Biological Monitoring of Chemical Exposure in the Workplace
Guidelines

Volume 1

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Contribution to the International Programme on Chemical Safety (IPCS)

Preface

Biological monitoring of chemical exposure in the workplace has become increasingly important in the assessment of health risk as an integral part of the overall occupational health and safety strategy. This activity requires accurate sampling and analysis with correct interpretation of results. Therefore, in 1992, the WHO Office of Occupational Health, in collaboration with the International Programme on Chemical Safety (IPCS), set up a global project entitled "Methods and Quality Assurance of Biological Monitoring of Chemical Exposure". The objective of this project was to provide occupational health professionals in member states with reference principles and methods for determination of biomarkers of exposure with emphasis on promoting appropriate use of biological monitoring and assisting in quality assurance. This monograph represents the first outcome of the above-mentioned project. It is a guideline for biological monitoring of exposure to selected metals, solvents, pesticides and other chemicals. All available information for selected chemicals has been assessed and validated.

A selection of individual chemicals has been made, using criteria related to the frequency of use, toxicity, routes of absorption, availability of information on human metabolism, relationship between exposure and biomarker and existence of Occupational Biological Reference Values (OBRV), which were developed by the Planning Meeting held in Kyoto, Japan, in October 1992.

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During project development and its implementation, WHO received technical cooperation from the International Labour Office (ILO), United Nations Environment Programme (UNEP), Organization for Economic Cooperation and Development (OECD), International Commission on Occupational Health (ICOH), International Union of Pure and Applied Chemistry (IUPAC), European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), International Council of Metals and the Environment (ICME), Nickel Producers Environmental Research Association (NIPERA), Cadmium Association, Eurometaux (European Metal Association), who are thankfully acknowledged.

Biological monitoring is an important tool in the prevention of occupational diseases related to those exposed to chemicals on a regular basis, particularly when multi-route exposure (inhalation, skin absorption, ingestion) or abnormal exposure takes place. However, it should be stressed that, in order to evaluate workplace conditions on a continuous basis, emphasis should be placed on environmental monitoring, complemented by biological monitoring.

In recommending the use of biological monitoring for practice and research purposes, reference should be made to the obligations of occupational health professionals, defined by the International Code of Ethics, in relation to the protection of confidentiality of health data.

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Chapter 1. General principles

1.1 Introduction

Occupational health maintenance requires a multidisciplinary approach to prevent health impairment that might result from excessive, acute or chronic exposure to chemical agents. Early detection of hazardous exposures may significantly decrease the occurrence of adverse health effects by reducing the level of exposure thanks to appropriate preventive measures. Monitoring of exposure is a procedure which consists of the routine assessment and the interpretation of biological and/or ambient parameters in order to detect possible health risks. This approach requires (a) the definition of permissible levels of exposure, that is, levels that according to the present status of knowledge are estimated to cause no adverse effects during the lifetime of the workers; and (b) the regular assessment of the possible health risks associated with exposure by comparing the current or integrated exposure with these permissible exposure limits.

1.2 Definitions

In 1980, the Commission of the European Communities (CEC), the US National Institute for Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA) organized a seminar on Assessment of Toxic Agents at the Workplace. The participants agreed upon the following definitions (1):

monitoring: systematic continuous or repetitive health-related activity designed to lead, if necessary, to corrective actions;

ambient monitoring is the measurement and assessment of agents at the workplace and it evaluates ambient exposure and health risk compared to an appropriate reference;

biological monitoring is the measurement and assessment of workplace agents or their metabolites either in tissues, secreta, excreta, expired air or any combination of these to evaluate exposure and health risk compared to an appropriate reference;

health surveillance is the periodic medico-physiological examinations of exposed workers with the objective of protecting health and preventing occupationally related disease. The detection of established disease is outside the scope of this definition.

The definitions of biological monitoring and health surveillance are separate components of a continuum which can range from the measurement of agents in the body through the measurements of metabolites, to sign of early disease. A problem left unresolved in these definitions concerns the precise place of certain biochemical tests, such

as zinc protoporphyrin (ZPP), δ-aminolaevulinic acid in urine, etc.

The following definition of biological monitoring of early effects has been proposed to fill this gap:

biological effect monitoring is the measurement of a reversible biochemical change caused by the absorption of the substance; the degree of change being below that associated with toxic injury and not associated with a known irreversible pathological effect (2).

The basis of these monitoring programmes is best explained by following up the fate of a chemical exerting systemic biological effects (i.e. a chemical which is not simply acting locally) from the environment to the target molecules in the body. Most of these chemicals do not cause a similar degree of toxicity in all organs but usually elicit the major toxicity in only one or two organs. These sites are referred to as the critical organs. The critical organ is not necessarily the site of the highest concentration of the chemical. Once absorbed and present in the circulation, the chemical is distributed to different compartments of the body. It may be eliminated unchanged (usually in urine or in expired air). Organic chemicals and also some inorganic chemicals (e.g. arsenic) may undergo various biotransformations which usually make them more easily excretable via urine or bile than the parent compound. The chemical or its metabolites may bind reversibly or irreversibly to target molecules. While binding to certain sites induces non-adverse effects, binding to some other specific sites may give rise to adverse health effects when the amount bound has reached a certain level and the repair mechanisms have been overwhelmed. This leads to the development of preclinical lesions at an early stage and to clinical lesions at a more advanced stage of intoxication. It should be noted, however, that storage of a chemical in non-critical sites is not always without potential risk since in some circumstances, it may be released in the circulation and transported to critical organs. For example, it has been suggested that lead stored in bones may be mobilized during pregnancy and transferred to the foetus.

For the same external dose, this progression from exposure to disease may vary between individuals. There are so many intermediate steps which may be different from one individual to the other (e.g. metabolic activation and/or inactivation, repair mechanisms, etc.) that the consequences of a similar exposure may differ between subjects (3–6).

Exposure can be assessed either by measuring the concentration of the agent in the workplace air by stationary or personal sampler (**ambient monitoring**), or by measuring some biological parameters (**biological monitoring**). Strictly speaking, biological monitoring of exposure to chemical agents means measurement of a substance or its metabolite in various biological media. Sometimes, the concept of biological monitoring is extended to include also the detection of early reversible non-adverse effect (**biological effect monitoring**) (e.g. δ-aminolaevulinic acid in urine or zinc protoporphyrin in blood for assessment of exposure to lead). The detection of an adverse effect (e.g. increased proteinuria) indicates that exposure is or has been excessive and therefore such a measurement is more logically included in a programme of **early detection of health impairment** due to industrial chemicals rather than in a biological monitoring

programme for evaluating exposure. The distinction between adverse and non-adverse biological effects is not always clear-cut and is sometimes arbitrary since it may be difficult to evaluate the health significance of an effect (7). As stated by Zielhuis and Henderson (8), it is rarely known whether the biological effects as such have to be regarded as adverse, either at the time of measurement or as predictor of an increased health risk or of an impaired state of health. In a symposium on biological monitoring sponsored by the US Environmental Protection Agency (EPA), the World Health Organization (WHO), and the Commission of the European Communities (CEC), it was proposed that a biological effect should be considered adverse if there is an impairment of functional capacity, a decreased ability to compensate for additional stress, a decreased ability to maintain homeostasis, and an enhanced susceptibility to other environmental influences or if such impairments are likely to become manifest in the near future (9).

In view of the inter-individual differences in susceptibility to xenobiotics, one might also consider **the detection of increased susceptibility to a chemical hazard**. Biological markers may be used to detect inherited or acquired limitations of an organism to respond to the challenge of exposure to specific or a group of xenobiotic substances. For example, slow acetylators (persons whose metabolic systems acetylate amines slowly) are at increased risk of developing bladder cancer when exposed to carcinogenic aromatic amines (3).

The main goal of biological monitoring of exposure is to ensure that the exposure of the workers does not entail an unacceptable health risk. It is essentially a preventive activity. The presence of a risk is recognized by reference to permissible levels in biological media, i.e. occupational biological action levels. Examples of such values are the Biological Exposure Indices (BEI) of the American Conference of Governmental Industrial Hygienists (ACGIH) (10) and the Biological Tolerance Values (BAT) of the Deutsche Forschungsgemeinschaft (DFG) (11).

1.3   The concept of internal dose

Biological monitoring of exposure attempts to estimate the internal dose on the basis of our knowledge of the fate of the chemical in the body. But depending on the chemical and the analysed biological parameter, the term internal dose may cover different concepts (3–6). Firstly, internal dose may mean the amount of chemical recently absorbed. Hence, a biological parameter may reflect the amount of chemical absorbed either shortly before sampling (for example, the concentration of a solvent in the alveolar air or in blood during the workshift) or during the preceding day (for example, the concentration of a solvent in alveolar air or in blood collected 16 hours after the end of exposure) or during past months when the chemical has a long biological half-time (for example, the concentration of some metals in blood). Internal dose may also mean the amount of chemical stored in one or in several body compartments or in the whole body (integrated exposure or specific organ dose). This usually applies to cumulative toxic chemicals. For example, the concentration of polychlorinated biphenyls in blood is a reflection of the amount accumulated in the main sites of deposition (i.e. fatty tissues).

Finally, with ideal biological monitoring tests, the internal dose means the amount of chemical bound to the critical sites of action (target dose or biological effective dose). Such tests can be developed, when the critical sites are easily accessible (e.g. haemoglobin in case of exposure to carbon monoxide or to methaemoglobin forming agents) or when the chemical interacts with a blood constituent in a similar way as with the critical target molecule (e.g. haemoglobin alkylation reflecting binding to DNA in the target tissue). In the latter situation, the amount bound to the blood constituent is used as a surrogate of the biologically effective dose.

1.4   Classification of biological monitoring approaches

The biological tests currently used for monitoring of exposure to industrial chemicals can be classified in three categories (3–6).

1.4.1   Determination of the chemical or its metabolites in biological media or exhaled air

The great majority of the tests currently available for biological monitoring of exposure to chemicals rely on the determination of the chemical or its metabolites in biological media. The biological media most commonly used are urine, blood and less frequently exhaled air. It is also possible to analyse biological materials, such as faeces, adipose tissue, hair, nail or saliva.

According to their specificity, these tests can be classified into two subgroups. The **selective tests** are based on the direct measurement of the unchanged chemicals or their metabolites in biological media. The unchanged substance is measured when it is not or is poorly biotransformed, when there is no knowledge about the metabolites (no toxicokinetics data), when the level of exposure is too low for a significant amount of metabolite to be produced, when a high degree of specificity is required (a metabolite may be common to several substances), or when sensitive methods for detecting the metabolites are not available.

Techniques are also being developed (neutron activation analysis/X-ray fluorescence) for directly monitoring in vivo the concentration of some metals in tissues, such as cadmium in liver and kidney and lead in bones (12). So far, these techniques are not applicable routinely.

Non-selective tests are used as non-specific indicators of exposure to a group of chemicals. As an example of non-selective exposure tests, one can cite the determination of diazo-positive metabolites in urine for monitoring exposure to aromatic amines, the analysis of thioethers in urine to assess exposure to mutagenic and carcinogenic substances and the determination of the mutagenic activity of urine. Because of their lack of specificity (for instance, thioether excretion may be increased by non-mutagenic or carcinogenic exogenous or endogenous substances and is influenced by smoking) and the existence of a large individual variability, these tests usually cannot be used to monitor exposure on an individual basis. It is, however, possible that when an adequate control

group is used as reference, they may be useful as qualitative tests to identify exposed groups.

1.4.2 Quantification of (reversible, non-adverse) biological effects related to the internal dose

This second category of tests includes those based on the quantification of non-adverse effects which are related to the internal dose. Most of these tests are non-specific. The development of these tests usually requires some knowledge of the mechanism of action of the chemical. An example of these tests is the use of the inhibition of pseudo-cholinesterase activity in serum to assess exposure to organophosphorus compounds. Others include the inhibition of the erythrocyte enzyme δ-aminolaevulinic dehydratase and the increase of zinc protoporphyrin which are generally considered as indicators of lead exposure, although they are not agent specific as such. Also the urinary excretion of beta-hydroxycortisol or D-glucaric acid may be used as an indicator of exposure to chemical inducing mono-oxygenase enzymes.

1.4.3 Measurement of the amount of active chemical interacting with the target and non-target (surrogate) molecules

Contrary to the preceding exposure tests, those belonging to this third category directly or indirectly estimate the amount of chemical interacting with the sites of action. When they are feasible, i.e. when the target site is easily accessible, these tests have the potential to assess the health risk more accurately than any other monitoring procedure. The determination of carboxyhaemoglobin is an example that has been used in occupational medicine for a long time. Progress in this monitoring approach is to be expected namely with the development of a new generation of tests based on immunological or GC-MS techniques. The latter have the potential of detecting with a great specificity (agent specific parameter) and sensitivity subtle alterations induced in the target and non-target molecules (e.g. DNA, haemoglobin, albumin) by reactive chemicals (e.g. mutagens and carcinogens). The DNA adducts can be measured either in hydrolysates of DNA molecules (e.g. in white blood cells) or in degradation products of DNA released in body fluids (e.g. urine). Much research is still needed before these tests can be introduced in the routine biological monitoring of industrial workers.

1.5 Comparison of ambient and biological monitoring of exposure

Biological monitoring of exposure may offer several advantages over environmental monitoring to evaluate the internal dose and hence to estimate the health risk. Biological monitoring takes into consideration absorption by routes other than the lungs. Many industrial chemicals can enter the body by absorption through the skin or the gastrointestinal tract. Personal hygiene habits vary from one person to another. The lack of care in personal hygiene can lead to significant ingestion of the substance (hand contamination, smoking, eating or drinking in the work area). The incorrect use of protective clothing

(e.g. gloves) can result in increased skin contamination and absorption. Because of its capability to evaluate the overall exposure (whatever the route of entry), biological monitoring has the advantage that it can be used to test the effectiveness of various protective measures, such as gloves, masks, and barrier creams.

Moreover, it is well known that great inter-individual variation exists in the absorption rate of a chemical through the lungs, the skin or the gastrointestinal tract as well as in the capacity for metabolizing and excreting the substance. In some cases, even if strict personal hygiene measures can be implemented so that the pollutant can enter the organism only by inhalation (in addition to the amount transported by mucociliary clearance from the lungs to the gastrointestinal tract), there is no reason to always postulate the existence of a relationship between the airborne concentration and the amount absorbed. Many physico-chemical and biological factors preclude the existence of such a correlation (e.g. type of compound [for example, exposure to a same ambient level of soluble or insoluble metal compound does not result in the same biological level], particle size distribution [inhalable or respirable fraction], variation in work load influencing ventilatory parameters and cardiac output and hence the alveolar air or blood concentrations of volatile organic solvents, etc.). A biological parameter may take all these various toxicokinetic factors into consideration.

Biological monitoring also reflects non-occupational background exposure (leisure activity, residency, dietary habits, smoking, etc.) which may also be expressed in the biological level. The organism integrates the total external (environmental and occupational) exposure into one internal load.

For all of the above reasons, it is clear that for many industrial pollutants, the measurement of the concentration in air may not necessarily prevent an excessive intake by the exposed workers.

Ambient monitoring is usually done to identify and quantify specific contaminants present in the environment, to determine compliance status with respect to various occupational health standards or to evaluate the effectiveness of engineering controls installed to minimize workers' exposure. Depending on the type of air sampling system selected – stationary (area) or personal – the estimate of the risk may be carried out on a group or on an individual basis.

Ambient monitoring is more suited than biological monitoring for the detection of acute exposure to dangerous chemicals. It can often be quickly applied to potentially hazardous conditions. If hazardous conditions are found, preventive measures can be instituted before severe adverse health effects occur. Ambient monitoring is usually more practical than a biological method to identify emission sources and evaluate the efficiency of engineering control measures. A single ambient monitoring operation may prevent overexposure of many individuals. Individual measurements, such as biological monitoring may entail higher costs than air monitoring. Moreover, in case of exposure to chemical substances that exhibit their toxic action at the site of contact (e.g. eye mucosa or lung irritants, respiratory tract carcinogens) and are poorly absorbed, a biological parameter

reflecting the internal dose is not necessarily related to the health risk. Only few bio-logical tests have been proposed for the identification or the monitoring of chemicals present at the interface between the environment and the body (skin, gastrointestinal mucosa, respiratory tract mucosa). The analysis of nickel in the nasal mucosa which is at present only an experimental technique, and the counting of asbestos bodies in spu-tum could be considered as examples of such tests. Except for these very few tests, di-rect toxic effects at this interface between the environment and the body, can only be prevented by keeping the airborne and surface concentrations of the substance below a certain level.

There is sometimes greater difficulty in obtaining biological samples (e.g. blood collec-tion may be considered as invasive by workers) than air samples.

Finally, for many industrial chemicals, the toxicokinetics (metabolism of the substance) and toxicodynamics (quantitative relationships among external exposure, internal expo-sure, adverse effects) data are still too limited to propose a valid and practical biological method for assessing the risk of overexposure. From the above considerations, it is clear that both ambient monitoring and biological monitoring of exposure represent two complementary approaches for health risk assessment in industry.

1.6   Information required for the development of biological monitoring approaches and the establishment of occupational biological action levels

The understanding of the cascade of events between external exposure to toxic sub-stances and the biological changes leading to adverse effects is fundamental for the de-velopment of relevant biological monitoring methods.

1.6.1 Toxicokinetics

The amount of a substance measured in body fluids is the result of a continuous inter-play between absorption, biotransformation, storage and excretion, and these processes may be influenced by several endogenous (e.g. genetic constitution, anthropometric factors, health status) and exogenous (e.g. work load, simultaneous exposure to several substances, drugs, alcohol intake, smoking habits) factors (13). Knowledge of these factors is essential for selecting the appropriate biological markers (e.g. parent com-pound or metabolite), the biological medium to be analysed and the sampling time (see below) and for the interpretation of the result.

The biological half-time of a substance in an organ, a tissue or the body as a whole, is the time needed to excrete half the amount of the substance. Some substances may have several half-times corresponding to the elimination from different organs or tissues but generally one main half-time predominates. For chemicals that have a long biological half-time in various body compartments, the time of sampling may not be critical. Some

chemicals show an accumulation during the work-week. For other chemicals, that are rapidly eliminated from the organism the sampling time may be of major importance. The shorter the half-time, the more critical the sampling time. The relationship between the half-time of a substance in blood or urine and sampling strategy is shown in figure 1.1 and the optimum times for sampling are proposed in table 1.1 (2).
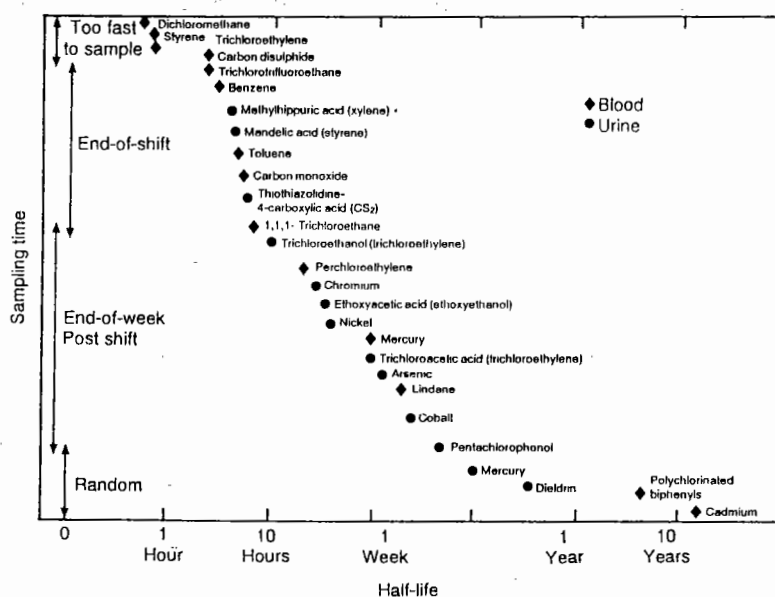
Figure 1.1. The relationship between the half-life of a substance in blood or urine and sampling strategy. (from: Biological monitoring for chemical exposures in the workplace. Guidance Note EH56, Health and Safety Executive. Crown copyright is reproduced with the permission of the Controller of HMSO.)

Table 1.1. The relationship between the half-time of a substance in blood or urine and sampling strategy (2)

Half-time	Optimum sampling time
< 2 hours	half-time too short for suitable monitoring
2–10 hours	end of shift or beginning of next shift
10–100 hours	end of shift, end of work-week
> 100 hours	time of sampling not critical

Similarly, the knowledge of the kinetics and the half-time is of importance when interpreting the representativeness of the sampling. Occupational biological reference values most often refer to exposure according to the ambient exposure limit values, i.e. mean

biological concentrations that are likely to be found after an 8-hour time-weighted average exposure. However, external exposure at the workplace is rarely constant in the time course of the workshift. It is important to understand if a parameter reflects the very recent exposure (the exposure just before the sample collection), the time-weighted exposure during the preceding day(s), long-term exposure or integrated exposure. For example, n-hexane concentration in exhaled air reflects the exposure just before sampling, cobalt level in urine is influenced by the exposure to soluble cobalt compounds during the previous shifts and PCB level in adipose tissue is an indicator of the body burden.

