 Finnish biomonitoring action levels for VOCs (all urinary concentrations have been adjusted to relative density of 1.024).

Substance	Parameter	Biomonitoring action level	Upper reference limit values	Sampling time
Benzene	Benzene in blood	20 nmol/l 200 nmol/l	5 nmol/l (nonsmokers)	morning[3)] midday[4)]
	t,t-Muconic acid in urine	40 µmol/l	0.5 µmol/l	post shift[2)]
Carbon disulfide	2-Thioxothiazolidine-4-carboxylic acid in urine	2 mmol/mol creat.[1)]		post shift
N,N-Dimethyl-formamide	*N*-(hydroxymethyl)-*N*-methylformamide in urine	650 µmol/l		post shift
Ethylbenzene	Mandelic acid in urine	5.2 mmol/l[1)]	0.2 mmol/l	post shift
Fluorotrichloro-methane (FC-11)	FC-11 in blood	100 nmol/l	5 nmol/l	morning
Methanol	Formic acid in urine	240 mmol/mol creat.	50 mmol/ mol creat.	morning
2-Butanone	2-Butanone in urine	60 µmol/l	1.5 µmol/l	post shift
n-Hexane	2,5-Hexanedione in urine	5 µmol/l	1 µmol/l (mild hydrolysis)	post shift
Phenol	Phenol in urine	3200 µmol/l[1)]	150 µmol/l	post shift
Styrene	Sum of mandelic and phenyl-glyoxylic acid in urine	1.2 mmol/l	0.2 mmol/l	morning
Tetrachloroethylene	Tetrachloroethylene in blood	6 µmol/l	0.1 µmol/l	morning
Toluene	Toluene in blood	1000 nmol/l[1)]	50 nmol/l	morning
1,1,1-Trichloroethane	1,1,1-Trichloroethane in blood	2.0 µmol/l	0.1 µmol/l	morning
Trichloroethylene	Trichloroacetic acid in urine	360 µmol/l	50 µmol/l	post shift
Trichlorotrifluoro-ethane(FC-113)	FC-113 in blood	50 nmol/l	5 nmol/l	morning
Triethylamine	Triethylamine in urine	0.85 mmol/l	0.02 mmol/l	post shift
Xylenes	Methylhippuric acids in urine	10 mmol/l	0.2 mmol/l	post shift

[1)] indicative limit value; [2)] post shift: specimen collected after the working hours; [3)] morning: specimen collected before the commencement of the work-shift (and exposure), i.d. apprx. 16 h after the last exposure and toward the end of the working week; [4)] midday: specimen is collected after the first half of the working day, 60 +/-10 min after the exposure

Table 14 Biological equivalent concentrations for VOC exposure, established in Italy by [154].

Substance	BM analyte	LBE*	Sampling time
Acetone	Acetone in urine	56 mg/l	end of exposure
Benzene			
exposure 0.1 ppm	Benzene in urine	575 ng/l (nonsmokers)	end of exposure
exposure 1 ppm	*t,t*-Muconic acid in urine	850 µg/g creatinine	end of exposure
exposure 1 ppm	*S*-Phenylmercapturic acid in urine	52 µg/g creatinine	end of exposure
Butan-2-one	Butan-2-one in urine	2 mg/l	end of exposure
Carbon disulfide	Carbon disulfide in urine	12.5 µg/l	end of exposure
Carbon tetrachloride	Carbon tetrachloride in urine	3.1 µg/l	end of exposure
Dichloromethane	Dichloromethane in urine	455 µg/l	end of exposure
Halothane (2-Bromo-2-chloro-1,1,1-trifluoro-ethane)	Halothane in urine	6.8 µg/l	end of exposure
Isopropyl alcohol	Acetone in urine	17 mg/l	end of exposure
n-Hexane	n-Hexane in urine	6 µg/l	end of exposure
4-Methylpentan-2-one	4-Methylpentan-2-one in urine	2.2 mg/l	end of exposure
Styrene	Styrene in urine	80 µg/l	end of exposure
Tetrachloroethylene	Tetrachloroethylene in urine	100 µg/l	end of exposure
Toluene	Toluene in urine	194 µg/l	end of exposure
1,1,1-Trichloroethane	1,1,1-Trichloroethane in urine	595 µg/l	end of exposure
Trichloroethylene	Trichloroethylene in urine	185 µg/l	end of exposure
Xylenes	Xylenes in urine	110 µg/l	end of exposure

*LBE Limiti Biologici Equivalenti

Table 15 Biological exposure indices for VOC exposure recommended in the United Kingdom [155,156].