Pharmacokinetics models may provide valuable tools when establishing relationships between external exposure and internal dose. Physiologically based pharmacokinetic models have been developed taking account of the variability of numerous exposure and physiological factors, such as the intensity and duration of exposure, the physical work load, the body build, the liver and renal function (14).

1.6.2 Toxicodynamics

Knowledge of the early adverse effects caused by a chemical is fundamental for a screening procedure in the assessment of health risks and is usually a prerequisite for an accurate assessment of the tolerable level of the biological exposure parameter. Knowledge of non-adverse biological changes induced by a toxicant or its active metabolites may also be useful for the development of biological monitoring methods. For example, the determination of serum pseudocholinesterase or δ-aminolaevulinic acid dehydratase inhibition can be used for assessing the risk of overexposure to some organophosphorus pesticides and lead, respectively. The determination of the dose-effect relationship (i.e. the concentration of the substance at which the effect is expected to occur) as well as the dose-response relationship (the percentage of individuals showing these effects at each dose level) is fundamental in risk estimation when proposing occupational biological action levels.

1.6.3 Levels in occupationally unexposed population

One has to distinguish the "reference values" observed in non-exposed people and the "reference values" (i.e. biological action levels) established for the exposed people, i.e. values allowing the uptake of a certain amount of a chemical agent but regarded as acceptable for the preservation of the subject health.

The knowledge of appropriate reference values (i.e. the level of the parameter in a comparative population not exposed to the substance in question) is useful when establishing biological action levels in occupationally exposed subjects and interpreting the result.

The parameter under investigation may be present to some extent in the general population without any occupational exposure. This level may be influenced by endogenous or exogenous factors; i.e. homeostasis mechanisms for essential elements, genetic factors, diseases, drug consumption, smoking habits, household product exposure, dietary and

environmental factors. Failure to know the major sources of variation of a parameter limits its validity.

It is important to note that background levels can be below the level of detection; for example, methylenedianiline or methylene bis(chloroaniline) are not detected in biological media of unexposed people.

As a result of environmental (soil, air, water, food) pollution coming from industrial emissions, traffic or natural abundance in the earth's crust, the reference values measured in groups without occupational exposure may greatly vary in the different parts of the world. For instance, the levels of arsenic, cadmium, selenium, lead, fluoride in body fluids or tissues can be very different (15). Hence, when this background level is high, there is not much room for additional occupational exposure in order to prevent health impairment.

As recently highlighted by King (16), in the past when occupational exposures were greater than they are today, occupational exposures were sufficiently high to be distinguished from the levels of unexposed. Today, the difference has been reduced in some cases to a level where statistical analysis of grouped data is necessary to determine the difference between exposed and non-occupationally exposed populations. Moreover, this problem of low level of exposure is further confounded by the uncertainties of much of the historical data on both "normal" and occupational levels. For instance, only over the past few years have instruments been available for the accurate measurement of chromium and nickel in blood, and all the previous data have to be discarded. In practice, each laboratory should be required to give, on each report, the "normal" level and range obtained by itself using the identical procedures as used for the samples (16).

1.7   Implementation of sampling

1.7.1 Biological media

The majority of the available biological tests rely on the analysis of breath, blood or urine (17–19). The choice of the medium depends on several factors, such as the kinetics (appearance and half-time of the biological parameter), the convenience of sample collection, or the possibility of sample contamination.

1.7.1.1 Blood

Blood constitutes the main vehicle for the transport and distribution of chemicals in the body. Therefore, most systemically active substances or their metabolites can be found in blood. It can be used for measuring most inorganic chemicals and for organic substances which are poorly biotransformed and have a sufficient half-time. Moreover the determination of an unchanged substance in blood may have a greater specificity than the determination of its metabolites in urine. Blood is also useful for the measurement of substances that bind to macromolecules, for example, surrogate molecules such as

haemoglobin. Some practical considerations have to be taken into account since depending on the substance, the analysis should be performed on whole blood, plasma, serum or erythrocytes. The appropriate anticoagulant (if necessary) must be selected. The biological parameter to be assessed can be either equally distributed between the different blood constituents or can accumulate in a particular blood compartment (e.g. red cells). Haemolysis of red blood cells, a frequent phenomenon occurring during blood sampling, transport, storage or mishandling may lead to erroneous results of analyses performed on plasma or serum. Some chemicals or their metabolites can be transported in blood free or bound to proteins. The analytical method must take this property into account.

The blood concentration of many volatile solvents has frequently the same significance as that in alveolar air. It reflects either the most recent exposure when blood is collected during exposure or the exposure during the preceding day if blood is collected 16 hours after the end of exposure. The blood concentration of some cumulative organic chemicals (e.g. polychlorinated biphenyls) mainly reflects the body burden, the blood level of these chemicals being related to their concentration in the main storage compartment.

1.7.1.2 Urine

Urine is easy to collect, the procedure is non-invasive and large volumes can be collected. It is usually suitable for monitoring water soluble metabolites of organic chemicals and several inorganic chemicals (metals). These tests are more readily accepted by the workers as they are less invasive than blood collection. In the case of exposure to substances with short biological half-times or with fluctuating airborne concentrations, the level of a metabolite in urine collected at the end of the shift is usually a better indicator of the average exposure during the shift than the concentration of the substance itself in exhaled air or blood samples. The latter (concentration of the substance in exhaled air or blood) is effectively more influenced by the very recent exposure.

The concentration of a substance in urine generally reflects its mean plasma level during the period of urine accumulation in the bladder but for some substances the amount stored in the kidneys may also influence the urinary level. Except in case of exposure to substances with long half-times, measurements performed on a 24-hour specimen might be more representative than those performed in spot samples. However, 24-hour samples are not frequently carried out in routine biological monitoring programmes. In the case of exposure to rapidly excreted substances, such as solvents, end of shift samples are more appropriate. It should, however, be realized that the urinary concentration of a metabolite greatly depends on the rate of urine production and its measurement in either too dilute (large beverage intake) or too concentrated (low beverage intake, perspiration due to hard work or high environmental temperature) urine specimens can lead to misinterpretation. The determination of urinary creatinine and/or density is usually advisable to exclude overdiluted and over-concentrated samples. Correction of the results for the dilution of the urine may be necessary for some substances but needs to be considered on its merits for each individual substance (20, 21). Since creatinine excretion depends to a certain extent on urinary flow, it has been suggested to correct creatinine concen-

tration in "spot" urine for the effect of varying hydration (22). Although the measurement of the elimination rate of a chemical may better reflect the internal dose than its concentration, quantitative urine collection during a defined time interval is rarely done in industry and is difficult to achieve. Mainly for metals, urine contamination during collection may also represent an important source of errors.

The renal excretion is governed by three mechanisms: glomerular filtration, tubular secretion and tubular reabsorption. The alteration of one of these mechanisms may greatly influence the elimination of a substance.

1.7.1.3 Breath

Alveolar air analysis is mainly limited to the estimation of exposure to volatile organic compounds (solvents). A distinction has to be made between "mixed-exhaled air" which is obtained during normal breathing and represents a gas mixture coming from the dead space and the alveolar structures and "end-exhaled air" which is collected at the end of the expiration and mainly reflects alveolar air concentration. Generally, the concentration in "mixed-exhaled air" is larger than the concentration in "end-exhaled air" during exposure and smaller than that in the "end-exhaled air" during the post-exposure period. This method is non-invasive but presents a risk of external contamination during collection. Moreover, the concentration of the solvent in alveolar air may fluctuate very rapidly with the changes of exposure intensity. The time of sampling is very critical and determines whether the measurement reflects either the very recent exposure level (sample collected during or immediately at the end of the shift) or the exposure during the previous day (sample collected 16 hours after the shift).

Several factors influence the alveolar air concentration of an inhaled substance. A relatively higher alveolar concentration is expected for substances of low solubility in blood and of low metabolism. An increase in cardiac output will cause a decrease in relative alveolar concentration which is appreciable in case of highly soluble and highly metabolized substances but practically unnoticeable in case of poorly soluble and poorly metabolized substances. The effect of an increase in alveolar ventilation on relative alveolar concentration is also quite slight and practically insignificant for a poorly soluble solvent, whereas it is appreciable (increase of alveolar concentration) for highly soluble solvents. Similarly, a progressive increase in relative alveolar concentration with duration of exposure may be measured during the shift in the case of poorly soluble and/or metabolized substances (23).

1.7.1.4 Other biological media

Analysis of milk or adipose tissue has sometimes been performed for assessing the body burden of lipophilic compounds (such as organochlorine pesticides) or to assess the risk of transfer of toxic substances to the newborn. The excretion of a substance in faeces mainly reflects the level of oral intake and has no practical interest in occupational settings. The influence of external contamination constitutes a serious source of error when one attempts to assess the internal dose of a substance by measuring its level in hair or nail. Appropriate washing procedures have not yet been elaborated. Some contaminants

may be excreted through sweat, sputum or saliva. However, various methodological problems (e.g. low rate of secretion, external contamination) limit the use of these media for the routine surveillance of workers. Techniques are being developed for measuring in vivo the concentration of lead in bone and that of cadmium in liver and kidney. At present, these techniques are used for research only and are not generally applied to routine monitoring.

1.7.2 Sampling time and representative sampling

A sampling strategy must be based on the time of appearance and disappearance of the parameter in the body fluids, therefore knowledge on the kinetics of the selected biological marker permits selection of the most appropriate time of sampling (see section 1.6).

1.8 Analytical methods

1.8.1 Analytical reliability criteria

The selection of an analytical method must be based on several criteria:
- Accuracy is the closeness of agreement between the analytical result and the true value. Trueness is the closeness of agreement between the average value of a large number of test results and the true value. Precision is the closeness of agreement between independent analytical results. Repeatability and reproducibility are the two extremes of precision, the first describing the minimum and the second the maximum variability in results (24). Analytical reliability criteria are discussed further in Chapter 2 of this volume.
- Specificity: the capacity of the method to measure the parameter under investigation exclusively.
- Sensitivity: the change in the response of the analytical method as a function of the change in the parameter level.
- Detection limit: the lowest concentration of the determinant which will be detected by the procedure.

1.8.2 Practicability

When planning the use of analytical methods, one must consider not only its reliability but also its practicability, i.e. the speed of the method (the time needed for the analysis of one specimen alone and the number of specimens which can be analysed per unit of time under routine conditions: this includes the time used for maintenance, reagent preparing, calibration, total operating time), the equipment and the technical skill required, the precautions and procedures required for safety, the work load, the specimen handling, the cost, and the space needed.

Regardless of whether a method is in routine use or is just developed, its performance in the analysis of field specimens should be monitored through rigorous quality assurance, as discussed in the next chapter.

1.9 Summary

- The biological determinant either gives an estimate of exposure or/and predicts health risk.
- The biological determinant should specifically assess the exposure to the chemical agent under investigation. Non-specific tests, however, may be used in association with specific but less quantitative tests (confirmatory tests).
- The biological determinant should be sufficiently sensitive to be able to identify individuals occupationally exposed to low level of chemicals and it should vary quantitatively with the intensity of exposure and the risk of occurrence of any adverse health effect.
- The biological determinant should be stable enough to allow storage of the biological sample for a certain period of time.
- The biological determinant should yield information on potential risks additional to the information obtained by ambient sampling.
- The collection of the biological sample should not provide too much discomfort or involve any health risk for the subject (ethical aspect).
- The accuracy (trueness and precision) and the analytical range of the method have to be sufficient. As some methods are quite time-consuming or sophisticated, the cost of the analyses in time and money may need to be balanced against the value of the biological monitoring data to be obtained.

1.10 Interpretation of the results

The biological monitoring test must be interpreted according to our current knowledge of the relationships between external exposure, internal exposure, and the risk of adverse health effects and on which basis the biological reference values have been established. The finding of a biological level above the reference value may only be a qualitative indication of exposure to a substance. If the quantitative relationship between external exposure and the internal dose is known, the biological parameter can be used as an index of exposure but provides little information on the health risk. In other terms, biological monitoring performed under these conditions is much more an assessment of the exposure intensity than of the potential health risk. In some situations, a quantitative relationship has been identified between internal dose and adverse health effects. The biological parameter can, in these cases, be considered as an indicator of health risk. It is also possible to derive a biological permissible value from this dose-effect relationship. When the internal dose is quantitatively related to both adverse effects and external exposure, the biological parameter provides information on both exposure and health risk. Sometimes, the relationship between internal dose and effect is unknown, but the internal dose can be related to external exposure and indirectly to the adverse effects. A biological permissible value can be estimated indirectly from the exposure limit in air. It is clear, however, that this method of deriving the biological limit value is much less reliable than a direct estimation based on the relationship between internal dose and adverse effect. Finally, if all the parameters are quantitatively related, both the biological and environmental exposure limits can be directly estimated (3–6).

So far, the majority of published works have focused on the internal dose–external exposure relationships established in volunteers or in industrial workers. The relationships between internal dose and early adverse effects, which are essential for deriving meaningful biological limit values, are comparatively less well documented.

In some cases where there is currently no known relation between the biological index and exposure (e.g. when the main route of exposure is through the skin) or health effect, it could be appropriate to set a biological monitoring guideline that is related to what level is being currently achieved across industry. A possible approach would be to set a guideline that was being achieved in 90% of employees. This approach may sometimes be supplemented by animal pharmacokinetic and effects data which are more easily generated. The relationship between internal concentration and adverse health effects may be known in the future only if biological monitoring is conducted in the present. In the future at least, epidemiological studies could be carried out to assess whether the present levels of exposure were low enough.

The results of a biological monitoring programme can be interpreted on an individual basis. This is usually performed by the occupational health physician who must also take into account several possible individual confounding factors. For instance, liver function impairment may be associated with a decrease in xenobiotics biotransformation. Several drugs may either increase or decrease liver microsomal enzymes activity and hence influence xenobiotics biotransformation. Likewise, alcohol consumption may interfere with the metabolism of various substances (e.g. methanol, toluene, xylene, styrene) in two opposite ways. Moderate chronic intake of ethanol usually stimulates drug metabolizing enzymes and hence the biotransformation of other absorbed chemical agents, whereas during or shortly after a large alcohol intake entailing a high concentration of alcohol in the body, there appears to be an inhibitory effect on the metabolism of xenobiotics (25). Perturbation of renal clearance, large or restricted beverage intake, may be responsible for misinterpretation of urinary results. Tobacco smoke containing many substances (e.g. cadmium, carbon monoxide) can also be a serious confounding factor. For example, smoking influences thioethers concentration in urine and the mutagenic activity of urine. Exposure from diet, environment and leisure activities may sometimes be of importance.

In the occupational setting there is often exposure to a mixture of substances (26). This may entail variations in terms of toxicokinetic and toxicodynamic processes; when interpreting the results, one has to consider the possible physico-chemical interactions between the substances, the effect that one agent may have on the absorption, metabolism, excretion of the other, the possibility of interactions between the parent compound and the metabolites. The effect may be (i) independent, where the substances exert their own toxicity independent of each other, (ii) additive, where the combined effect of the two chemicals is equal to the sum of the effects produced by the individual agents, (iii) synergistic, where the combined effect of the two chemicals is much greater than the sum of the effects of each agent given separately, (iv) antagonistic, where two chemicals administered together interfere with each other, or (v) potentiating, where a substance of low or no toxicity enhances the toxicity of another chemical.

Results are generally interpreted by comparison to adequate reference values. However, because of the difference in individual susceptibility, the threshold values above which an adverse effect will occur will differ between the subjects. A biological reference value for occupationally exposed people is not, therefore, an assurance that it will protect all the exposed persons from adverse health effects. In some susceptible individuals, a biological response may occur even with exposure below these reference values.

When there is considerable inter-individual variability for a certain parameter, the post-exposure level may be better interpreted by comparison to the individual pre-exposure level (the baseline value); for example, the cholinesterase activity of red blood cells, used as an index of exposure to organophosphates or carbamates should preferably be expressed as a percentage of the individual baseline activity. Similarly, for cumulative industrial chemicals it is recommended that the baseline internal dose be established before the subjects are exposed to these substances.

The results can also be interpreted on a group basis by considering their distribution. If all the observed values are below the biological permissible value, the working conditions are satisfactory. If all or the majority of the results are above the biological permissible value, the overall exposure conditions must certainly be corrected. A third situation may also occur: the majority of the workers may have values below the biological permissible level but a few of them have abnormally high values. Several interpretations can be put forward. One interpretation is that the subjects exhibiting the high values perform activities exposing them to higher levels of the pollutant, in which case the biological monitoring programme has identified job categories for which work conditions need to be improved. Another interpretation is that these workers do not perform different activities and, in this case, their higher internal dose must result from different hygiene habits or non-occupational exposure, or genetic polymorphism.

1.11 Published biological action levels

The ACGIH (10) and the DFG (11) publish annual lists of biological monitoring guidelines for those substances for which there is sufficient information available. Biological Exposure Indices (BEI) are reference values intended as guidelines for the evaluation of potential health hazards in the practice of occupational hygiene. BEIs represent the levels of determinants which are most likely to be observed in specimens collected from healthy workers exposed via inhalation exposure to TLV-level concentrations. BEIs do not indicate a sharp distinction between hazardous and non-hazardous exposure. Due to biological variation it is possible for an individual's measurements to exceed the BEI without incurring a health risk. If, however, measurements in specimens obtained from a worker on different occasions persistently exceed the BEI, or the majority of measurements in specimens obtained from a group of workers at the same workplace exceed the BEI, the cause of the excessive values must be investigated and proper action taken to reduce exposure.

Biologische Arbeitstofftoleranzwerte (BAT), Biological Tolerance Value for a Working

Material is defined as *the maximum permissible quantity* of a chemical compound, its metabolites, or any deviation from the norm of biological parameters induced by these substances in human beings. According to current knowledge these conditions generally *do not impair the health* of the employee, even if exposure is repeated and of long duration. BAT values are conceived as ceiling values for healthy individuals. They are established as a rule for blood and urine, with due regard to both the effects of the chemical compound and an appropriate margin of safety. Determining in these respects are health protection criteria which are well-founded on industrial health and toxicological experience.

BEIs and BATs reflect different philosophies in setting guidelines, and interpretation of the results of monitoring in terms of these limits is different. The setting of a BEI equivalent to the TLV for the same material implies that the exposure data on which the TLV was set were collected under conditions in which there was predominantly airborne exposure; i.e. the relationship between effect and airborne concentrations could be well established. If there is significant but varying potential for skin absorption there will be poor correlations between ambient air measurements and biological indicator and any such correlation obtained will vary with the occupational setting in which the data were collected. A further difficulty is that although the definition allows that a proportion of employees exposed at the TLV will exceed the BEI, there is no indication how far they may exceed this value and not incur a health risk. If the TLV is well-founded and will protect the majority of workers exposed at that level, then the biological monitoring levels found in most of them should be acceptable. If the biological monitoring levels occurring at the TLV are normally distributed, then 50% of the employees should exceed the BEI. The extent by which they can safely do so will be reflected in the spread of this distribution. The BEI figure represents the mean value but additional presentation of the 90 or 95% upper confidence limit would give an upper limit of acceptability for an individual result. The information to calculate or estimate this upper limit is not usually available.

The problem with the BAT values are those encountered when any regulatory authority tries to set "No Observable Effect Levels" or "No Observable Adverse Effect Levels". There are usually insufficient good quality data available to set such levels with any confidence. This is especially so for long-term exposures.

The BAT definition is essentially a health-based one while the description given in the ACGIH Introduction to the BEIs is related to an equivalent hygiene limit which can correspond to any one of a number of different biological end-points. However, later in the BEI Introduction it states that "The BEI is based either on the relationship between intensity of exposure and biological levels of the determinant or on the relationship between biological levels and health effects." Ideally, it should be possible to identify the concentrations of hazardous materials or their metabolites in biological fluids below which there is no or negligible risk of clinical damage. It would then be feasible to identify those individuals who by their occupational exposure or by special circumstances relating to their personal practices exceed these concentrations and are therefore significantly at risk.

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Chapter 2. Quality assurance

2.1 Introduction

Quality assurance is an important feature of all analytical work and it comprises several features that are common to all chemical analyses. In the present text, emphasis is on the features, and especially sources of error, that are more or less specific to biological monitoring (in contrast to e.g. clinical chemistry and toxicology), such as contamination, importance of the timing of sample collection or need for international collaboration in external quality assurance. Because much less space is given to features shared by other fields of analytical work, the text is thus intentionally somewhat out of balance. The importance of different sources of error varies markedly between different analyses, and therefore a fairly large number of specific examples are given in the text. This of course does not mean that all known sources of error in all different biological monitoring analyses are covered – they are just illustrative examples.

This chapter deals mainly with analytical quality; however, one should note that laboratory quality is a wider concept, and encompasses not only accuracy (for definitions, see below) of analytical results, but also throughput time, and cost (level of personnel education and training, instrumentation, quality of chemicals) and assistance in the interpretation of the results. This is especially important in biological monitoring, where several different approaches and analytical methods may be selected that are widely different as to their practicability and cost. Often, more accurate results are obtained when more expensive methodology and instrumentation is selected. Especially, the limit of detection is dependent on the cost involved. The selection of approach and methodology must be made based on the needs of the occupational health services for accuracy: the methods chosen must be accurate enough, but must not involve unnecessary sophistication and cost.

Two WHO working parties have made efforts to define and unify the use of the terminology concerning quality of health and environment-related laboratories (1, 2). According to this terminology, **quality assurance** refers to all steps which may be taken to ensure that laboratory results are reliable. It covers the utilization of scientifically and technically sound practices for laboratory investigations, including the selection, collection, storage and transport of specimens and the recording, reporting and interpretation of results. It refers also to training and management designed to improve the reliability of investigations. Quality assurance in biological monitoring has been dealt with in a WHO document (3). From the point of view of an analysis, quality assurance can be divided into two stages, initial assessment of an analytical method (as to its practicability,

precision, trueness, [linearity, specificity, recovery, calibration standards, blanks, inter-ference]) (4), and subsequent quality assessment.

Quality assessment refers to the quality of the analytical results. It has two **compo-nents: internal quality control,** which is a set of procedures used by the staff of a laboratory for continuously assessing results as they are produced in order to decide whether they are reliable enough to be released, and **external quality assessment**, which is a system of objectively checking of laboratory performance by an external agency (1).

In the early 1980s, the Organization for Economic Co-Operation and Development (OECD) developed OECD principles of **good laboratory practice (GLP)** in order to facilitate mutual acceptance of test data on the harmful effects of chemicals, and thus to limit unnecessary duplication of testing, with the aim to avoid creation of technical bar-riers to free international trade. The GLP principles are concerned with the organiza-tional process and the conditions under which laboratory studies are planned, per-formed, monitored, recorded and reported (5). These principles then developed to rec-ommendations on **quality systems** (i.e., organizational structure, responsibilities, pro-cedures, processes and resources for implementing quality management) that are gen-erally applicable to laboratories doing analyses, chemical or other (6). These recom-mendations were recently adapted for use in clinical chemistry laboratories (7), whose functions closely resemble those of biological monitoring laboratories.

2.2   Sources of possible errors

2.2.1 Pre-analytical

2.2.1.1 Environmental sources of variation

Toxic chemicals in body fluids are not only derived from exposure at work: diet, and free-time activities may constitute important additive or alternative sources of chemi-cals. To assess adequately the occupational exposure to a chemical, such non-occupa-tional exposures must be considered. Therefore, a part of the occupational exposure as-sessment should be the determination of the concentrations of the biomarker in persons not exposed to the chemical at work, to obtain a reference interval for the occupationally non-exposed. The reference interval for the non-exposed – where the interest is focused on the upper reference limit (URL) -- is different in different geographic locations. This has been extensively documented e.g. in the context of lead and cadmium (8, 9). More-over, changes may occur with time, as seen for example in the concentrations of lead in blood in several countries in recent years (10, 11) – or for e.g. cadmium in Japan (12).

Most environmental chemicals enter the body via the diet: concentrations of heavy metals, organochlorine pesticides, phenolic compounds and several organic acids in the urine or blood reflect their levels, or their precursor levels, in the diet. Personal habits, notably smoking, are known to influence the concentrations of exogenous chemicals in body fluids, for example cadmium and benzene (11, 13). Drugs may also be sources of

toxic chemicals in the body: e.g. aluminium from antacid drugs can elevate urinary aluminium levels (14–17).

2.2.1.2 Kinetic sources of variation

Concentrations of extrinsic chemicals in body fluids are often not constant, but change with time, and often show an exposure-related fluctuation. The half-times of chemicals thus become an important determinant of the concentration observed, and to obtain representative results, the time of sampling for chemicals with a short half-time must be rigorously standardized (18).

In addition, chemicals and other environmental factors may change the fate of toxic compounds in the organism: Alcohol drinking may change the metabolism of e.g. trichloroethylene, xylene, styrene and toluene (19–24). Solvents may affect each other's metabolism: xylene and toluene (25), toluene and benzene (26), and xylene and ethyl benzene (27) exhibited a mutual inhibition in simultaneous exposure and toluene inhibited the metabolism of hexane to hexanedione (28).

2.2.1.3 Sampling

(a) *Blood specimens*

Physiological changes may induce marked intra-individual variation in the actual concentrations of blood constituents (29–31), and although data on such variation in the concentrations of toxic chemicals in blood are scanty, it seems inevitable that physiological factors should have similar effects also on measurements of their concentrations.

Distribution of water depends on posture: standing up leads to a loss of plasma water, and to an apparent increase of approximately 10% in the concentration of non-diffusible blood constituents like proteins or cells. Posture-dependent changes may be much more marked in disease states (32).

Similar changes in water distribution take place locally, such as occurs when a tourniquet is used for blood specimen collection (29–31). Xenobiotics with limited water solubility are transported in the blood bound to proteins, lipoproteins or cells. Changes in posture and application of tourniquet will therefore change their apparent concentrations.

Meals affect the blood composition; in particular, the concentration of triglycerides increases after a meal. This may affect the distribution of lipid soluble solvents, such as dichloromethane, xylene, or inhalation anaesthetics (33–35).

Concentrations of many body constituents show a diurnal variation. From the point of view of biological monitoring, it is important to note that the excretion rate of water, and creatinine excretion, are among such chemicals (36). Diurnal variation has also been demonstrated for some chemicals that are analysed for biological monitoring purposes,

such as manganese, mercury, lead, δ-aminolevulinic acid, and hippuric acid (36–39).

Exercise causes changes in blood serum constituents, possibly due to leakage of intra-cellular components. Continuous training may cause hemodilution; this may lead to apparently low values of blood components. Changes caused by physical strain may even be seen in urinary excretion of trace elements, as exemplified by a 5-fold increase of the urinary chromium excretion after 2 hours of running (40).

During pregnancy the plasma volume increases by a third; this elicits changes in the concentrations of many blood components (30, 41); lactating may also affect serum composition, as exemplified by chromium (42).

Errors caused by physiological variation may be decreased by standardization of specimen collection (31, 43).

Many pathological conditions may affect concentrations of chemicals in blood or serum. Several organic chemicals (styrene, xylenes, toluene, benzene) are metabolized in the liver, and trace elements (e.g. lead, arsenic, zinc and copper) are excreted primarily through the liver. Liver disease and cholestasis thus probably decrease their clearance (e.g. 44). On the other hand, kidney damage causes retention of chemicals (e.g. Al, F), for which the main excretory route is the kidneys (45, 46). Similarly, the kinetics of compounds excreted through the lungs are probably changed due to chronic bronchitis or emphysema (47).

(b) Urine specimens

Usually, only spot urine specimens are available for analysis. Because the concentration of many analyses is dependent on the rate of urine excretion, which varies to a great extent, some standardization for urinary excretion rate has long been used (48–50). In some cases, no correction method is applicable, but even in such cases it may be advisable to assess the urine dilution/concentration in order to be able to identify the specimens which cannot be interpreted meaningfully since they are too diluted or concentrated. The most widely used approaches have been based on relative density, the concentration of creatinine in urine, and the length of the urine collection period, i.e., excretion rate. The best correction method is chemical-specific and the search for the optimal correction requires studies of multiple samples from several individuals under different conditions, such as during water deprivation and loading, variable physical work load, and varying dietary composition. Araki and co-workers have extensively studied the correction of urinary concentrations to a standard urinary flow rate of 1 mL/min (51–53). Although this approach cannot be applied in routine biological monitoring, it provides a generic method to compare the behaviour of the excretion of different chemicals in the urine by the following equation:

$$C_S = C_O \times F^b, \quad (1)$$
where C_S = standardized concentration, C_O = observed concentration, F = urine flow rate mL/min, and b = chemical specific constant from the experimental equation
$$\log C_O = a - b \times \log F \quad (2)$$

It can be seen from the equation (1) that when the constant b approaches 0, no correction is required, i.e., the observed concentration is independent of urinary flow rate. This, however, does not seem to be true for any chemical studied (including, especially, creatinine) (53). When b approaches 1, the corrected concentration is proportional to urine flow rate, and correction to relative density is rather accurate. This seems to be the case for mercury (53). On the other hand, when b approaches 0.67, the b constant for creatinine (53), correction to creatinine excretion would seem most appropriate in routine biological monitoring. Manganese, cadmium, and hippuric acid are candidates for this approach (however, see inter-individual variation below). Chromium and copper are intermediate, and thus correction to either creatinine or relative density may be applied. For several chemicals, inter-individual variation of the constant b is very large (for example, cadmium, manganese), and consequently, no general correction method will give accurate results. Similarly, for methylethylketone, no correction improved the correlation between urinary methylethylketone, and its concentration in the air (54, 55). When the urine is very dilute (relative density < 1.010 or urinary creatinine < 0.3 g/L [< 2.65 mmol/L]) or concentrated (relative density > 1.030 or urinary creatinine > 3.0 g/L [> 26.5 mmol/L] it is unlikely that any correction will give accurate results.

(c) Hair

Trace elements in the hair originate from the matrix proper, from sebum, sweat, and from extrinsic sources (dust, shampoos, colouring chemicals) (56). The differentiation of the source has proved very difficult. It was not possible to differentiate between extrinsic and intrinsic arsenic, cadmium, lead, mercury or methyl mercury with any washing procedure (57, 58). Different washing procedures resulted in different, procedure-specific plateau concentrations of chromium, manganese, iron, copper, zinc, and cadmium. It could not be determined, which of the plateaus represented the intrinsic metal concentration of the hair (59–61).

Hair trace element analysis does not seem appropriate for the biological monitoring of occupational exposure.

(d) Exhaled air

Collection of exhaled air is non-invasive, and since air as a matrix for the analysis is relatively simple, exhaled air would seem appropriate as a sample for biological monitoring (62) – and indeed, it has been used, and even biological action levels for solvents in breath have been proposed (63). However, since the concentration of solvents in breath is very much different in different stages of exhalation (alveolar vs. dead-volume air), sampling must be thoroughly standardized (62). Also, there are often marked problems in the stability of breath specimens.

2.2.1.4 Storage and transportation of samples

Several processes may affect the stability of samples during storage, and especially during transport. Often it is impossible to guarantee stable conditions during mailing and it has to be verified that the samples stay stable in practically any condition, in temperatures higher than 40°C as well as lower than 0°C. Also, low temperatures may be a

hazard: the specimen containers may not accommodate for the increased volume of aqueous solutions that take place upon freezing. It is apparent that the specimen containers must be resistant to physical impacts, such as dropping on concrete floors, etc.

(a) Evaporation

Organic solvents which are routinely analysed in biological specimens for assessing occupational exposure include benzene, methylethylketone, tetrachloroethylene, toluene, and 1,1,1-trichloroethane. Some of them are quite volatile, but no systematic study on how rapidly they are lost from biological samples is available. Pure solvents have been reported to be lost from screw-capped containers (64); the amount lost was dependent on the material of the tube, and especially that of the stopper. Toluene and ethyl benzene were rapidly lost from blood specimens in vials kept open, even at 4°C (65). Benzene, toluene, trichloroethylene and ethyl benzene remained stable for at least three weeks in bovine blood, when they were stored in full glass vials with ground glass stoppers (66). Similarly, blood toluene was reported to remain stable in full glass vials, closed with aluminium or Teflon® lined stoppers both at 4°C and at room temperature. However, toluene was rapidly lost from plastic blood collection vials, Monovette® syringes, and glass vials with rubber stoppers (65). Toluene was also lost from Vacutainer® tubes at room temperature and at 4°C (67).

(b) Precipitation

Urine, when voided, is often an oversaturated solution of e.g. urates and phosphates. In addition, cells and cell conglomerates may act as centres for crystallization. Therefore, urine tends to precipitate on storage. Cooling may cause additional precipitate formation. Bacterial infection in the urinary tract may increase the tendency to precipitation. Trace elements in the urine may coprecipitate with other elements, or adsorb onto the surface of the precipitates. Precipitation is seldom a problem in the analysis of organic chemicals in the urine.

[63]Nickel, added to urine samples, adsorbed on the precipitate that formed. This loss of nickel was less than 1 % at pH 1, but 6 % at pH 6 (68). When urine was stored at pH< 2, no loss of cadmium on to the forming precipitate was observed (69).

Centrifugation of urine decreased the mercury content of the liquid phase (70). Full recovery of mercury added in urine could only be achieved, if the specimen was vigorously shaken before analysis. None of the preservatives tested could prevent this loss (71). Arsenic, copper, antimony, chromium, mercury, selenium and zinc were concentrated in the precipitate upon storage for 2 days of acidified urine, whereas manganese, cobalt, caesium and rubidium remained in the supernate fraction (72).

Marked differences thus exist between losses of different metals on precipitates in urine. This loss must always be accounted for when sampling urine specimens. Precipitation is a factor to be remembered also when preparing quality control specimens in a urine matrix: the homogeneity of the quality control specimens has to be investigated carefully.

(c) Adsorption

Although adsorption of metal cations onto surfaces of different types of glass, or plastics from distilled, fresh, and even sea water is a well recognized problem, much less data are available on the adsorption of elements onto surfaces from blood or urine samples. Stoeppler (68) did not detect any loss of added ^{63}Ni from urine samples onto polyethylene container walls. Concentrations of nickel or chromium in urine samples, spiked with small concentrations of the metals and stored for 6 months at 4°C did not show a decrease (73). The International Union of Pure and Applied Chemistry (IUPAC) reference method for nickel in urine calls for acidification of urine quality control samples with nitric acid and storage in polypropylene tubes with a screw-cap at -20°C (74). No adsorption of cadmium onto container walls (type not specified) was observed from urine acidified to pH<2 (69).

Polypropylene tubes were found to be suitable for storage of serum for aluminium analysis (75, 76), whereas Vacutainer® (glass) tubes gave rise to either increases (leaching) or losses (adsorption) in the aluminium content (75).

No change was seen in the concentration of cadmium or manganese in blood during 5 days in Vacutainer® tubes (77). Contradictory results have been reported on the stability of blood samples for lead analysis. Although several studies indicate that lead can be stored in different plastic (polypropylene, polyethylene, polystyrene, polycarbonate) or glass (Pyrex, soda glass) vials especially in frozen state (78–81) without losses, some studies have also reported marked losses (82–84).

An elevated concentration (50 µg/L) of chromium in serum remained unchanged for less than 4 days at room temperature, less than 3 weeks in a refrigerator, but more than 18 months at -10°C in polyethylene or polycarbonate tubes (85).

A solution of cobalt 0.3 mg/L in approximately 0.1 mol/L salt solution, at a neutral pH, wastable for 28 days in polystyrene tubes (86). Blood specimens for cobalt analysis could be stored in plastic tubes for one week in a refrigerator; in longer storage deep freezing was reported to be necessary (87).

In contrast to the vast body of data available on the adsorption of trace elements on vial surfaces from specimens of fresh and salt water, such information on biological specimens is scanty. However, it seems that adsorption is not equally important a source of error for biological specimens as it is in the storage of water specimens. Also, few organic chemical metabolites excreted in the urine would seem so little soluble in water that adsorption on the tube walls would constitute a problem.

(d) Chemical instability

Many organic chemicals have limited life-span in biological matrices. Biological materials may contain enzymes that degrade chemicals; in urine samples, bacteria may be effective in the biotransformation of a variety of organic molecules. Phenylglyoxylic acid was unstable in urine stored at room temperature, or in refrigerator, and so were

hippuric and methylhippuric acids while benzoic, methylbenzoic, and mandelic acids remained stable for weeks (88–91). Phenol and tri-, tetra- and pentachlorophenol, as well as 2-thiothiazolidine carboxylic acid were quite stable in refrigerator in urine (92–94). The instability of ß$_2$-microglobulin in acid urine seriously limits its usefulness in biological monitoring (95) (see Bernard, this volume). In blood samples, stored for the analysis of toluene by head space gas chromatography, n-hexanal was formed, which interfered with the analysis of toluene (65).

Although chemical instability is usually considered a problem of organic chemicals, even e.g. mercury solutions in water, and in urine, are notoriously unstable (for references, see 94).

(e) Loss of water

Loss of water by evaporation results in apparent increases in the concentrations of the chemicals studied. Water may be lost through the container walls, or through leaky closures; therefore the results may be quite variable from one container to another. Generally, errors introduced by water loss are minor, and do not affect everyday routine analyses. However, in specimens with long storage times (especially quality control materials!) such losses may become significant. Annual losses of water from polyethylene, polypropylene, high density polypropylene, Teflon-FEP, and glass containers were less than 0.5% (64, 69). Losses from polyvinyl chloride were approximately 0.5%, those from polycarbonate approximately 2% and from polymethylpentane 1% annually (96).

(f) Redistribution

The concentration of manganese is more than 20 times higher in erythrocytes than in plasma (37, 97, 98). The ratio for lead is 50–100 (99–105); for zinc it is approximately 10 (106). It is thus evident that destruction of cells leads to gross elevation of the concentration of such elements in plasma/serum. Plasma may be separated from cells more rapidly than serum, and the procedure is more gentle toward the cells. Therefore, plasma is to be preferred over serum, when elements with unequal distribution in blood are analysed, although addition of anticoagulant adds a risk of contamination (see below). Hemolysis may give rise to analytical interferences e.g. by liberation of considerable amounts of iron into the serum: iron interferes with e.g. the analysis of nickel by electrothermal atomic absorption (74).

2.2.1.5 Contamination

Contamination is by far the most important source of error in the analysis of trace elements. Trace elements are ubiquitous in the earth's crust, and tend to enter the samples in all phases of the performance of the measurement. The increasing sensitivity of analytical methods has resulted in the use of decreasing volumes of specimens, and therefore, to decreasing amounts of the analyte in question. Recognition of contamination has also dramatically changed our views on the true concentrations of many trace elements in biological samples. Pre-analytical contamination may be derived from the air, from the clothes and skin of the subject or the collector of the sample; from specimen con-

tainers or from chemicals added to the sample vials e.g. in order to prevent coagulation, or to preserve the sample. The effect of contamination on the analysis is dependent on the level of the analyte: when the true concentrations are very low, even smallest contamination may completely invalidate the analysis, while at high analyte levels, a minor contamination need not be very serious. Contamination is usually not a problem when a metabolite rather than the parent compound is being measured.

Breath and blood samples for biological monitoring of solvents may also be prone to contamination: the specimens should be collected in an area, where the air does not contain the solvents to be measured.

(a) Workplace air and skin

Contamination from workplace air or from the clothes of the worker causes the most drastic errors in measurements of toxic elements in body fluids. The reason is the high concentrations of the chemicals in the workplace, where they tend to be orders of magnitude higher than, e.g. in the laboratories.

In addition to air-borne dust, contamination on the skin may result from sweating: Hohnadel and co-workers (107) detected high concentrations of nickel, copper, zinc and lead in sweat collected during a sauna bath. The significance of the quantities found is not easily interpreted. However, as water from the sweat evaporates, the amounts remaining on the skin may be rather high. Capillary blood samples, which are collected from the fingertip, are more prone to contamination than venous blood samples.

The skin has to be thoroughly cleaned before drawing blood samples for toxicological analysis. Washing with 0.1 mol/L hydrochloric acid, followed by rinsing with ethanol, removed lead contamination from the skin while washing with only ethanol or ethanol and water, was not sufficient for this purpose (108). In a study of plasma lead, Everson and Patterson (101) washed the skin successively with soap water, alcohol, acetone, hydrochloric acid, and low-lead water. The risk of contamination from the workplace air is even greater when urine specimens are collected and analysed for the unchanged compound. They should not be collected in the workplace, but at a separate site, and only after making sure that no dust from the clothes of the worker may reach the specimen container.

(b) Needles

By neutron-activating disposable stainless steel needles and analysing the resultant radioactivity in blood specimens drawn through these needles, Versieck and co-workers (109, 110) showed that significant amounts of chromium, nickel, cobalt and manganese were leached in the first 20 mL of the blood. Damage caused by the activation process did not explain the findings. Concentrations of chromium in the blood obtained through disposable stainless steel needles was four times higher than that in blood obtained through a plastic catheter (111); the first 20 mL of blood collected through a stainless steel needle contained 8 times more chromium than the next three 20-mL portions (112). Siliconisation of the needle seemed to abolish chromium contamination (113,

114). Siliconisation of the inner surface of the needle may decrease also the nickel contamination. Serum nickel values in blood samples obtained through disposable needles were twice as high as those from samples obtained through plastic catheters (115), while no such effect was seen when siliconised needles were used (116). Elevated serum manganese concentrations were also reported after collecting samples through stainless steel needles (117), while no leaching of aluminium, cadmium or cobalt was detected from needles (76, 117–120). However, Parkinson and co-workers (121) have reported that stainless steel venipuncture needles they used were randomly contaminated with aluminium.