Substance	BM analyte	Health guidance value	Sampling time
Butan-2-one	Butan-2-one in urine	70 µmol/l	post shift
2-Butoxyethanol	Butoxyacetic acid in urine	240 mmol/mol creatinine	post shift
N,N-Dimethylacetamide	*N*-Methylacetamide in urine	100 mmol/mol creatinine	post shift
4-Methylpentan-2-one	4-Methylpentan-2-one in urine	20 µmol/l	post shift

Table 16 Biological exposure indices for VOC exposure recommended in Poland [157].

Substance	BM analyte	Biomonitoring exposure indices	Sampling time
Carbon disulfide	2-Thioxothiazolidine-4-carboxylic acid in urine	3 mg/g creat.	end of exposure at any day
Ethylbenzene	Mandelic acid in urine	20 mg/h	excretion rate during the last 2 h of shift
Methanol	Methanol in urine	6 mg/l	end of exposure at any day
n-Hexane	2,5-Hexanedione in urine	2.5 mg/l adjusted to urinary density of 1.016	end of exposure at end of work week
Phenol	Phenol in urine	8.8 mg/h	excretion rate during the last 2 h of shift
Styrene	Mandelic acid in urine Mandelic and phenyl-glyoxylic acid in urine	16 mg/h 25 mg/h	excretion rate during the last 2 h of shift
Tetrachloroethylene	Tetrachloroethylene in blood	800 µg/l	10-20 min after 4-6 h of exposure
Toluene	Benzoic acid	80 mg/h	excretion rate during the last 2 h of shift
	Toluene in blood	300 µg/l	10-20 min after end of exposure
Trichloroethylene	Trichloroacetic acid in urine	20 mg/l	end of exposure at any day
Trimethylbenzene	Sum of 2,4-/2,5-/3,4-dimethylbenzoic acid in urine	170 mg/h	excretion rate during the last 2 h of shift after 4 days of exposure
Xylenes	Methylhippuric acids in urine	1.4 g/l adjusted to urinary density of 1.024	end of exposure at any day

8. CONCLUSIONS AND RECOMMENDATIONS

We draw the following conclusions:

- Biological monitoring of exposure to VOCs may be helpful for an assessment of the total VOC uptake and health effects they may induce. Representativeness of the results depends crucially on the kinetics of the VOCs in the body.
- Due to the in general complex kinetics of the VOCs, the time of specimen collection has to be selected very carefully. For a comparison with existing limit values, the standardized sampling

recommendations have to be followed strictly.

- Biological monitoring of VOC exposure is primarily based on VOCs in whole blood or metabolites in urine. Especially for occupational exposure, analytical instrumentation and extensive experience exist in many international scientific laboratories for its reliable routine application.
- At typical workplace and environmental concentrations the metabolic interaction between different VOC components and the level of biological indicators of exposure do not seem to be a major source of error. Simultaneous consumption of alcohol has been shown to affect the concentrations measured.
- Different activities of VOC metabolizing enzymes (e.g., GST T1, NAT 2) due to genetic polymorphism may be of relevance for a person's individual susceptibility to VOC exposure (e.g., methyl bromide, aromatic amines).

We recommend further studies in the following areas:

- Improvement of analytical methodology especially for VOCs in whole blood at low level environmental exposure and the development of a reliable and standardized procedure to prepare matrix calibration standards for VOC quantification.
- Reference materials for VOCs in blood and for urinary VOC metabolites should be prepared in order to help the laboratories to improve the analytical reliability as well as the comparability of biological monitoring data.
- Scientific work for establishment of reference values and intervals (e.g., 95 fractiles) for industrially unexposed persons should be continued with improved analytical methodology.
- Combining effects of simultaneous exposure to different VOCs should be studied in more detail.
- The issue of biochemical (e.g., protein/DNA adducts) and biological effect markers should be followed in future work in order to improve the interpretation of such data especially for carcinogenic compounds.