(c) Anticoagulants and preservatives

Information on the contamination resulting from the use of anticoagulants is very scanty. Lead in heparin does not contribute significantly to the concentration of lead in whole blood, whereas for lead in plasma it does, as the concentration of lead in plasma is very low (101, 122). Sodium citrate and lithium heparin were reported to contain too high concentrations of aluminium (123), while potassium EDTA from one source – but not from another – could be used for plasma aluminium analyses (123, 124). Potassium EDTA from a further source was found suitable for the analysis of cobalt in whole blood (87): however, in an analysis of a large number of trace elements, and several anticoagulants, contamination was most frequently encountered, when EDTA was used (124).

Although analytical data on many metals in anticoagulants are not available, it would seem that all anticoagulants are a likely source of contamination.

Hydrochloric and nitric acids have been used as preservatives for urine specimens for metal analyses, and mineral acids are extensively used in graphite furnace analysis of trace elements in biological specimens. Historically, concentrations of trace elements in commercially available acids have been incompatible with analysis of trace elements in biological samples (125). However, present commercial ultra pure hydrochloric, nitric, sulphuric and perchloric acids have been reported to be suitable for trace element analysis in urine without further purification (74, 126, 127).

(d) Glass and plastic ware

Glass (c.f. 128) and various plastics (96, 129, 130) contain varying amounts of practically all elements, and these may leach into water, acids, as well as blood and urine specimens. Even high purity quartz test tubes were a source of contamination of aluminium, chromium, and molybdenum (114). It seems that no commercially available container should be used for storage of specimens for trace element analysis without prior cleaning.

Evacuated blood collection tubes, and especially their rubber stoppers, have been notorious for their contamination with metals, which was first recognized with zinc, lead and cadmium (75, 80, 108, 131–135). At present, many manufacturers market evacuated tubes specifically destined for lead, or more general trace element analysis. In many in-

stances they have been found suitable for trace element analysis, but there may still be differences between the different brands, and analyses at lowest levels may still be contaminated (75–77, 80, 131, 132, 134–137).

Plastic tubes may also be a source of organic chemicals: Ethylbenzene and xylene leached to blood specimens from Monovette® tubes (138). Similarly, we could not use evacuated blood collection tubes for blood benzene analysis because of leaching from the rubber stopper of a chemical with identical chromatographic behaviour with benzene (Pekari K, Finnish Institute of Occupational Health, unpublished). Further, we noted a high PCB contamination in a set of plastic blood collection vials (Luotamo M, Finnish Institute of Occupational Health, unpublished).

It seems advisable that the laboratory doing the analysis should provide the specimen containers; this helps to diminish contamination risks. Commercial evacuated tubes may introduce contamination to blood specimens. Although this risk seems smaller if dedicated tubes manufactured specifically for trace metals are used, absence of contamination should be verified before starting the use of commercial tubes.

2.2.2 Analytical

Analytical error is very much different for different analytical approaches; only general principles, with some examples specific of certain biological monitoring methods are discussed here. The terminology used here is that proposed by the International Organization for Standardization (ISO) (139–141), and endorsed by International Union of Pure and Applied Chemistry (IUPAC), Association of Official Analytical Chemists (AOAC), and Scandinavian Society for Clinical Chemistry (NFKK) (7, 142), and is somewhat different from the traditional (143).

Accuracy is the closeness of agreement between the analytical result and the true value; it is the composite of **trueness** and **precision.** Trueness is the closeness of agreement between the average value obtained from a large series of analyses, and the true value. Precision is the closeness of agreement between independent analytical results. Trueness and precision may be described in terms of **bias** and **imprecision.**

2.2.2.1 Bias

Bias in an analysis is caused by **contamination** or shortcomings in analytical **specificity, recovery,** or **calibration.**

(a) Contamination

Contamination is inadvertent adding to the specimen of an amount of the analyte. Contamination in pre-analytical phases has been discussed above. Contamination during the analysis may be derived from reagents (trace elements in mineral acids) used in the analysis, as well as from parts of the analytical instrumentation, such as pipette tips or glass and plastic ware (see above). Use of metals, such as nickel as matrix modifiers may contaminate the furnace and thus make the use of an instrument difficult to subse-

quent nickel analysis (144). Carryover from a sample with high concentration to the following sample -- with a lower concentration -- in the analytical run is an important possibility for contamination. Some ubiquitous elements, such as aluminium, zinc, and nickel -- as well as common laboratory solvents -- present special contamination problems, and it may be necessary to work in special clean laboratories. Also organic chemicals that are present in biological specimens at very low concentrations, may be problems from the point of view of contamination. For example, the cleaning solution used to wash the floors in the laboratory performing the analysis was found to contaminate the analyses of TCDD from human adipose tissue (145). Analysis of blank samples may aid in the identification of contamination from the sample containers, laboratory ware and reagents.

(b) Specificity

Specificity is the ability of the analytical method to measure exclusively the chemical wanted, i.e., the analyte. The specificity of different analytical methods varies widely: typically calorimetric or photometric methods are less specific than methods using chromatographic separation (e.g. hippuric, methylhippuric and mandelic acids, phenol, hexanedione (146–150) or atomic absorption (lead 151). Specificity may be achieved by specific detection (e.g. specificity of the mass of the molecule in mass-spectrometric analysis, or specificity of the intra-atomic energy states in atomic absorption), or by sample purification (extraction, chromatographic methods), or usually by a combination of both (e.g. head space capillary chromatographic separation followed by photo ionisation detection of benzene in blood (152). Lack of specificity depends on the presence of disturbing chemicals and therefore is very much dependent on the matrix: a method that is fully specific in one matrix may be unspecific in another matrix. Thus, for example, the high iron content of blood, and high calcium content of urine, interfere with the analysis of nickel by ICP-mass spectrometry -- which is usually considered a very specific method (153). Pre-treatment may inadvertently decrease the specificity of an analysis: Acid hydrolysis at a pH<2 led to formation of 2-acetylfuran in urine specimens, which cochromatographed with 2,5-hexanedione in capillary gas chromatography (154). Molecular absorption is a form of non-specificity that is very important in flameless atomic absorption spectrometry: remnants of organic material that burn at the time of atomization are detected as absorption. This has been avoided by instrumental developments, such as background correction by tungsten or D_2 lamps, or Zeemann effect. In addition, matrix modification may be useful. For example, urine chromium analysis using D_2 background could only be achieved by the use of matrix modification by acid, which allowed ashing at higher temperatures because of decreased volatility of chromium (127).

(c) Recovery

Recovery is the proportion of the analyte in the sample that reaches the final step in the analysis, and gives rise to the signal that is measured. It is seldom 100 per cent, and the reasons for losses are many: extraction depends on the equilibrium of the analyte in two phases, and is by definition less than complete. Dry ashing, and even wet ashing, may lead to evaporation loss of the metal to be analysed; metals may adsorb on vessel walls

or silica crucibles; in wet ashing, the analyses may coprecipitate with e.g. calcium sulphate and be lost from further analysis. High temperature in the ashing stage of graphite furnace atomic absorption spectrometry, specially in the case of metals with low boiling points, such as cadmium or lead, and in the absence of matrix modifiers, may lead to evaporative losses.

Recovery tends to be different for standards prepared in pure solvents from that observed in the specimen in a biological matrix. This will lead into biased results, unless the differences in recovery are specifically considered and corrected for. Recovery may also be different for different samples (e.g. urine specimens with different pH, osmolality, protein/glucose content, blood samples with different fat contents).

Recovery may be assessed by analysing samples with added concentrations of the analyte (spiking), and the effect of defective recovery may be counteracted by preparing the standards in a matrix identical to that of the samples, by the use of internal standardization, and by using the method of standards' addition. The prerequisite of the efficacy of internal standardization is that the selected internal standard behaves in the analysis in a fashion that is identical to the analyte; an ideal case is the analyte itself labelled with an isotope label (13carbon, 2deuterium), which have been extensively used in e.g. dioxin analysis from human specimens.

Interference may be considered a special case of low recovery: a component of the matrix decreases the signal of the analyte in the measurement apparatus. Such interference is the main reason, why voltammetric techniques are difficult to apply for biological samples. Similarly, silicates, aluminium and phosphates tend to decrease the AAS signal of cadmium, and iron and nickel that of chromium. Interference may be decreased by more intensive sample purification before the analysis.

(d) Calibration

Analyses are seldom carried out in an absolute way, i.e., purification of the analyte from the sample, and weighing. Rather, the analysis depends on the comparison of the signal of the analyte in the matrix to be studied to that in a standard (solution). Thus the accuracy of the end result is crucially dependent on the accuracy of the concentration of the calibration standard, i.e., on the purity of the chemical, and the accuracy of the dissolution and dilution process. Further, instability of the standard solution may lead into bias in the analysis. The traceability of the standard chemical and the instruments to prepare the standard solutions is a way to assure the accuracy of the standard solutions.

2.2.2.2 Imprecision

Imprecision is caused by slight variations in the way the analysis is performed: weighing, volumetric delivery, timing and temperature of enzymatic reactions, variation in the timing and forcefulness of mixing in extraction, instrument function (gas flow and pressure in GC and AAS, pumping rate of liquid chromatographs, instrumental drifts and shifts, reading of analogue meters, etc.). Imprecision for an analysis is not constant: it is smallest when replicate analyses are made in **repeatability conditions** (141), that is, in

the same laboratory by the same person, same instrument, same batch of reagents and standards, as successive samples in the same analytical series. Imprecision is larger in **reproducibility conditions:** using same methodology in different laboratories with different operators using different equipment (141). Imprecision is also to a great extent affected by the training and skill of the analyst. The distinctive feature of imprecision is that it is random, i.e., equally likely to produce "too high" and "too low" results. On the other hand, imprecision is typically dependent on the concentration of the analyte being measured: it is highest at the limit of detection, decreases with increasing concentration, but tends to rise again, when the concentration becomes very high. Imprecision need not be related to the bias of the analysis. Imprecision can be expressed in statistical terms as standard deviation, or relative standard deviation (coefficient of variation).

2.3 Quality management

ISO Guide 25 (6) formulates the general requirements for the competence of analytical laboratories; they are derived from the OECD guidelines for good laboratory practice for laboratories testing chemicals for toxic and harmful properties (5). The ISO guide gives detailed instructions on the requirements on the laboratory organization and management, and instructs on the main components of **quality management,** that is, quality system, audit and review. The **quality system** is spelled out in the laboratory **quality manual** and its attachments; compliance of procedures specified in the quality manual is verified in **audits** at specified intervals and the appropriateness of the quality system adopted is **reviewed** at least once a year by the management. Quality management has been proposed as a prerequisite for the recognition of the laboratory competence to carry out its tasks, and thus for laboratory accreditation (155).

Quality manual is a set of documents describing the organizational structure, responsibilities, procedures and processes by which the laboratory achieves its objectives – and carries out the quality management (7, 156). The quality manual has three main sections: 1) quality policy statement, i.e., a statement by the head of the laboratory, indicating the commitment to implement and maintain a high standard of quality in the laboratory; 2) description of the organization and the responsibilities and authority of the various responsible persons; and 3) work instructions, i.e., description of the actual measurement procedures and other detailed administrative and technical procedures which are necessary for the laboratory work. A guideline for a quality manual for the clinical laboratory has been published (7), and may be useful for biological monitoring laboratories in the development of their own quality manuals.

Audit is a verification, performed at appropriate internals, of the compliance of the work done in the laboratory with the quality manual, and its descriptions of work procedures. Audits shall be carried out by trained and qualified staff who are, wherever possible, independent of the activity to be audited. Where the audit findings cast doubt on the correctness or validity of the laboratory's results, the laboratory shall take immediate corrective action and shall immediately notify, in writing, any client whose work may have been affected (6).

Review of the quality system shall be carried out at least once a year by the laboratory management to ensure its continuing suitability and effectiveness and to introduce any necessary changes or improvements (6).

2.3.1 Internal quality control

Internal quality control (IQC) is a part of quality management; it comprises the set of procedures undertaken by the laboratory for continuous monitoring of operations and results in order to decide whether the results are reliable enough to be released; IQC primarily monitors the batch wise accuracy of results on quality control materials, and precision on independent replicate analysis of test materials (142). A guideline for the internal quality control in a clinical laboratory has been published (157). Internal quality control procedures most often depend on repetitive analysis of samples designed for IQC purposes. In its simplest form, it means continuous follow-up of the differences of results of duplicate samples. The range of the results gives an indication of the imprecision of the method. More information may be derived from several replicate analyses of the same homogeneous control specimens; they provide information in addition to imprecision, also on changes in bias. As a minimum, one such analysis should be included in every analytical run. This form of internal quality control has the potential problem that since the result to be expected will be known (open controls), the analyst may tend to bias the reporting of the results toward this expected figure. With the increasing use of digital instrumentation with analyst-independent reporting, this bias is no more crucial. Also, increasingly automated sample handling throughout the analysis decreases the possibilities that the control specimens receive a special treatment. The fundamental premise of internal quality control schemes (as well as of external quality assessment programmes) is that the control specimens and the routine samples are treated in an identical fashion.

In clinical chemistry laboratories, daily average of analytical results may also be used as a form of internal quality control: it is likely that the average does not change on a daily basis. This, however, seems inappropriate in biological monitoring, since the proportion of "abnormal" results (those in excess of the upper reference limit) tends to be high, individual results may be exceedingly high, and the total number of daily analyses tends to be low.

The material used for internal quality control (control material, 158) should have a matrix similar, if not identical, to that of the routine samples. For several analyses, this can be achieved by pooling surplus samples. For a number of analyses in biological monitoring, no stable quality control materials with an appropriate matrix are available; these include head-space analyses of volatile solvents in the blood. In these cases, the only option is a formulated aqueous control specimen – prepared and used separately from the calibration standards. It goes without saying that all efforts should be made to ensure the non-infectivity of the control materials. Use of certified reference materials (see below) as control materials in internal quality control is not appropriate, and mostly economically not feasible. For some analyses, commercial reference materials are also available (Table 2.1) and may be used in internal quality control.

As indicated above, part of internal quality control necessarily will be done on an "open" basis. However, it is advantageous to also do occasional "blind" quality control analyses, in order to avoid conscious and non-conscious "improvement" of analytical results.

One purpose for the internal quality control is to give an alert when an analysis is no longer functioning as expected (as defined in the initial assessment of the method, see above). This alert is based on the finding that a result obtained in a control analysis does not belong to the random variation that is specific of the method; in addition, unacceptable variation between the duplicates of a specimen, or non-linearity of the calibration curve may be used as an indication of abnormal analytical performance. Modern laboratory data acquisition systems may be programmed to give such an alert automatically. They also generate automatically quality control charts, which display visually the results of control samples as a function of time (or analytical run number). For manual analyses, it is mandatory that quality control charts are developed for each analysis (with the set criteria of acceptability for control analyses indicated) and continuously updated.

2.3.2 External quality assessment

External quality assessment, also called proficiency testing, is also a part of the quality management and comprises methods of checking laboratory performance by inter-laboratory tests. Such activities have their roots in informal exchange of specimens between laboratories in order to verify and improve laboratory performance, and, on the other hand, in a large number of ad hoc comparative studies on laboratory performance, usually on a specific analysis. External quality assessment is not very well developed in biological monitoring of exposure (see examples below). Formalized guidelines for the optimal operation of continuously operated proficiency testing schemes have been published (142, 159, 160).

2.3.2.1 Structure of external quality assessment scheme

The optimal structure of the scheme for any one analyte is as follows (142): 1) The co-ordinator organizes preparation, homogeneity testing and validation of test material; 2) Co-ordinator distributes test samples on a regular schedule; 3) Participants analyse test portions and report results centrally; 4) Results are subjected to statistical analysis, and the performance of the individual laboratories is assessed; 5) Participants are notified of their performance; 6) Advice is made available for poor performers, on request; 7) Co-ordinator reviews performance of the scheme.

2.3.2.2 Test materials

An important feature of the scheme is the selection and preparation of the test material. The test material must be similar to the materials that are routinely analysed as far as the matrix, and concentrations of the analyte are concerned. It must be homogeneous, stable, and non-infective. In biological monitoring, the exact chemical identity of the analyte must be carefully considered in relation to the analysis performed, and the aim of

the analysis. Thus it is imperative that e.g. in the specimen where "phenol" or "total phenol" will be analysed for the quality assurance of the biological monitoring method of exposure to phenol, the analyte is in the form that it appears in the urine of workers exposed to phenol (i.e., phenylglucuronide and phenylsulphate, rather than phenol). Similarly, for urinary arsenic analysis, the speciation of arsenic (As^{3+}, As^{5+}, methylarsonic acid, dimethylarsinic acid, arsenobetaine, arsenocholine), has to be considered. Similar considerations apply to several other biological monitoring analyses (e.g. blood mercury, urinary hexanedione, urinary hippuric and methylhippuric acids).

2.3.2.3 Frequency of sample distribution

The frequency for the distribution of samples in a programme depends on the difficulty in executing effective analytical quality control, laboratory throughput of analyses, consistency of results from previous rounds, cost/benefit of the scheme, and availability of suitable materials for test schemes. In practice, the optimal frequency is probably between once in two weeks and once in four months (142). A frequency greater than once in two weeks would for most biological monitoring analyses seem to be not cost-effective; it would also encourage replacement of internal quality control by the external quality assessment scheme – for which purpose this frequency is too low (one internal quality control specimens should at a minimum be included in every analytical run, see above).

2.3.2.4 Assigned values

The external quality assessment scheme should provide the participant laboratories with an assigned (target) value for the analysis and criteria for acceptability only after the analyses have been performed. The assigned values may be determined by a consensus from expert laboratories, by a consensus of the participating laboratories, by analysis of a certified reference material for comparison, or directly by formulation, i.e., by adding a measured amount of the analyte into the matrix, which does not contain it at all.

(a) Formulation

Formulation is problematic for many biological monitoring analyses. For trace elements, appropriate matrix with zero concentration is seldom available; for several organic compound metabolites the exact composition of the chemical species to be analysed may vary (see above). In addition, the formulation approach has the generic problem that the behaviour in the analysis of the added analyte may be different from such analyte that has reached the matrix *in vivo* (binding to lipids, proteins, small molecular weight carriers). However, formulated samples have been extensively used for studying the analytical recovery (a pair of samples is shipped, one with "natural" content of the analyte, the other spiked with a known amount).

(b) Comparative analysis of certified reference material

Comparative analysis of certified reference material is also of limited usefulness in arriving at a target value. This is because there are few certified reference materials available (Table 2.1; see reference materials below). A further limitation is that for most

analyses of interest for biological monitoring, no definitive ["no known source of inaccuracy or ambiguity"] or even reference ["negligible inaccuracy in comparison with imprecision"] (161) method is available, and thus, even if a certified reference material were available, the true concentration cannot be determined with certainty.

(c) Consensus of participating laboratories

Consensus of participating laboratories, mostly as a robust mean or mode (i.e., a value derived by exclusion of outliers), is probably the most widely used approach to arrive at a target value in external quality assessment for biological monitoring analyses. For several analyses, however, the accuracy of this approach is quite questionable because of the limited number of participating laboratories. (For example, in the FIOH quality assurance scheme for organic solvent metabolites, the number of laboratories reporting results on urinary hexanedione analysis has constantly been less than 10.)

(d) Consensus of expert laboratories

Consensus of expert laboratories has been used in the German external quality assurance scheme. In few countries can this approach be effectively used, because the number of laboratories doing biological monitoring analyses for chemicals other than, say, lead, cadmium and mercury, and of course even more of "expert" laboratories, is limited. International collaboration is the only viable way to make use of either "consensus" approach.

2.3.2.5 Reporting of results and analysis of performance

The participating laboratories should receive the analysis of their own performance, in comparison to that of the others, within as short a delay as possible. If the delay becomes too long, the result may not have the improving effect on laboratory performance; also, long delays decrease the interest of the participants in the scheme. The report should include the results entered for the laboratory in question (for checking purposes), the target value and the way it was achieved, and the distribution of the results from all participating laboratories, also by analytical method, if feasible. Both a graphical presentation, and exact results obtained, should be reported. It is very informative not only to report the laboratory performance for the actual round of sample distribution, but also as a cumulative score, as is done in e.g. the UK Trace Element External Quality Assurance Scheme (TEQAS), and National External Quality Assurance Scheme (NEQAS).

For international quality assurance schemes, the co-ordinator can hardly give binding limits of acceptability, but an indication of performance criteria is very useful. This would optimally be derived from the requirements of data interpretation, i.e., needs of occupational health services. An alternative approach is to make the judgement based on attainable analytical performance of the methodology used, or simply on the performance of the laboratories participating in the scheme.

2.3.2.6 Existing quality assessment schemes in biological monitoring

For the analysis of lead in blood, several external quality assurance schemes are/have

been operative (for example, US Public Health Services/CDC, State of New York Department of Health, Commonwealth of Pennsylvania Department of Health, College of American Pathologists, UK EQAS, Centre de Toxicologie du Québec, Puerto Rico Department of Health, Wisconsin State Laboratory of Hygiene, Commission of the European Communities, and the Chinese Academy of Preventive Medicine (162, 163).

A Danish quality assurance scheme that was operative 1987–1992, concluded that the laboratory performance for the analyses of cadmium and chromium in blood was not satisfactory; for lead, the reported values were in good concordance with the expected ones (164).

The German Society of Occupational Medicine has offered inter-comparison programmes for toxic chemicals in blood/serum since 1982; the programme consists of one annual round, and the number of participants is altogether approximately 90 (165, 166). In the rounds in 1991 and 1992, the programme was expanded to include also four chlorinated solvents in blood, six PCB isomers, and six other chlorinated environmental contaminants.

The Centre de Toxicologie du Québec has operated an inter-laboratory comparison programme for blood cadmium and lead, serum aluminium, selenium, copper and zinc, urinary arsenic, fluoride, mercury, cadmium and chromium. For elements that had been in the programme for more than six years, no trend with time was observed in the laboratory performance; however, for chemicals newly added, such as urinary and blood cadmium, there was an improvement with time (167).

In Japan, an industrial health regulation obliges the employers to do biological monitoring for all the workers exposed to lead or toluene, xylene, tricloroethylene, tetrachloroethylene, 1,1,1-trichloroethane, styrene, dimethylformamide or n-hexane. Since 1980, the Japan Federation of Occupational Health Organizations (since 1987, under the supervision of the Quality Control Committee supported by the Ministry of Labour) has conducted an annual quality assurance round among laboratories performing such analyses. In 1991, 36 to 67 laboratories participated in 10 types of such analyses (B-Pb, B-FEP, U-ALA, urinary hippuric acid, urinary methylhippuric acid, urinary total trichlorocompounds, U-TCA, U-mandelic acid, monomethylformamide, and 2,5-hexanedione (168–170).

As a part of the UK External Quality Assessment Scheme, a programme for the quality assurance of lead in blood was started in 1973. At present, some 80 laboratories take part in these analyses, and a further approximately 40 laboratories in the blood cadmium analyses. The programme started by using liquid human blood as the material, but moved to liquid bovine blood in 1992; specimens are sent to participating laboratories every two weeks (171).

The Robens Institute of Health and Safety runs an external quality assurance scheme (TEQAS) for copper, zinc, gold, aluminium and selenium in serum (matrix horse or calf serum), lead and cadmium in blood (human blood), and mercury and cadmium in urine

(human urine). The scheme includes a monthly specimen, and the number of participating laboratories is 20–110 for different elements (172, 173).

The Finnish Institute of Occupational Health has arranged a quality assurance scheme for metabolites of organic solvents in urine. At present, four sets of specimens are sent annually for the analysis of methylhippuric acids, hexanedione, trichloroacetic acid, mandelic acid and phenol (174).

2.3.3 Reference materials

Two different sets of reference materials may be discerned:

Reference material is a material, one or more properties of which are sufficiently well established to be used for the assessment of a measurement method; a certified reference material whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body (140).

Several national and international organizations manufacture, and offer for sale, certified reference materials (CRMs). Some such materials that are of interest in biological monitoring have been listed in Table 2.1. Available biological reference materials for trace elements and organic contaminants were listed in 1990 by International Atomic Energy Agency (IAEA) (175); a general computerized data base (COMAR) on available reference materials for chemical analysis is maintained by the Federal Institute for Materials Research and Testing in Berlin, Germany. It contains data on more than 6,900 certified reference materials, of which approximately 1% are biological. The preparation of a certified reference material is a time consuming, meticulous and expensive endeavour and consequently it is and will not be possible to satisfy the demand for all types and quantities of CRMs. For this purpose, CRMs must be used properly, i.e., effectively, efficiently, economically (140). Certified reference materials may be used to verify the precision and trueness of analytical methods; their use is an important part of the initial assessment of analytical methods (4), but the use of certified reference materials for the day-to-day verification of the precision of an analytical method is not usually cost-effective. Best information from the use of a CRM is obtained, when the CRM has identical matrix, and similar concentration with the samples that will be analysed using the method studied. It is especially important to note that getting results within the specified acceptability limits from a certified reference material at a high concentration level by no means guarantees the accuracy of the method at a lower level. The certified concentration value of a CRM is the best estimate of the true value; this value has an uncertainty that depends on the process by which the certified value was obtained. This uncertainty sets a limit for the accuracy of a method that can be verified by using this CRM.

Non-certified reference materials are also on sale (Table 2.1). It is often difficult to know how well the stated concentration value estimates the true value, and therefore these reference materials are best suited for precision studies; their analysis only gives a rough estimation of the trueness of the analysis.

Table 2.1. Reference materials for biological monitoring

Source, Label	Material	Analyte(s) with certified concentrations	Analytes with Non-certified concentrations
NIST[1] SRM 2670	Freeze-dried urine	Cu, Se,, normal levels	Al, As, Be, Cd, Au, Pb, Mn, Hg, Ni, Pt, normal levels
NIST SRM 2670	Freeze-dried urine	As, Cd, Cr, Cu, Pb, Hg, Se, elevated levels	Al, Be, Au, Mn, Ni, Pt, V, elevated levels
NIST SRM 2671a	Freeze-dried urine	Fluoride, normal & elevated levels	
NIST SRM 2672b	Freeze-dried urine	Mercury, normal & elevated levels	
NIST SRM 955a[2]	Blood	Lead	
NIST 909[3]	Human serum	Cd, Cr, Cu, Pb, V	
BCR[4] CRM 194 (also LGC B1-94)	Lyoph. bovine blood	Cadmium, lead; high normal level	
BCR CRM 195 (also LGC[5] B1-95)	Lyoph. bovine blood	Cadmium, lead; elevated level	
BCR CRM 196 (also LGC B1-96)	Lyoph. bovine blood	Cadmium, lead; grossly elevated level	
IAEA[6]-A13	Lyoph. animal blood	Selenium, Rubidium	Ni, Pb
LGC C91-32	Bovine blood	Cadmium, lead; high-normal level	
LGC C91-33	Bovine blood	Cadmium, lead; elevated level	
LGC 91-34	Bovine blood	Cadmium, lead; grossly elevated level	
LGC91-02	Lyoph. human urine	As,Cu, Se	Be, Cd, Cr, Mn, Ni, Pb
LGC 91-03	Lyoph. human urine	As, Be, Cd, Cr, Cu, Mn, Ni, Pb, Se	
LGC 91-31	Bovine serum	Cu, Se	Al, Co, Mn, Mo
LGC N15-98	Bovine serum	Al, Cd, Co, Cr, Cs, Cu, Mn, Mo, Rb, Se	As, Hg, Ni, Pb, Tl, V
NRCCRM[7];GBW 09102	Lyoph. human urine	As, Cu, Se	Be, Cd, Cr, Mn, Ni, Pb
NRCCRM; GBW 09103	Lyoph. human urine	As, Be, Cd, Cr, Cu, Mn, Ni, Pb, Se	
NRCCRM; GBW 09132	Bovine blood	Cadmium, lead; high-normal level	
NRCCRM; GBW 09133	Bovine blood	Cadmium. lead; elevated level	
NRCCRM; GBW 09134	Bovine blood	Cadmium, lead; grossly elevated level	
NRCCRM; GBW 09131	Bovine serum	Se	Co, Mn, Mo, Al
NRCCRM; GBW 09136	Lyoph.animal blood	Erythrocyte protoporphyrin, normal level	
NRCCRM; GBW 09137	Lyoph. animal blood	Erythrocyte protoporphyrine, elevated level	

Lyphochek 1 & 2 [8]	Lyoph. human urine		Al, As, Cd, Cr, Co, Cu, F, Pb, Mn, Hg, Ni, Sb, Se, Tl, ALA, Hippuric acid, Mandelic acid, Pentachlorophenol, Phenol, Trichloroacetic acid
Kaulson Laboratories, Inc [9]	Lyoph. bovine blood		Pb; low, medium & high levels
Kaulson Laboratories, Inc	Lyoph. human blood		Pb; low, medium & high levels
Kaulson Laboratories, Inc	Lyoph. urine		Pb; low, medium & high levels
Seronorm Trace elements WB [10]	Lyoph. human blood		Al, As, Cd, Co, Cr, Cu, F, Hg, Mn, Ni, Pb, Tl
Seronorm Trace elements S	Lyoph. human serum		Al, As, Cd, Co, Cr, Cu, F, Hg, Mn, Ni, Pb, Tl
Seronorm Trace elements U	Lyoph. human urine		Al, As, Cd, Co, Cr, Cu, F, Hg, Mn, Ni, Pb, Tl
Referensmaterial AB [11]	Lyoph. human blood		Cd, Co, Cr, Mn, Pb; normal + 5 different elevated levels
Trace Laboratories Ltd [12]	Bovine blood		Pb, Cd; different elevated levels

1) Standard Reference Materials Program, Room 204, Building 202, National Institute of Standards and Technology, , Gaithersburg, MD 20899, USA 2) In preparation 3) Not in NIST catalogue 1992-1993; 4) BCR = European Community Measurements and testing, former Community Bureau of Reference, EEC; 5) International Atomic Energy Agency, P.O. Box 100, A-1400 Vienna, Austria; 6) Office of Reference Materials, Laboratory of the Government Chemist, Queens Road, Teddington, Middlesex TW11 0LY, U.K.; 7) NRCCRM = National Research Centre for Certified Reference Materials, Peoples' Republic of China. Available from Promochem GmbH, Postfach 12 46 D-4230 Wesel, Germany; 8) Bio-Rad ECS Division, 1000 Alfred Nobel Drive, Hercules, California 94547, U.S.A.; 9) Kaulson Laboratories, Inc. 687-691 Bloomfield Avenue, West Caldwell, NJ 07006 U.S.A.; 10) Nycomed Diagnostica, P.O. Box 4284 Torshov, 0401 Oslo 4, Norway; 11) Referensmaterial AB, Lobeliavägen 6, S-52333 Ulricehamn, Sweden; 12) Trace Laboratories Ltd, Temple Row House, 269 City Road, Edgbaston, Birmingham B16 0NB, UK.

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Chapter 3. Selected metals

3.1 Cadmium

3.1.1 Introduction

Cadmium is widely dispersed in the environment. In non-occupationally exposed persons, food and tobacco consumption represent the main sources of exposure to cadmium. Cadmium is widely used in industry where workers are exposed to fumes and dust. In human exposure the two main target organs are the kidney and the lung.

A considerable body of knowledge exists with regard to exposure, metabolism and health effects from cadmium exposure and no attempt is made to cover the extensive scientific literature in full. This chapter focuses on the biological monitoring of internal exposure and on biological effect monitoring to assess exposure.

3.1.2 Physical-chemical properties

Cadmium (Cd; CAS 7440-43-9) is a soft, blue white malleable metallic element in Group II B of the Periodic table.

Chemical and physical properties include:

Atomic mass	112.4
Boiling point	765°C
Melting point	320.9°C
Vapour pressure	0.013 kPa at 320.9°C

Solubility: Metallic cadmium is soluble in ammonium nitrate, dilute nitric acid, hot sulphuric acid and insoluble in water. Cadmium sulphate, nitrate and halides are water soluble. Cadmium oxide (CdO) and cadmium carbonate are soluble in dilute acids and ammonia solutions and insoluble in water. The sulphide, selenide, and telluride are coloured (yellow, red, and black) and are insoluble in water.

Further information on physical and chemical properties may be obtained in e.g. CRC Handbook of Chemistry and Physics (1) and in the IARC Monographs, Volume 58 (2).

Conversion factors:
1 μg/L = 8.90 nmol/L; 1 μmol/L = 112 μg/L
1 μg/g creatinine = 1 μmol/mol creatinine

3.1.3 Possible occupational and non-occupational exposures

(a) Occupational exposures

Potential exposure to cadmium and cadmium compounds may occur in a variety of occupational settings. The major sources of occupational exposures are the smelting and refining of zinc, lead and copper ores, electroplating, manufacture of cadmium alloys, pigments and plastic stabilizers, production of nickel-cadmium batteries and welding (2). A list of occupations involving potential exposure to cadmium and cadmium compounds is given in table 3.1.1.

Table 3.1.1. Occupations with potential exposure to cadmium and cadmium compounds

High risk	Moderate/low risk
Alloy makers*	Brazing workers
Battery workers*	Coating workers
Pigment workers*	Diamond workers
Plastic workers*	Electroplaters
Smelters and refiners*	Electrical contact workers
Welders*	Enamelling workers
	Engravers
	Glass workers
	Laser workers
	Metalizers
	Paint workers
	Pesticide workers
	Phosphorus workers
	Printing shop workers
	Semiconductor and superconductor makers
	Sensor makers
	Solar cell makers
	Solder workers
	Textile printers
	Thin film makers
	Transistor makers

* Activities at highest risk because atmospheric concentrations of cadmium can reach high levels and because the number of workers employed is significant (3).

Workers may be exposed to cadmium in these occupations via inhalation of either finely ground particulates (e.g. pigment dusts) or cadmium oxide fumes generated during heating or welding of cadmium-containing materials. Airborne concentrations of cadmium can vary considerably according to the type of industry and the specific work conditions in each plant (4). IARC Monograph Volume 58 (2) summarizes data on exposure to cadmium and cadmium compounds and the results of biological monitoring in different occupational situations.

(b) Non-occupational exposures

People in the general environment are exposed to cadmium via food, drinking water, air,

dust, and smoking. For non-smokers food is the main source of cadmium intake. Gastro-intestinal absorption from food is generally much less efficient than from water or air. Most foodstuffs contain less than 0.08 µg/kg wet weight but considerably higher concentrations may be found in liver and kidneys of adult domestic animals and in certain types of seafood. The elevated cadmium content of rice from Japan is noteworthy, because rice is a major part of the Japanese diet (5).

In most countries the average daily intake of cadmium via food is about 10–25 µg/d (6, 7). In Japan the daily intake of cadmium via food is between 37–44 µg/day (5).

Smoking is a significant source of exposure. It is estimated that a smoker who smokes 20 cigarettes per day has a daily absorbed intake of 2–4 µg and accumulates 0.5 mg cadmium in one year (6–8).

3.1.4 Summary of toxicokinetics

There are a number of reviews on the toxicokinetics of cadmium in man (6–10).

3.1.4.1 Absorption

(a) Inhalation

This is the major route of absorption for occupational exposure to aerosols, dusts and fumes. Pulmonary absorption ranges from 7–10% of inhaled cadmium. The degree of absorption depends upon particle size and solubility of the cadmium compound.

(b) Dermal

Limited percutaneous absorption of cadmium salt in solution has been demonstrated in laboratory animals, but is considered to be negligible for humans.

(c) Gastrointestinal

In some circumstances ingestion of cadmium dust may also be significant. Cadmium may be ingested directly from contaminated hands (mainly when workers eat or smoke at the workplace) or indirectly following the clearance of large particles deposited in the upper respiratory tract.

Cigarette smoking adds to the amount of cadmium deposited in the lung. Furthermore gastrointestinal absorption is the major route for non-occupational exposure. The average normal gastrointestinal absorption in humans ranges from 3–7% of ingested cadmium. Various dietary factors, such as iron, calcium and protein deficiency may increase the gastrointestinal absorption rate to as high as 20% (11, 12).

3.1.4.2 Metabolic pathway and biochemical interaction

Once cadmium has been taken up from the gastrointestinal tract or the lungs it is transported in the blood to the liver, initially bound to albumin and high molecular weight

protein in the plasma. This form of cadmium is rapidly taken up by the liver where the cadmium is liberated from the proteins and induces the synthesis of metallothionein, a low-molecular-weight sulphur-rich protein that binds cadmium quite effectively. This protein sequesters cadmium in the liver cells and thereby acts as a detoxifying agent. A small portion of the metallothionein-bound cadmium re-enters the blood. The cadmium metallothionein complex is distributed to all organs in the body, but particularly in the kidneys. Cadmium metallothionein in blood plasma is effectively filtered through the glomeruli in the kidneys into the primary urine. Subsequently, it is reabsorbed by the tubular cells. In the tubular cells, lysosomes containing proteolytic enzymes rapidly degrade the cadmium metallothionein complex and release cadmium into the cytoplasm. The tubular cells have a certain capacity for metallothionein production of their own, thereby preventing toxic effects from non-metallothionein-bound cadmium. If the metallothionein-producing capacity of the tubular cells is exceeded, the first signs of kidney toxicity will appear (13, 14). The kidney toxicity is due to a competition of Cd with tubular zinc-dependent enzymes involved in the catabolism/reabsorption of low molecular weight proteins.

3.1.4.3 Distribution

Most of the cadmium in circulation in the blood is found in the red blood cells, bound to metallothionein. Between 40–80% of retained cadmium has been estimated to be stored in the liver and kidneys, with one third in the kidneys. The distribution pattern is largely dependent on the time elapsed since uptake, or the duration of long-term exposure. In man, a long time after a single exposure or after exposure lasting several decades, most of the cadmium is in the kidneys, the target organ. The cadmium concentration is highest in the renal cortex. In the kidney, and particularly in the renal cortex, the cadmium level increases with age and is greater in smokers than in non-smokers. When renal dysfunction develops the cadmium level in the kidney decreases owing to increased excretion of cadmium in the urine.

3.1.4.4 Elimination

Cadmium is eliminated from the body very slowly. The main route of elimination in humans is renal, with only limited elimination via the faeces. Only 0.01 to 0.02 % of the body burden is excreted per day in urine or faeces. Renal damage from cadmium results in increased urinary cadmium excretion. Transplacental transfer and secretion into milk are very low.

One of the most important aspects of the metabolism of cadmium in humans is its long biological half-time. Mathematical models have been developed for evaluating the metabolism of cadmium in man. According to a one-compartment model, which considers only the renal cortex, a half-time of 20 years has been calculated (15). A more elaborate model describes the flow of cadmium between eight different tissues (16, 17); this model gives half-times of 8–14 years for the different compartments (7, 18).

3.1.5 Summary of toxic effects

A large number of reviews have been published (7–10, 19–21). Cadmium may cause acute and long-term effects. In man, the principal acute manifestations are gastrointestinal disturbances following ingestion and chemical pneumonitis following inhalation of cadmium oxide. Cadmium fumes when inhaled in sufficient concentrations are toxic to the epithelial and endothelial cells of alveoli and cause acute pulmonary oedema.

In man, the principal toxic effects resulting from long-term exposure to cadmium are renal dysfunction and lung impairment. Long-term inhalation exposure to cadmium dust or fumes in the cadmium industry has been shown to produce emphysematous lung changes (20, 22).

The kidney is considered to be the critical organ following long-term cadmium exposure, the organ where the first signs of adverse effects are seen (18, 23–25). The early stages of interference by cadmium with renal function are usually characterized by an increased excretion of low molecular weight proteins (e.g. β-2 microglobulin, retinol-binding protein (RBP), α_1-microglobulin). As a result of tubular dysfunction in some subjects an increased excretion of high molecular weight proteins (e.g. albumin, transferrin) due to increased glomerular permeability is seen (mixed proteinuria). The latter effects are irreversible (26–29).

Other severe effects of cadmium poisoning are osteomalacia and/or osteoporosis seen in itai-itai patients living in heavily cadmium-polluted areas of Japan and in a number of severe industrial intoxications in Europe (30, 31).

The possible role of occupational cadmium exposure for the development of cancer has been re-evaluated in the IARC monograph, Volume 58 (2, 32). There is sufficient evidence of the carcinogenicity of cadmium and cadmium compounds in humans. In the overall evaluation, cadmium and its compounds have been classified in group 1 – carcinogenic to humans.

3.1.6 Biological monitoring indices

There are several recent reviews on biological monitoring of occupational cadmium exposure (4, 8, 9, 33, 34). The main biological monitoring indices are shown in table 3.1.2.

For the biological monitoring of cadmium exposure at the workplace, cadmium concentrations in blood and urine are the most important parameters (4, 8, 9, 33, 34). A number of studies indicate that the level of cadmium in blood can be considered as an indicator of current exposure, whereas cadmium in urine, in conditions of low-level exposure (as found in the general population) and absence of renal damage, reflects the cadmium body burden (35).

Table 3.1.2. Available biological monitoring indices

Indicator	Suitability for use	Ref.
Cadmium in blood (CdB)	Indicator of recent exposure	4, 8, 9, 33, 34
Cadmium in urine (CdU)	Non-invasive	4, 8, 9, 33, 34, 35
	For relationship between Cd(U) and exposure and body burden – see section 3.1.7.1	
Cadmium in kidney and liver	Direct determination of body burden, non-invasive but instrumentation costly and requires specialized personnel. Not suitable for routine monitoring	36–39
Urinary proteins	Used for effect monitoring	26, 42–47

The varying relationships between blood and urine concentrations during cadmium exposure have led to the recommendation that both blood and urine concentrations are measured and the results considered together. CdU has been shown to increase significantly with age parallel to the accumulation of cadmium in the kidney (40, 41).

Measurements of selected proteins and enzymes in urine can be used to assess the effects of cadmium on kidney function. The earliest indicator of cadmium-induced renal tubular dysfunction is the presence of elevated levels of low-molecular weight proteins (e.g. β_2-microglobulin, retinolbinding protein) in urine (42, 43). β_2-microglobulin (β_2-M) has been widely used as an indicator, but retinolbinding protein (RBP) appears to be a more reliable due to its greater stability (44, 45).