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9. LIST OF ACRONYMS

Acronym	*Explanation*
ACGIH	American Conference of Governmental and Industrial Hygienists (USA)
BAT	Biologischer Arbeitsstoff-Toleranz-Wert (biological tolerance value, evaluated by Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft/Germany)
BEI	Biological Exposure Indices (evaluated by American Conference of Governmental and Industrial Hygienists/USA)
BEM	Biological effect monitoring
BM	Biological monitoring
CEC	Commission of the European Communities
DGAUM	Deutsche Gesellschaft für Arbeitsmedizin und Umweltmedizin e.V. (German Society of Occupational and Environmental Medicine)
EKA	Expositionsäquivalente für krebserzeugende Arbeitsstoffe (exposure equivalents for carcinogenic substances, evaluated by Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft/ Germany)
FG	Fat group
FIOH	Finnish Institute of Occupational Health
GC/ECD	Gas chromatography/electron capture detection
GC/FID	Gas chromatography/flame ionization detection
GC/MSD	Gas chromatography/mass selective detection
GC/NPD	Gas chromatography/nitrogen selective detection
IFCC	International Federation of Clinical Chemistry
JFOHO	Japan Federation of Occupational Health Organizations
K-EDTA	Potassium salt of ethylenediaminetetraacetic acid
KISCO	Korea Industrial Health Corporation
MAK	Maximale Arbeitsplatzkonzentration (maximum workplace concentration, evaluated by Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft/ Germany)
MG	Skin and muscle group
NIOSH	National Institute of Occupational Safety and Health (USA)
OSHA	Occupational Safety and Health Administration (USA)
PBPK	Physiologically based pharmacokinetic model
RP-HPLC/UV	Reversed-phase high-performance liquid chromatography with UV detection
SPE-adsorption	Solid-phase extraction - adsorption chromatography
SPE-RP	Solid-phase extraction - reversed phase
VOC	Volatile organic compounds
VPG	Vessel-poor group
VRG	Vessel-rich group
WHO	World Health Organization

10. LIST OF TRADITIONAL NAMES AND THEIR EQUIVALENT IUPAC NAMES

Names in the literature	*IUPAC names*
2-butanone	butan-2-one
4-chlorocatechol	4-chlorobenzene-1,2-diol
4,5-dihydroxy-2-hexanone	4,5-dihydroxyhexan-2-one
dimethylformamide	*N*,*N*-dimethylformamide
ethylene glycol dinitrate	ethylene dinitrate
ethylene glycol monobutyl ether	2-butoxyethanol
ethylene glycol monobutyl ether acetate	2-butoxyethyl acetate
ethylene glycol monoethyl ether	2-ethoxyethanol
ethyl methyl ketone	butan-2-one
fluorotrichloromethane	trichlorofluoromethane
n-hexane	hexane
2,5-hexanedione	hexane-2,5-dione
hippuric acid	*N*-benzoylglycine
mandelic acid	α-hydroxybenzeneacetic acid
methyl *tert*-butyl ether	*tert*-butyl methyl ether
methyl butyl ketone	hexan-2-one
methylformamide	*N*-methylformamide
2-methylhippuric acid (*o*-toluric acid)	*N*-(2-methylbenzoyl)glycine
3-methylhippuric acid (*m*-toluric acid)	*N*-(3-methylbenzoyl)glycine
4-methylhippuric acid (*p*-toluric acid)	*N*-(4-methylbenzoyl)glycine
methyl isobutyl ketone	4-methylpentan-2-one
trans, *trans*-muconic acid	*trans*, *trans*-hexa-2,4-dienoic acid
phenylglyoxylic acid	α-oxobenzeneacetic acid
S-phenylmercapturic acid	*N*-acetyl-*S*-phenyl-L-cysteine
tetrachloroethylene	tetrachloroethene
thiodiglycolic acid	2,2'-sulfanediyldiacetic acid
trichloroethylene	trichloroethene

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