The first phase of Cd-induced tubular dysfunction, as characterized by a limited microproteinuria, e.g. β_2-M and/or RBP > 300 µg/g creatinine, but < 1000–1500 µg/g creatinine can be reversible when the worker is removed from exposure (46, 47). More elevated microproteinuria, however, is irreversible and the forerunner of progressive loss of renal function (26).

Injury to the proximal tubule can be detected by measuring the urinary activity of cytolytic enzymes. Of these, the lysosomal enzyme N-acetyl-β-D-glucosaminidase (NAG) has proved to be one of the most valuable (48).

Table 3.1.3 shows the most important indicator proteins and enzymes for effect monitoring. The three proteins, β_2-microglobulin, retinolbinding protein and α_1-microglobulin are discussed more fully in sections 3.1.9, 3.1.10 and 3.1.11.

Table 3.1.3. Important renal indicator proteins and enzymes (4)

Type	Proteins or enzymes	Molecular weight (kDa)
High molecular weight	Immunoglobulin G	150
	Transferrin	95
	Albumin	67
Low molecular weight	α_1-microglobulin	30
	Retinolbinding protein (RBP)	25
	β_2-microglobulin	12
Enzymes	Alanine-aminopeptidase (AAP)	-
	N-acetyl-β-D-glucosaminidase (NAG)	140–150

3.1.7 Cadmium in urine index

3.1.7.1 Toxicokinetics

Cadmium in urine (CdU) is bound mainly to metallothionein. The elimination half-time in humans, reflecting whole body clearance, has been estimated at 10–30 years. There is a three-phase relationship between CdU and exposure and body burden (49). In the first phase, cadmium accumulates in the renal cortex and is bound to metallothionein, but not all the available binding sites are saturated. During this phase cadmium is excreted in amounts related, in part, to levels in the kidney. Thus, CdU levels reflect integrated past exposure.

In the second phase, exposure results in the saturation of binding sites and an increase in CdU. During this phase CdU reflects both past (body burden) and recent exposure. In the third phase, renal tubular dysfunction has developed which results in increased CdU excretion. It may take more than a year following initial exposure to cadmium for the body burden to be increased sufficiently to result in a rise in the CdU above background levels (16, 48).

3.1.7.2 Biological sampling

(a) Sampling time and specimen

The time of sampling is not critical, given the long half-time of cadmium.

(b) Contamination possibilities

Risk of contamination is a serious problem. Contamination of urine from work clothes is likely and overalls etc. used during work must be removed in a separate room before entering the sample collection room (outside workplace). Showering before urine collection is recommended.

(c) Sampling device and container

Urine specimens (10–50 mL) should be collected in acid-cleaned (nitric acid, supra pure

quality diluted with bidistilled water 1+10) plastic containers (preferably high-density polyethylene containers); do not use plastics with a Cd-softener. Coloured plastic and rubber should be avoided.

(d) Preservatives, shipment and stability

No preservatives have to be added to the sample. Urine samples can be stored for two weeks at 4°C or deep frozen (-20°C) for several months.

3.1.7.3 Recommended analytical method(s)

The most widely used technique for the determination of cadmium is graphite furnace atomic absorption spectrometry (GFAAS). The determination can be performed after chelation and extraction (50); after isolation by anion-exchange chromatography (51); or after dilution and acidification with nitric acid and subsequent direct determination (52, 53). A method based on chelation/extraction is recommended by the German Research Foundation (50).

(a) Principle of the method

Cadmium in the urine is complexed with the chelating agent, N,N-hexamethylene-dithiocarbamic acid, hexamethylene ammonium salt (HMA/HMDC). The cadmium-HMDC complex is extracted in a mixture of organic solvents (diisopropylketone-/xylene) and determined by electrothermal atomic absorption spectrometry (ETAAS). Calibration is carried out with standards of defined cadmium concentrations in aqueous solution containing glutathione (50).

(b) Reagents required

Glutathione solution: 670 mg glutathione is dissolved in ultra pure water in a volumetric flask and made up to 1 L (2.2 mmol/L). The solution is stable for about 14 days.
Extraction solvent: 680 mg HMA/HMDC, (Merck, Darmstadt, Germany, GR-grade) is warmed up gently with 15 mL xylene in a 50 mL volumetric flask until dissolved. After cooling the solution is made up to 50 mL with diisopropylketone.
Standards: Cadmium concentrations between 0.5 and 10 µg/L (4.5–90 nmol/L) in glutathione solution.

(c) Equipment

Atomic absorption spectrometer with background correction and graphite furnace, graphite tubes. Mono-element cadmium hollow cathode lamp or cadmium ED lamp. Centrifuge.

(d) Procedure

For sample treatment 1 mL of the acidified urine (around 1 mL acetic acid (100 mL urine)) is diluted with 4 mL ultra pure water. Then 2 mL extraction solvent is added and the tube stoppered. The tubes are shaken vigorously for at least 5 min on a shaker and then centrifuged for 10 min (minimum 3000 g). An aliquot of the organic phase is in-

jected into the graphite furnace. The calibration standards and the glutathione solution (blank) are treated and analysed as for the urine samples.

Atomic absorption spectrometer
Wave length   228.8 nm
Background correction   Deuterium lamp or Zeeman
Graphite furnace
The following data are intended to serve as a guide for the temperature-time programme to be used with the graphite furnace, an optimization of the programme must be carried out for each individual instrument:

Table 3.1.4. A guide for the temperature-time programme to be used with the graphite furnace

Analytical step	Step duration		Temperature
	Ramp time (Sec)	Hold time (Sec)	(°C)
Drying	25	30	180
Charring	10	30	350
Atomisation	2	8	2200 miniflow (30mL/min)
Heating	1	2	2700
Inert gas:	Argon		
Injected volume:	25 µL		

(e) Criteria of analytical reliability (50)

i) Trueness
Trueness, based on recovery studies, was 106%.

ii) Precision
The within-series precision of the analysis was 3.3–4.0% relative standard deviation. The between-day precision was 10.5–6.8% relative standard deviation.

iii) Detectability
The limit of detection of cadmium in urine was 0.2 µg/L (2 nmol/L).

(f) Quality assurance

Results of intercomparison programmes have shown that there are still considerable problems with the determination of cadmium in urine (54–56). It is important to employ a rigid quality control programme both internally and externally (56–58).

For **internal quality control** commercially available control specimens from e.g. Bio Rad Lab., Anaheim, California, USA – Lyphocheck[R] Urine Metals Control; Nycomed AS, Oslo, Norway, – Seronorm[TM] Trace Elements Urine and certified urine specimens from e.g. National Institute for Standards and Technology (NIST) Gaithersburg, Maryland, USA can be used.

For **external quality control** (inter)national intercomparison programmes are available from e.g. Centre de Toxicologie du Québec, Canada and the German Society of Occupational and Environmental Medicine, Erlangen, Germany.

(g) Source of possible errors

i) Pre-analytical
Contamination of the urine samples by cadmium during sample collection and storage must be avoided.

ii) Analytical
Reagents with purest grade commercially available must be used. Reagent blanks must be measured in each run of analysis.

(h) Reference to the most comprehensive description of the method

Angerer J, Schaller KH. Analyses of Hazardous Substances. In: Biological Materials, Vol 2, VCH-Verlagsgesellschaft Weinheim, Germany, 1988.

(i) Evaluation of the method

This method has been recommended by the German Research Foundation (50).

3.1.7.4 Other analytical methods

For the determination of cadmium in urine many modifications of the GFAAS method exist (51, 53, 59–61). Direct measurement of cadmium in urine by GFAAS is prone to severe matrix and spectral interferences. D'Haese (62) overcame these effects by coating the L'vov platform with ammonium molybdate, reducing the atomization time, introducing a post-atomization cooling step, carefully selecting ashing and atomization temperatures, and using an appropriate procedure for matrix modification. For calibration of direct GSAAF methods, matrix-matched calibration standards must be used.

Other techniques used for the cadmium determination in urine are flame-AAS, flame atomic fluorescence spectrometry (63), differential pulse anodic stripping voltammetry (64, 65) and inductively coupled plasma emission spectrometry (66).

3.1.7.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

Excretion in urine increases with age for persons not occupationally exposed to cadmium. Smokers have higher urinary cadmium excretion compared to non-smokers. People living in cadmium-polluted areas have higher urinary cadmium levels than those living in non-polluted areas (34). Mean urinary cadmium levels measured in several countries in Europe, in the USA and in Japan range from about 0.4 to 6.1 µg/L (3.6–54 nmol/L) (2), but in most countries average urinary cadmium is 0.5–1.0 µg/L (4.5–9 nmol/L) (67).

(b) Published biological action levels

WHO (1980) recommends that the individual urinary cadmium excretion should not be allowed to reach 10 μg/g creatinine (10 μmol/mol creatinine). They recommended that specific control measures be applied as soon as the individual concentrations of cadmium in urine exceed 5 μg/g creatinine (5 μmol/mol creatinine). Consequently, this level should be regarded as a health-based biological limit or biological action level (68).

The American Conference of Governmental Industrial Hygienists (ACGIH) has adopted a biological exposure index (BEI) of 5 μg/g creatinine (5 μmol/mol creatinine) (67). Interpretation of biological monitoring with respect to that BEI, particularly for individual workers, requires a programme of periodic biological, medical and environmental monitoring. (For exposure standards from the U.S. Occupational Safety and Health Administration (OSHA) see also section 3.1.8.5b).

In Germany, exposure equivalents for carcinogenic substances (EKA-values) for cadmium in urine have not been evaluated. Until a Technical Exposure Limit (TRK-value) has been definitely established, the BAT-value valid until 1989 of 15 μg/L (134 nmol/L) for cadmium in urine can be used as a guideline for monitoring occupational exposure.

(c) Non-analytical interference

As discussed above, diet and the environment can be important contributors to urinary cadmium levels.

3.1.8 Cadmium in blood index

3.1.8.1 Toxicokinetics

Lauwerys et al. 1979 (48) observed a linear increase in blood cadmium for up to 120 days, followed by a plateau, in four new employees exposed to high levels of cadmium. The kinetics of cadmium in blood require at least a two-compartment model. Jarup et al. (69) studied five workers for 15 years after cessation of exposure, and using a two-exponential regression model calculated a fast decay half-time of 75–130 days, and a slow decay half-time of 7.4–16 years (11–16 years without renal dysfunction).

3.1.8.2 Sampling

(a) Sampling time and specimen

Most cadmium in blood is bound to the red blood cells and cadmium is usually measured in whole blood. Samples can be taken at any time. Finger pricks should not be performed, due to contamination and analytical problems.

(b) Contamination possibilities

Contamination during blood collection should be avoided by performing the venipuncture outside the workplace. Random analyses of blanks should be carried out to check

the venipuncture equipment and blood collection tubes for contamination.

(c) Sampling device and container

Blood collection should be performed by venipuncture devices using only cadmium-free needles, sampling devices and containers, e.g. Vacutainer[R], Monovettes[R]. K-EDTA has proved to be particularly suitable as an anti-coagulant.

(d) Preservatives, shipment and stability

Experience has shown that a storage period in the refrigerator of up to 3 weeks does not lead to any deterioration in the results of analysis. For longer storage the samples should be kept at a temperature of -20°C.

3.1.8.3 Recommended analytical method

Graphite furnace atomic absorption spectrometry (GFAAS) is the method of choice for the determination of cadmium in whole blood.

The most commonly used method is based on the work of Stoeppler and Brandt (70) and published more comprehensively by the Karolinska Institute, Stockholm, Sweden (71) and the German Research Foundation (72). (For review see also 53).

(a) Principle of the method

The cadmium content of whole blood is determined by electrothermal atomic absorption spectrometry. After adding of Triton[R]-X-100, the sample is deproteinized with 1 mol/L nitric acid. The solid constituents are removed by centrifugation and the cadmium concentration is determined from the supernatant. The standard addition procedure is used for the quantitative determination (70, 72).

(b) Reagents

Chemicals for the determination must be of supra pure or comparable quality.
Nitric acid, 1 mol/L
Triton[R] X 100, 2.5% aqueous
Calibration standards: 0.5, 1.0, 2.0 µg Cd/L in nitric acid, 1 mol/L (5, 10, 20 nmol Cd/L nitric acid).

(c) Equipment

Atomic absorption spectrometer with background correction.
Graphite furnace.
Mono element cadmium hollow cathode lamp or a cadmium ED lamp. (Minimum 2500 g).
Shaker; e.g. Vortex
Centrifuge

(d) Procedures

A 0.5 mL sample of whole blood is pipetted into each of four plastic centrifuge tubes and mixed with 100 µL of a 2.5% Triton-X-100 solution. The mixtures are then agitated on a shaker for 30 seconds and 1.5 mL nitric acid (1 mol/L) or 1.5 mL of one of the three indicated standards is pipetted into each of the four tubes using an adjustable pipette. The addition of the three standard solutions gives a set of samples with different known levels of added cadmium. During addition of the HNO_3 the sample is mixed for approximately 15–30 seconds on the shaker for protein precipitation. After precipitation, centrifugation is carried out for 15 min (minimum 2500 g). The supernatant is then carefully removed and transferred to four new plastic disposable tubes. In each series of tests at least one reagent blank is included, using double-distilled water instead of blood and spiked three times.

The proposed time-temperature programmes are intended to serve only as a guide.

Atomic absorption spectrometer
Wave length   228.8 nm
Background correction   Deuterium lamp or Zeeman
Graphite furnace

Table 3.1.5. Proposed time-temperature programmes

Analytical step	Step duration (Sec)	Temperature (°C)
Drying	20	130
Sliding thermal charring	16	280*
Postcharring	60	280*
Atomization with miniflow	4	2100
Heating	4	2500
Cooling	30	room temperature
Inert gas	Argon	
Injected volume	20µL	

(e) Criteria of analytical reliability

i) Trueness
Trueness based on recovery studies is similar to values found for urine measurements.

ii) Precision
The within-series precision was 5.5–12.3% relative standard deviation at 0.65–5.5 µg Cd/L (5.8–49 nmol/L).

iii) Detectability
The limit of detection of cadmium in blood was 0.2 µg/L (2 nmol/L).

(f) Quality assurance

Results from intercomparison programmes have shown that there are still considerable

problems with the determination of cadmium in blood (55, 56, 73, 74).

For **internal quality control** commercially available control specimens from e.g. Nycomed AS, Oslo, Norway – Seronorm™ Trace Elements Whole Blood and certified blood specimens from e.g. National Institute for Standards and Technology (NIST), Gaithersburg, Maryland, USA; Community Bureau of Reference (BCR), EC, Brussels, Belgium can be used.

For **external quality control** (inter)national intercomparison programmes are available from e.g. Centre de Toxicologie du Québec, Canada and the German Society of Occupational and Environmental Medicine, Erlangen, Germany (54, 55), the UK Health and Safety Executive Programme (UKEQAS), Birmingham, UK and the Danish external quality assessment scheme (DEQAS), National Institute of Occupational Health, Copenhagen, Denmark (73).

(g) Sources of possible errors

i) Pre-analytical
Special care must be taken to avoid contamination. Cleaning procedures of containers and other labware must be strictly followed.

ii) Analytical
The deproteinization step is a serious problem in the analysis of blood as the difficulties of performing reproducible deproteinization particularly affect the precision and accuracy of the method. Therefore, the deproteinization procedure should be standardized as much as possible and the analyses should be performed by skilled laboratory staff.

(h) References to the most comprehensive description of the method

Vahter M. Assessment of Human Exposure to Lead and Cadmium through Biological Monitoring. Swedish National Institute of Environmental Medicine and Karolinska Institute, Stockholm, Sweden, and Division of Environmental Health, World Health Organization, Geneva, 1982.

Angerer J, Schaller KH. Analyses of Hazardous Substances in Biological Materials, Vol 1, VCH Verlagsgesellechaft, Weinheim, Germany, 1985.

(i) Evaluation of the method

The present method has been compared with several independent analytical procedures. Parallel analysis of 66 blood samples by an inverse voltammetric method gave a good agreement (r = 0.988; y = 1.03x-0.03) (70). This is also the case for the comparison with other atomic spectrometric methods (72).

3.1.8.4 Other analytical methods

Direct measurement of cadmium in blood by GFAAS with background correction is prone to severe matrix and spectral interferences. To overcome these effects an opti-

mized ashing and atomization programme and an appropriate procedure for matrix modification is necessary (see section 3.1.7.4) (62, 75).

Other methods used in occupational health and environmental studies to determine cadmium in whole blood include anodic stripping voltammetry (ASV), ICP-MS, neutron activation analysis and emission spectrometry. But most of these techniques require extensive sample pre-treatment (e.g. digestion) which introduces the risk of contamination or losses, are cumbersome and require highly skilled laboratory staff. Recently, Ostapczuk (76) published a method using potentiometric stripping analysis (PSA) for the determination of cadmium in blood. In contrast to ASV this technique is not subject to background interferences from organic electroactive constituents. Therefore, it is sufficient to dilute the blood sample with an appropriate supporting electrolyte (0.5 mol/L HCl). For 1 mL of blood and a 1-min deposition time, the detection limit is 1 μg/L (9 nmol/L) for a deposition time of 10 minutes the detection limit is improved to 0.1 μg/L (1 nmol/L).

3.1.8.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

Alessio et al. (77) reviewed papers published in the international literature between 1976 and 1991 on reference values for cadmium in blood of persons not occupationally exposed. Four high-quality studies were subsequently considered (78–81). The results of the evaluation of the data applying two different statistical methods are presented in table 3.1.6.

Table 3.1.6. Blood cadmium concentrations in μg/L (1 μg/L = 8.9 nmol/L) for persons not occupationally exposed (77)

Group	Statistical method Brune et al. (1991) (82)	Statistical method Alessio et al. (1992) (77)
Non-smokers (n=1502)		
GM	0.62	0.56
GSD	1.74	1.75
75th percentile	1.79	0.84
90th percentile	2.85	1.19
Smokers (n=785)		
GM	1.58	1.50
GSD	1.99	1.97
75th percentile	2.89	2.47
90th percentile	5.64	3.78

GM: geometric mean, GSD: geometric standard deviation

To the present day, cadmium exposure of general populations in Japan is still higher than in other countries. Ikeda (5) reported about cadmium levels in blood from 2,000 subjects living in 49 non-polluted areas in Japan. The geometric mean for cadmium in blood was 3.2 μg cadmium/L (28 nmol/L) in men, 3.7 μg cadmium/L (33 nmol/L) in women.

Blood cadmium concentrations increase with age, cigarette smoking and intake from environmental pollution. The influence of sex and age on blood cadmium appear to be overshadowed by the impact of smoking, which causes a dose-related increase in blood cadmium levels with increasing number of cigarettes smoked per day (71).

The contribution of tobacco smoking to cadmium concentrations in the blood of workers with occupational exposure to cadmium is, therefore, significant, particularly at low levels of exposure. Cadmium in the blood reflects recent exposure (over months) more than it does long-term exposure. According to the kinetic model proposed by Kjellström and Nordberg (16), a fraction of blood cadmium is related to body burden, with the remainder determined by recent exposure. Knowledge of past blood cadmium levels, past exposure and current environmental monitoring are needed to accurately interpret an elevated level of cadmium in blood (83–85).

(b) Published biological action levels

WHO (1980) proposed 10 µg cadmium/L (89 nmol/L) for cadmium in blood as the individual critical level if the exposure is regular and long-term. They recommended that control measures be applied as soon as the individual level of cadmium in blood exceeds 5 µg/L (45 nmol/L). Consequently, this level should be regarded as a health-based biological action level (68).

The American Conference of Governmental Industrial Hygienists (ACGIH) has adopted a biological exposure index (BEI) of 5 µg/L (45 nmol/L) for cadmium in blood (67). Interpretation of biological monitoring with regard to that BEI, particularly for individual workers, requires a programme of periodic biological, medical and environmental monitoring.

The Occupational Safety & Health Administration (OSHA) of the USA has published a comprehensive worker exposure standard requiring biological monitoring at least annually for blood cadmium, urinary cadmium, and urinary β_2-microglobulin. If a worker's blood cadmium exceeds 5 µg/L (45 nmol/L), urinary cadmium exceeds 3 µg/g creatinine (3 µmol/mol creatinine), and urinary β_2-microglobulin exceeds 300 µg/g creatinine, the employer is required to seek and correct the cause of the elevated exposure and increase the frequency of biological monitoring to every half year. Medical removal is required if a worker's blood cadmium exceeds 15 µg/L (133 nmol/L) or urinary cadmium exceeds 15 µg/g creatinine (15 µmol/mol creatinine). There are additional intermediate decision points. (Code of Federal Regulations, Title 29, Part 1910.1027 Cadmium, US Government Printing Office, Washington, DC 20402-9328.)

In Germany exposure equivalents for carcinogenic substances (EKA-values) for cadmium in blood have not been evaluated. Until a Technical Exposure Limit (TRK-value) has been definitely established, the BAT-value (valid until 1989) of 15 µg/L (133 nmol/L) can be used as a guideline for monitoring occupational exposure.

3.1.8.6 Research needs

Though much progress has been achieved in recent years a few topics need further investigation to ensure a reliable approach to the problem of biological monitoring, namely: standardization of analytical techniques for both internal dose and effect indicators; provision of internal and external quality control measures for both internal dose and effect parameters; examination of the relationship between metallothionein-bound and "free" cadmium in the kidney and the occurrence of kidney damage.

To establish appropriate occupational exposure limits it is important that further studies are carried out to determine what are the critical effect markers (NAG excretion, microproteinuria) and to define their dose-response curves at low levels of exposure.

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3.1.9 β_2-microglobulin in urine

3.1.9.1 Toxicokinetics

β_2-microglobulin (β_2-m) is a small protein (Mr 11.8 k D) which was discovered in 1968 by Berggård and Bearn (1) in urine from patients with tubular dysfunction. It is synthesized by virtually all nucleated cells and is present on their membrane as the light chain of the class I histocompatibility antigens. As a result of the metabolism and degradation of HLA, β_2-m is dissociated from the heavy chain and appears in its free form in extracellular fluids including plasma. Turn-over studies in humans have shown that the production of β_2-m in normal subjects is 150 to 200 mg daily. Owing to its small size, the protein is rapidly eliminated from plasma by glomerular filtration (half-time = 2.1 hours) and serum concentrations are only 1–2 mg/L in healthy subjects. As a corollary, there is an inverse relation between the serum concentration of β_2-m and the glomerular filtration rate, which explains why serum β_2-m progressively rises with age. Serum β_2-m may also increase in several lymphoproliferative disorders, such as acquired immunodeficiency syndrome, systemic inflammatory disorders or malignancies (2).

More than 99.9% of β_2-m filtering through the glomeruli is reabsorbed by the proximal tubules, then catabolized in the lysosomal system. The amount of β_2-m excreted in urine is consequently very low, averaging 70–80 µg/g creatinine (8–9 mg/mmol creatinine) in healthy subjects (3, 4). It is important to point out that the tubular reabsorption of proteins involves an adsorptive endocytotic process which is saturable. For β_2-m it is estimated that saturation is reached when the serum level exceeds 5 mg/L (5). Above this threshold the urinary excretion of β_2-m is invariably increased and no longer reliably reflects the integrity of the proximal tubules. A rise of the serum level above this threshold may be encountered in renal insufficiency and in several non-renal disorders as indicated above.

3.1.9.2 Biological sampling

The usefulness of the urinary β_2m assay for the early detection of tubular injury has been extensively documented in a number of studies carried out on populations exposed to occupational or environmental toxins, especially cadmium, and on patients treated with nephrotoxic drugs (6, 7). The determination of urinary β_2-m has been used in occupational medicine for more than two decades for the diagnosis of cadmium nephropathy. The sensitivity of β_2-m assay compared to that of other tests (e.g. total proteinuria) stems from the fact that under normal conditions tubular reabsorption of β_2-m is nearly complete. The β_2-m test is greatly limited, however, because of the instability of β_2-m in acid urine. When the urinary pH falls below 5.6, a time- and pH-dependent degradation of β_2-m occurs due to the activity of acid proteases (3, 8–11). The degradation is very rapid at 37°C, so it may already occur in the bladder. Even neutralization of the pH after urine collection is not a fully satisfactory solution to the problem. The degradation of β_2-m is enhanced in pathological urines and may start from higher pH (from 6.0) because in that situation tubular impairment is associated with an increased leakage of proteolytic enzymes from damaged tubular cells.

(a) Sampling time and specimen

The ideal procedure to collect always reliable urine samples is to administer sodium bicarbonate to the subject in order to achieve a urinary pH > 6. In practice, the administration of sodium bicarbonate is rarely feasible. The β_2-m test must then be applied on a spot urine sample (mid-stream ideally) which is immediately neutralized after collection. If possible, the pH of the sample should be measured before adding the buffer. First morning samples should be avoided because of the high probability of β_2-m being degraded during the night. Since the urinary excretion rate of β_2-m is to some extent diuresis-dependent (12), it is advisable also to avoid extreme variations in the urinary flow. This is especially justified when the results are corrected for creatinine concentration which, at extreme values (e.g. <0.3 and >3 g/L [<2.65 and >26.5 mol/L]), is no longer linearly related to the diuresis.

(b) Contamination possibilities

It should be noted that β_2-m can also be degraded by elastase-like proteases in neutral or slightly alkaline urines infected by bacteria. The presence of urinary tract infection or pyuria thus also requires consideration of the possibility of a loss of β_2-m (13).

(c) Sampling device and container

A convenient procedure is to transfer an aliquot of the urine into a tube containing 10% (v/v) of 1 mol/L phosphate buffer pH 7 supplemented with 1% NaN_3.

(d) Preservative, shipment and stability

An anti-bacterial agent must always be added to the urine. It can be added to the buffer used for neutralizing the urinary pH immediately after collection or, in the case of a 24-hour urine collection, it must be added to the container (e.g. 10 mL of a 10 % NaN_3 solution in a two-litre bottle).

It is always preferable to ship the samples on dry ice. However, if the urine is buffered at a pH >6 and contains a preservative (e.g. 0.1 % NaN_3), a shipment at ambient temperature should not significantly affect the stability of β_2-m.

In urine with pH > 6 and containing a preservative, β_2-m is stable for several days at room temperature and at least two weeks at 4°C. At -20°C, the protein is stable for several years. In practice, if the assay cannot be performed within 2 or 3 weeks after collection, samples should be stored in the freezer. Freezing/thawing cycles, however, should be reduced to a minimum.

3.1.9.3 Recommended analytical methods

A number of methods have been described for measuring β_2-m in human biological fluids. However, a quantitative determination of the protein in normal urine can be performed only by using assays with a detection limit below 20 µg/L, such as radioimmunoassay (RIA) (14–16), enzyme immunoassay (EIA) (17–21), latex immunoassay (LIA) (22–26) or related techniques (27). Test packs based on RIA or related assays (e.g.

Kabi-Pharmacia, Sweden; Diagnostic Products Corporation, Belgium) are commercially available. The oldest one is the Phadebas^U β_2-microtest from Kabi-Pharmacia. This test has been used for more than two decades for screening of tubular proteinuria in occupational or environmental medicine. For those laboratories not equipped with a gamma-counter or not licensed to use radioisotopes, the EIA or Immulite test^u (e.g. from Diagnostic Products Corporation, Grimbergen, Belgium) are possible alternatives presenting the advantages of a longer stability of reagents and of photometric measurement.

The immunoprecipitation methods developed for the assay of β_2-m in serum (radial immunodiffusion, nephelometry, turbidimetry) (28–30) generally have limits of detection above the normal range of urinary β_2-m and are therefore not suited to the early detection of tubular damage. But an equally simple and cheap test is the turbidimetric (or photometric) version of the LIA method developed by Bernard et al. (23, 25). This method relies on the agglutination by β_2-m of latex particles on which a specific antibody has been adsorbed; after a period of incubation, the resulting agglutination is measured by turbidimetry. This method uses commercially available reagents with a very low cost and, for the measurement, a standard photometer. The adsorption of the antibody on to latex particles as described recently (31) is very simple and can be completed in less than 15 min. A turbidimetric LIA method is described below. This immunoassay can accurately and precisely quantify a wide range of proteins in plasma or urine including β_2-microglobulin, retinol-binding protein, alpha$_1$-microglobulin or albumin.

(a) Principle of the method

The method consists of incubating solutions containing the antigen (β_2-m or any other protein) with latex particles on which a specific antibody has been adsorbed. The antigen-antibody reaction results in an agglutination of the latex particles proportional to the concentration of the antigen. The remaining unagglutinated latex particles are then quantified by measuring the absorbance with a spectrophotometer at 360 nm.

(b) Reagents required

Polystyrene latex particles 0.845 (+ 0.0104 standard deviation) μm in diameter (ESTA-POR K080, Rhone-Poulenc Industries, Aubervilliers, France). These particles are supplied as an aqueous suspension at the concentration of 100 g/L and are used directly without washing.

Antibodies: The immunoassay uses immunoglobulin fractions of rabbit antisera. Excellent results have been obtained with the anti-β_2-m antibody from Dakopatts (Glostrup, Denmark).

Standards: The assay can be calibrated with standards of purified β_2-m or with urines with known concentrations of the protein. Stock solutions of standards are ideally prepared in a phosphate-buffered saline, pH 7.4 containing 0.1% bovine serum albumin and stored in aliquots at -18°C.

Buffers: The buffer used for diluting the standards and urine samples consists of a gly-

cine-buffered saline (GBS) containing per liter 0.1 mol of glycine, 0.17 mol of NaCl, 7.6 mmol of NaN_3 and 1 g of bovine serum albumin (BSA) and adjusted to pH 9 with NaOH.

Preparation of the latex reagent: The procedure described in the IUPAC method (26) for the adsorption of antibody on the latex particles requires a 60-min incubation followed by three centrifugations of 10 min at 25,000 g. This yields a reagent that can be stored for some months at 4°C and is stabilized with BSA immediately before the assay.

A very sensitive and stable reagent can also be prepared using the same reagents but in less than 15 min by using the following procedure in which coating of particles with antibody and their stabilization with BSA are combined. The antibody solution (e.g. from Dakopatts) is dispersed in 1 mL of a glycine-NaOH buffer containing per liter 10 mmol/L of glycine and adjusted at pH 9. Then 50 μL of the latex suspension (ESTAPOR 10%) are added. After 10 min incubation, the latex suspension is dispersed in 10 mL of distilled water containing 30 mg of bovine serum albumin (the best stabilization is achieved with the fatty acid-free bovine serum albumin from Calbiochem, San Diego, CA, USA, No. 126609). This preparation is then sonicated for 30 sec before the addition of 1 mL of a glycine-NaOH buffer, 1 mol/L, pH 10.1 (kept at 4°C). (If an ultrasonifier is not available, a vigorous vortex-mixing for 2–3 min can give the same result). This preparation which is sufficient for 100 assays is stable for one day at room temperature. It can also be be stored for a few days at 4°C but in this case it has to be resonicated for 10 sec (or vortex-mixed for 2–3 min) before use. The amount of antibody adsorbed onto the latex particles is a very critical parameter for both the sensitivity of the assay and the stability of the particles. It must be optimized for each new batch of antibody or of latex particles. The optimal antibody loading is the minimal amount required to obtain an agglutination curve with an adequate sensitivity. Above this optimum, further increasing the antibody loading does not enhance the sensitivity but destabilizes the particles. With the Dakopatts antibodies the optimal volume of antibodies to be adsorbed onto the particles (50 μl of a 10% ESTAPOR suspension) in the above-described procedure varies usually between 5 and 20 μL depending on the antigen-antibody system.

(c) Equipment required

Spectrophotometer. Ultrasonicator, or if not available, a vortex mixer.

(d) Procedure and calibration

Standards and unknown samples are diluted in the GBS-BSA buffer. It is recommended that the dilutions are made on the day of the assay, or no more than 24 hours before, to avoid changes in the pH of the GBS-BSA buffer. It is important that the same batch of buffer is used throughout the assay. The working range varies with the type of protein measured, the agglutinability of the particles (which depends on the affinity and the amount of adsorbed antibodies) and the method for quantifying the agglutination. For β_2-m it is usually between 1 and 128 μg/L. Urine samples must always be analysed at two different dilutions (to detect a possible postzone effect). Only clear urine samples

can be analysed directly. Turbid specimens must be centrifuged at 1500–2000 g before determination.

The assay procedure consists of incubating aliquots of 50 µL of standards or diluted urine samples with 50 µL of the latex reagent in polystyrene tubes which are then incubated at 37°C for exactly 30 min. Two tubes containing 50 µL of GBS-BSA buffer (blanks) must be regularly spaced in each series. Incubation is stopped by gently adding 2 mL of a GBS buffer adjusted to pH 10 (or a 0.27 mol/L NaCl solution containing 0.1% v/v Tween 20). The content of the tubes is very gently homogenized and poured into a cuvette with a light path of at least 1 cm. The absorbance is measured at 360 nm against the stop buffer. Plotting of the delta Optical Density (blank minus assay) versus the log of standard concentrations yields a sigmoidal-shaped curve with a nearly linear section extending over 5 or 6 points.

(e) Criteria for analytical reliability

The immunoassay has sufficient accuracy and precision to quantify β_2-m at the wide range of levels encountered.

(f) Quality control

Control samples consisting of pooled normal urines should be run with each series of samples to ensure consistent results. These control samples should be kept under the same conditions as the standards.

(g) Sources of possible errors

i) Pre-analytical
The most important source of error arises from the degradation of β_2-m in acidic urine. This has been discussed in detail above.

ii) Analytical
For a successful application of the assay, it is essential to use the recommended reagents and to work under the optimal conditions of the assay (e.g. stability of the latex particles, antibody loading, etc.)

(h) Reference to the most comprehensive description of the method

Herber RFM, Bernard A, Schaller KH. Standard method for the determination of β_2-microglobulin, retinol-binding protein and albumin in urine. Pure and Applied Chemistry, 1994.

(i) Evaluation of the method

The method has been proposed as a standard IUPAC method for the assay of β_2-microglobulin, retinal binding protein and albumin.

3.1.9.4 Other analytical methods

A number of other immunoassays are available (17–21, 22–27), including radioimmu-noassays (14–16), and these have been discussed briefly in section 3.1.9.3 above.

3.1.9.5 Guide to interpretation

Before interpreting any result of β_2-m determination in urine, it is important to ascertain that the protein has not been degraded by acid proteases or as a result of bacterial infec-tion. A β_2-m concentration below 20 µg/L in a normodiuretic urine sample is highly suggestive of a degradation of β_2-m and should be regarded as doubtful.

(a) Measured values in groups without occupational exposure

Abnormally elevated values of urinary proteins are usually defined as those higher than the 95th percentile or the geometric mean plus two geometric standard deviations (the concentration of β_2-m in urine shows a log-normal distribution of the values found in a group of healthy subjects matched for age and sex. Upper reference values of urinary β_2-m reported in the literature for healthy male subjects aged 20 to 60 years usually range from 200 to 300 µg creatinine (23–34 mg/mmol creatinine). In the urine of female subjects, these upper limits of normal must be increased by 15–20% due to the lower excretion of creatinine. These values correspond to a 24-hour urinary excretion of 300 to 450 µg. A urinary excretion of β_2-m exceeding 300 µg/g creatinine (34 mg/mmol creatinine) can be interpreted as an undisputable sign of a decreased tubular reabsorptive capacity provided the subject is not suffering from a lymphoproliferative disorder and has a normal or slightly impaired glomerular filtration rate (GFR>30 ml/min). Since the fractional excretion of β_2-m is about 0.03%, the % loss of the tubular reabsorptive ca-pacity can be roughly estimated by dividing the relative increase in the urinary output of β_2-m by 30.

(b) Published biological action levels

There are no published biological action levels.

(c) Sampling representative of recent or long-term exposure or biochemical effect

When applying sensitive markers of nephrotoxicity for screening purpose, it must be pointed out that in contrast to functional tests they do not have an en *officio* prognostic value. In some situations an increased β_2-microglobulinuria may merely signal bio-chemical, physiological or other changes, transient or not, that do not necessarily imply a loss of renal function (inhibition of β_2-m reabsorption by some drugs, febrile pro-teinuria, overflow proteinuria, etc.). By contrast, elevations of urinary β_2-m which are repeatedly observed at several months or years interval may have a less favourable prognosis for they may indicate the presence of permanent renal injury which may lead to irreversible degenerative changes. The health significance of an elevated value of β_2-m in urine must be established in each situation on the basis of prospective studies on populations at risk as this has been done in cadmium workers. Studies conducted in Belgium and Sweden (7) have indeed shown that the rise of β_2-microglobulinuria in-

duced by cadmium is irreversible and predictive of an acceleration of the age-related decline in the renal function. However, abnormally elevated values of β_2-m in urine may vary from a few hundreds up to more than 100,000 µg/g creatinine (11,300 mg/mmol creatinine). Observations made in Japan (32) suggest that the persistence of cadmium-induced β_2-microglobulinuria depends on the duration and/or intensity of cadmium exposure, as well as on the severity of tubular impairment. According to some authors (32), elevations of β_2-microglobulinuria would be irreversible only above a certain threshold estimated at about 1,000 µg/g creatinine (113 mg/mmol creatinine).

3.1.9.6 Research needs

Further studies are needed to assess the health significance of a slightly increased β_2-m excretion (between 300 and 1,000 µg/g creatinine [34 and 113 µg/mmol creatinine]).

There is some evidence (33) that the assay of anionic microproteins present in very low concentrations in serum (and hence in tubular fluid) (e.g. Clara cell protein) allows detection of very subtle defects in the tubular reabsorption of proteins that pass completely unseen when screening is based on the assay of β_2-m, retinol-binding protein or alpha$_1$-microglobulin. Research is needed to confirm these observations.

3.1.9.7 References

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3.1.10 Retinol-binding protein in urine

3.1.10.1 Toxicokinetics

Retinol-binding protein (RBP) is 22.2 kD microprotein first isolated from plasma by Kanai et al. (1) in 1968 and from urine by Peterson and Berggård (2) in 1971. The protein is electrophoretically heterogeneous with four pI values between 4.4 and 4.8. It belongs to the lipocalin protein superfamily, consisting mostly of lipophilic carrier proteins. The function of RBP is to transport vitamin A in the form of retinal from its storage site in the liver to epithelial tissues. This transport can be summarized as follows. RBP is synthesized in the endoplasmic reticulum of parenchymal liver cells where it binds one molecule of retinal. RBP is then secreted in plasma as a non-covalent complex 1:1 with transthyretin (formely called prealbumin). The size of this bimolecular complex is sufficient to prevent its elimination in urine. Upon recognition by a cell surface receptor in target tissues, RBP undergoes a conformational change abrogating its affinity for transthyretin. The free RBP is then rapidly eliminated from plasma by glomerular filtration and catabolized by proximal tubule cells. The concentrations of total RBP in serum fluctuate in a narrow range around 45 mg/L. Approximately 10–15% of RBP in plasma is not bound to prealbumin. This fraction increases as the glomerular filtration rate (GFR) declines to reach 80% or more in patients with end-stage renal diseases. The major part of urinary RBP is free even in patients with massive proteinuria (3–5).

The renal handling of RBP is very similar to that of β_2-microglobulin (β_2-m). Both proteins are reabsorbed by proximal tubule cells with an efficiency of about 99.97% and their reabsorption is saturated from about the same degree of renal insufficiency (GFR reduced about by 70%) (5). A common mechanism is thus presumably responsible for the tubular transport of β_2-m and free RBP, a conclusion supported by the fact that both proteins compete for their uptake by rat kidney. This probably explains why β_2-m and RBP can detect tubular dysfunction with equal sensitivity and specificity (5–8). Concentrations of RBP in urine are very similar to that of β_2-m averaging 50–70 µg/g creatinine (6–8 mg/mmol creatinine). The protein markedly increases in the urine of patients with tubular dysfunction and can reach values higher than 100,000 µg/g creatinine (11,300 mg/mmol creatinine) (8–11, 12).

In practice, however, the assay of urinary RBP proves more advantageous than that of

β_2-m for two reasons. First, contrary to β_2-m, RBP is stable in acid urine and requires no special precautions for the collection of the urine sample. The pH and temperature-dependent hydrolysis of RBP is observed only below a pH of 5, i.e. outside the biological range. This higher stability of RBP, demonstrated for the first time in the early 1980s (6–8), has been corroborated by a number of investigators and is now a generally accepted finding (10, 13, 14). Second, renal insufficiency is practically the only clinical situation susceptible to increase the serum level of free RBP above the saturation threshold of renal uptake.

3.1.10.2 Biological sampling

(a) Sampling time and specimen

Sampling time is not critical for the assay of urinary RBP. RBP can be determined on a 24-hour urine sample or on a spot (ideally mid-stream) urine sample. As for other low molecular weight proteins, the urinary excretion rate of RBP may be to some extent influenced by the diuresis. It is therefore advisable to avoid extreme variations in the urinary flow. This is especially true when the results are corrected for the urinary concentration of creatinine which at extreme values (e.g. < 0.3 and > 3 g/L [< 2.65 mmol/L, > 26.5 mmol/L]) is no longer linearly related to the diuresis.

(b) Preservative, shipment and stability

A preservative must be added to the urine (e.g. NaN_3 0.1%). If possible, the samples should be shipped on dry ice. However, shipment at room temperature should not significantly affect the stability of RBP provided the urine contains a preservative.

RBP is stable in urine for several days at room temperature and at least for two weeks at 4°C. At -20°C, the protein is stable for several years. In practice, if the analysis cannot be performed within 2 or 3 weeks after collection, it is preferable to store the samples frozen. Repeated freezing/thawing cycles should be avoided.

3.1.10.3 Recommended analytical methods

A quantitative determination of RBP in normal urine is only feasible with immunoassays with a detection limit below 10 µg/L such as latex immunoassay (9, 10), radioimmunoassay (11) or enzyme immunoassay (12, 15). Classical immunoprecipitation methods, such as radial immunodiffusion, turbidimetry or nephelometry usually lack sensitivity for an accurate quantitation of RBP in normal urine and in addition have a relatively high antibody consumption (16, 17). Some authors have succeeded in decreasing the detection limit of an immunonephelometric method (Behring laser nephelometer) to 30 µg/L but relative standard deviations exceed 25% for a RBP concentration of 90 µg/L (18).

Latex particle enhanced turbidimetric immunoassays are certainly better alternatives since they can reach a sensitivity and a precision comparable to the best RIAs or EIAs while presenting the advantages of speed, simplicity and low reagent cost. The turbidimetric version of the LIA developed in 1981 for RBP (9) and proposed as a standard

method by IUPAC (19) is particularly simple and has remarkably low reagent and equipment requirements (see sections 3.1.9.3a–f on β_2-microglobulin for a detailed description of the method).

All reagents (polyclonal antisera and standards) required for developing an immunoassay of RBP are commercially available. It is important to note, however, that free RBP and transthyretin-bound RBP may present different immunoreactivities in several immunoassays (9, 12). If these immunoassays are used to measure urinary RBP they must be calibrated with standards based on free RBP isolated from urine.

Reference to the most comprehensive description of the method

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3.1.10.4 Other analytical methods

The PETUNIA (20) (particle-enhanced turbidimetric assay) method offers similar performances to the turbidimetric LIA but with the advantage that it can be fully automated on a centrifugal analyser. In the PETUNIA method, however, antibodies must be covalently bound to the latex particles and not simply physically adsorbed as in LIA.

3.1.10.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

The reference limits for urinary RBP reported in the literature for healthy male subjects are close to that of β_2-m and also vary between 200 and 300 µg/g creatinine (23–34 mg/mmol creatinine). In the urine of female subjects, these upper reference limits must be increased by 15–20% to account for the lower excretion of creatinine. These values correspond to a 24-hour urinary excretion of 300 to 450 µg.

If the glomerular filtration rate is normal or only slightly impaired, a urinary excretion of RBP exceeding 300 µg/g creatinine (34 µg/mmol creatinine) can be considered unequivocably as a sign of a decreased reabsorptive capacity of the proximal tubule. Since the fractional excretion of free RBP is, like that of β_2-m, around 0.03% (5), the % loss of the tubular reabsorptive capacity can be roughly estimated by dividing the relative increase in the urinary output of RBP by 30.

(b) Published biological action levels

There are no published biological action levels.

(c) Sampling representative of recent or long-term exposure or biological effect

In urine with pHs greater than 6 the urinary excretion of RBP closely correlates with that of β_2-m, and therefore it can logically be assumed that both urinary proteins have the same health significance and that the recommendations made for β_2-m (see para-

graph 3.1.9.5) are also valid for RBP. An elevation of urinary RBP has *per se* no prognostic value and may have a variety of causes that do imply a loss of renal function. It is only when urinary RBP becomes increasingly abnormal over months or years that it may reflect the development of a progressive and irreversible nephropathy with a less favourable prognosis. In cadmium-exposed subjects (21), it is well accepted that an abnormal elevation of urinary RBP is irreversible and predictive of an accelerated decline of the renal function with age. The only uncertainty that persists is the health significance of a very slight elevation of urinary RBP (i.e. between 300 and 1000 µg/g creatinine [34 and 113 µg/mmol creatinine]) which would occur after a relatively short exposure to cadmium.

3.1.10.6 Research needs

There is a need for additional information on the health significance of a slightly increased urinary excretion of RBP (e.g. between 300 and 1000 µg/g creatinine).

As mentioned in paragraph 3.1.9.6 above, research on the use of assays for anionic microproteins (e.g. Clara cell proteins) in urine is needed to evaluate their use for the early screening of tubular proteinuria (22).

3.1.10.7 References

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3.1.11 Alpha$_l$-microglobulin

3.1.11.1 Toxicokinetics (or physiology)

Alpha$_1$-microglobulin (α_1-m) also called protein HC (heterogeneous in charge, human complex forming) is a glycoprotein with a Mr of 26.1 kD by sedimentation equilibrium centrifugation and of 31 kD by SDS-PAGE (1–4). The protein, isolated from urine and plasma by several research groups in the seventies (5–7), has two outstanding features: a yellow-brown colour due to a strongly attached chromophore of unknown nature and a considerable charge heterogeneity at least as extensive as that of immunoglobulins (pI = 4.3–4.8).

α_1-m, like RBP, is a member of the lipocalin superfamily of hydrophobic ligand binding proteins. The liver is probably the main site of synthesis. Apart from severe liver disease where it is low and renal failure where it is high, the plasma level of α_1-m undergoes little change in many forms of inflammatory or neoplastic disorders. The function of α_1-m is hitherto unknown but there are several pieces of evidence suggesting that it is involved in immunoregulation (1–4).

α_1-microglobulin occurs free in serum and bound to several high molecular weight

proteins such as IgA and albumin. These forms differ considerably in structure and anti-genic properties making quantitative immunochemical determination of total α_1-m in serum difficult (2, 3, 8, 9). This explains the large variations reported for the normal level of total α_1-m in serum. Free α_1-m in plasma (normally around 20 mg/L) is elimi-nated mainly by glomerular filtration and as a corollary rises in parallel with the de-crease of the glomerular filtration rate (10, 11). The renal handling of α_1-m is, however, less well characterized than that of β_2-microglobulin or retinol-binding protein. It is well documented that its urinary excretion increases markedly in situations associated with a proximal tubular dysfunction such as in chronic cadmium poisoning (12–15). However, the glomerular sieving coefficient (i.e. the protein concentration ratio between the glomerular ultrafiltrate and the plasma) of free α_1-m is unknown. However, since the size of the protein is close to the landmark separating low and high Mr proteins (40 kD) it is likely that its urinary excretion partly depends on the integrity of the glomerular filter and may thus increase when the latter is compromised. The efficiency of the renal uptake of free α_1-m, which critically determines its sensitivity to tubular insult, is also unknown as well as the serum concentration above which its tubular transport is likely to be saturated (16).

Several authors have proposed α_1-m as the best marker of proximal tubular dysfunction. The reasons for this are its resistance to acid proteases and its high concentration in urine rendering its determination much easier than that of RBP or β_2-m. However, its relative advantage over RBP is unknown. In addition, the few studies which have com-pared the urinary excretion of α_1-m to that of other microproteins in subjects with vari-ous types of renal disorders suggest that it might be less sensitive as a marker of tubular function than RBP and probably also less specific in that α_1-m is more often elevated in both tubular and glomerular diseases (15–19). For instance, in patients with burn injury-induced tubular damage, Yu et al. (15) have reported that correlations between α_1-m and β_2-m or RBP were much less than those for β_2-m and RBP. The three proteins usually followed the same pattern of increase but the magnitude of change of RBP and β_2-m were two to three times greater than that of α_1-m. For these reasons, urinary α_1-m could be more of value for screening of tubular or mixed proteinuria rather for a very sensitive and specific assessment of the proximal tubule function integrity (19).

3.1.11.2 Biological sampling

(a) Sampling time and specimen

Sampling time is not critical for the assay of urinary α_1-m. Alpha$_1$-microglobulin can be determined on a 24-hour urine sample or on an untimed (ideally mid-stream) urine sample. No information is available on the influence of diuresis on the urinary excretion rate of α_1-m. It is advisable, however, to avoid extreme variations in the urinary flow when the results are corrected for the creatinine content of urine which at extreme val-ues (e.g. < 0.3 and > 3 g/L [<2.65 mmol/L and >26.5 mmol/L]) is no longer linearly re-lated to the diuresis.

(b) Preservatives, shipment and stability

A preservative must be added to the urine (e.g. NaN_3 0.1%). If possible, the samples should be shipped on dry ice. However, provided the urine contains a preservative, shipment at room temperature should not significantly affect the stability of α_1-m.

α_1-m is stable for several days at room temperature and at least for two weeks at 4°C. At -20°C, the protein is stable for months and probably several years. In practice, if the assay cannot be performed within 2 or 3 weeks after collection, it is advisable to store the samples in the freezer.

3.1.11.3 Recommended analytical methods

Like other urinary microproteins, α_1-m can be determined in urine by radioimmunoassay (20), enzyme immunoassay (21), latex immunoassay (22) or other sensitive techniques. But what makes urinary α_1-m of interest is that it can also be accurately measured by very simple immunoprecipitation methods, such as radial immunodiffusion, or nephelometry. Three of these methods are commercially available from Behring: 1) the Rapitex test which is semi-quantitative latex agglutination test for a rapid screening of urine samples with a cut-off adjustable between 10 mg/L and 20 mg/L; 2) the LC-Partigen α_1-m test, a radial immunodiffusion method permitting the quantitative assay of α_1-m in the range from 3 to 55 mg/L in undiluted urine; and 3) the immunonephelometric test applicable on the Behring laser nephelometer with a working range of 3 to 83 mg/L.

All reagents (polyclonal antisera and standards) necessary to the development of an immunoassay of α_1-m are commercially available. As aforementioned, free α_1-m and α_1-m bound to high molecular weight plasma proteins (IgA and albumin) do not present the same immunoreactivities. It is thus important that immunoassays of urinary α_1-m be calibrated with standards based on free α_1-m isolated from urine.

3.1.11.4 Other analytical methods

Although no application has been published so far, urinary α_1-m can be easily determined by photometric latex immunoassays and in particular the IUPAC standard method (23) (see section on β_2-microglobulin for a detailed description) and probably also by the PETUNIA method (24).

3.1.11.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

The urinary excretion of α_1-m by healthy subjects aged 20 to 60 years is normally below 20 mg/g creatinine (30 mg/24 hours). The upper reference limits, expressed per g of creatinine, might be different between males and females because the latter excrete less creatinine and also less α_1-m (25) but so far no study has proposed cut-off values for the different sexes.

(b) Published biological action levels

There are no published biological action levels.

(c) Sampling representative of recent or long-term exposure or biological effect

In subjects with a normal glomerular filtration rate, a urinary excretion of α_1-m above 20 mg/g creatinine (2 mg/mmol creatinine) can be interpreted as the sign of a renal impairment localized in all likelihood at the tubular level. However, if the albuminuria is also increased, as this is frequently the case, it cannot be excluded that the elevation of urinary α_1-m results partly or wholly from an increased glomerular leakiness.

To our knowledge, no prospective study has been carried out in order to assess the predictive value of an increased urinary excretion of α_1-m. But since the response of this protein parallels that of other microproteins in renal disorders affecting predominantly the tubules (e.g. in cadmium nephropathy), one can logically think that this similarity extends also to the health significance of these proteins.

3.1.11.6 Research needs

More information about stability and sensitivity of the assay of urinary α_1-m compared to that of the RBP assay is needed.

As mentioned in paragraph 3.1.9.6 above, research on the use of assays for anionic microproteins (e.g. Clara cell protein) in urine is needed to evaluate their use for the early screening of tubular proteinuria (19).

3 1.11.7 References

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3.2 Chromium

3.2.1 Introduction

Chromium in the form of various alloys and compounds has been in wide-spread commercial use for more than 100 years. Early applications included chrome pigments and tanning liquors. In recent decades, chromium has also been widely used in chromium alloys and chrome plating.

Several million workers world-wide are exposed to airborne fumes, mists and dusts containing chromium or its compounds. Highest exposures to chromium (VI) may occur during chromate production, welding, chrome pigment manufacture, chrome plating and spray painting; highest exposures to other forms of chromium occur during mining, ferrochromium and steel production, and welding, cutting and grinding of chromium alloys.

Biological monitoring of these occupational exposures is feasible and should become more generally applicable in the future. This chapter focuses mostly on the use of biological indicators in the assessment of occupational exposure to chromium compounds.

3.2.2 Physical-chemical properties

Chromium (Cr. CAS: 7440-47-3) is a lustrous, silvery metal which is ductile, malleable and flexible.

Valences: Chromium can exist in formal oxidation states of –II to VI. The most stable oxidation states are 0, II, III and VI. Chromium (VI) and (III) are the most common valence types in the workplace.

Solubility: The solubility of inorganic chromium compounds varies from very water soluble to insoluble. Further information on solubility of the various chromium compounds and their physical and chemical properties may be obtained in e.g. International Agency for Research on Cancer (IARC) Monograph, Volume 49 (1).

Conversion factors:
1 μg/L = 19.2 nmol/L; 1 μmol/L = 52 μg/L
1 μg/g creatinine = 2.17 μmol/mol creatinine

Data are presented for a few occupationally relevant compounds:

Table 3.2.1. Physical properties of chromium compounds (1)

Chemical name	Atomic/molecular mass	Melting point (°C)	Boiling point (°C)	Solubility
Chromium (III) compounds				
Basic chromic sulphate	165.06	--	--	Soluble in water (approximately 700 g/l at 35°C)
Chromic nitrate	238.03	--	--	Soluble in water. Both hydrated
(7.5 hydrate)	(373.13)	(100)	Decomposes	forms soluble in water; the
(nonhydrate)	(400.15)	(60)	Decomposes at 100	nonahydrate is soluble in acids, alkali, ethanol and acetone
Chromic oxide	151.99	2435	4000	Insoluble in water, acids, alkali and ethanol
Chromium (VI) compounds				
Ammonium chromate	152.07	180	--	Soluble in water (405 g/l); insoluble in ethanol, slightly soluble in ammonia, acetone and methanol
Barium chromate	253.33	--	--	Very slightly soluble in water (4.4 mg/l at 28°C); soluble in mineral acids
Basic lead chromate	546.37	--	--	Insoluble in water; soluble in acids and alkali
Calcium chromate	156.09		--	Slightly soluble in water and
(dihydrate)	(192.10)	(200)		ethanol; soluble in acids. Hydrated form is soluble in water (163 g/l at 20°C; 182 g/l at 45°C), acids and ethanol
Chromium trioxide	99.99	196	Decomposes at 250°C	Soluble in water (625 g/l at 20°C; 674.5 g/l at 100°C), ethanol, diethyl ether and sulphuric and nitric acids
Lead chromate	323.18	844	Decomposes	Very slightly soluble in water (0.58 mg/l at 25°C); soluble in most acids and alkali but not in acetic acid or ammonia
Potassium chromate	194.20	968.3	Decomposes	Soluble in water (629 g/l at 20°C 792 g/l at 100°C; insoluble in ethanol
Sodium chromate	161.97	792	Decomposes	Soluble in water (873 g/l at 30°C) and methanol (3.44 g/l at 25°C); slightly soluble in ethanol
Strontium chromate	203.61	Decomposes	--	Slightly soluble in water (1.2 g/l at 15°C; 30 g/l at 100°C); soluble in hydrochloric, nitric and acetic acids and ammonium salts
Zinc chromate	181.37	--	--	Insoluble in cold water; decomposes in hot water; soluble in acids and liquid ammonia
Zinc chromate hydroxide	280.74	--	--	Slightly soluble in water; soluble in dilute acids, including acetic acid

3.2.3 Possible occupational and non-occupational exposure

(a) Occupational exposure

A wide range of chromium alloys and inorganic chromium compounds are encountered in the workplace. These chromium compounds vary greatly in their toxic and carcinogenic effects. For this reason, it is necessary to divide chromium and its inorganic compounds into a number of groups. The American Conference of Governmental Industrial Hygienists (ACGIH) (2) has proposed the following groupings:

1. Chromium metals and alloys: This group includes chromium metal, stainless steels, and other chromium-containing alloys.

2. Divalent chromium compounds (Cr^{2+}) (chromous compounds): This group includes chromous chloride ($CrCl_2$) and chromous sulphate ($CrSO_4$).

3. Trivalent chromium compounds (Cr^{3+}) (chromic compounds): This group includes chromic oxide (Cr_2O_3), chromic sulphate ($Cr_2[SO_4]_3$), chromic chloride ($CrCl_3$), chromic potassium sulphate ($KCr[SO_4]_2$), and chromite ore ($FeO.Cr_2O_3$).

4. Hexavalent chromium compounds (Cr^{6+}): This group includes chromium trioxide (CrO_3), the anhydride of chromic acid; chromates (e.g., Na_2CrO_4); dichromates (e.g. $Na_2Cr_2O_7$); and polychromates. Because of the wide range of solubility for compounds in this group, these have been divided into two subgroups based on solubility in water.

    a. Water-soluble, hexavalent chromium compounds: These include chromic acid, its anhydride, and the monochromates and dichromates of sodium, potassium ammonium, lithium, caesium, and rubidium.

    b. Sparingly soluble or water-insoluble, hexavalent chromium compounds: These include zinc chromate, calcium chromate, lead chromate, barium chromate, strontium chromate, and sintered chromium trioxide.

The major industries producing occupational exposure to chromium are stainless steel welding, chromate production, chrome plating, ferrochrome production, tanning and leather working, and chrome pigment production.

With respect to hexavalent compounds, the most important exposures are to sodium, potassium, calcium and ammonium chromates and dichromates during chromate production; to chromium trioxide during chrome plating; to insoluble chromates of zinc and lead during pigment production and spray painting; to water-soluble alkaline chromates during steel smelting; and welding and to other chromates during cement production and use. Trivalent compounds that are common in workplace air include chromite ore during chromate production and in the ferrochromium industry, chromic oxide during pigment production and use, and chromic sulphate during leather tanning (1, 3, 4, 5).

Further information on occupations with potential exposure to chromium can be found in reference 6. Exposure at the workplace can vary considerably according to the type of industry and the specific work conditions in each plant. IARC Monograph Vol. 49 (1) summarizes data on exposure to inorganic chromium (air and biological monitoring) in different occupational situations.

(b) Non-occupational exposure

Chromium is the twenty-first most abundant element in the earth's crust and occurs widely in the natural environment. It never occurs in the metallic state, very rarely in the hexavalent state, but most widely in the trivalent state as the oxide in combination with other metal oxides. Intake of chromium is mostly via food, although the chromium content of most foods is very low. Municipal water supplies and ambient air frequently contain traces of chromium. The average amount of chromium ingested daily by adults was determined to be about 25 μg (7).

Medical implants, e.g. hip prosthesis are commonly made of cobalt, nickel and chromium containing alloys. These implants can undergo corrosion and release significant amounts of metal ions, producing chromium concentrations in tissues and body fluids exceeding the upper normal limits (8).

3.2.4 Summary of toxicokinetics

There are several reviews on the toxicokinetics of chromium in man (9–13).

3.2.4.1 Absorption

(a) Inhalation

Absorption of chromium compounds occurs mainly through inhalation. Chromium may reach the respiratory tract in the form of vapours, mists, fumes or dusts, where it may be present in the hexavalent, trivalent or elemental state. The absorption is dependent on the valence and solubility of the particular chromium species. The degree of absorption decreases with increasing particle size and increases with increased water solubility of the chromium compound. Vapours or mists of hexavalent water soluble chromium lead to rapid absorption at all levels. Chromium (VI) is reduced in the lower respiratory tract by the epithelial lining fluid and by pulmonary alveolar macrophages. Trivalent chromium is poorly absorbed by the body regardless of the route of administration; the absorption is dependent on the nature of the compound (14–16).

(b) Dermal

Experimental data show that hexavalent chromium is absorbed through the skin. The rate of absorption is dependent on exposure time and chromium (VI) concentration in the administered solution (17). Data on the urinary excretion of chromium in chrome platers indicate that dermal absorption is significant (18). Dermal absorption of trivalent chromium sulphate could not be demonstrated in humans (14). However, a fatal chromium intoxication, due to skin absorption has been described, after accidental ex-

posure to a chromium sulphate tanning liquor. In general, hexavalent chromium entering the body through dermal exposure or any other route is reduced to trivalent chromium before excretion in urine (19, 20)

(c) Gastrointestinal

The amount of trivalent chromium absorbed by the gastrointestinal tract is very low. More than 99% of administered chromium was recovered in faeces following oral administration of chromium chloride to humans (21).

Oral intake of hexavalent chromium results in its rapid reduction by compounds of saliva and gastric juice to the poorly absorbed trivalent form. Following oral administration of sodium chromate to humans faecal excretion of chromium indicated that about 10% of the administered dose had been absorbed from the gastrointestinal tract (21).

3.2.4.2 Metabolic pathways and biochemical interaction

Absorbed chromium is transported in the body by blood. Chromium (III) is bound principally to serum proteins, especially transferrin; cellular uptake is very poor (11, 22). Chromium (VI) rapidly penetrates the erythrocyte membrane. Metabolism of chromium (VI) involves rapid cellular and extracellular (plasma) reduction to chromium (III) and predominantly binding to haemoglobin (23). No hexavalent chromium could be detected in urine of animals or humans after exposure to chromium (VI) (24, 25).

The ability of erythrocytes to reduce hexavalent chromium represents an important mechanism of detoxification and can vary from individual to individual.

Further detailed information on cellular uptake and metabolism of trivalent and hexavalent chromium is given by Cohen and Costa (22). The interactions of chromium with nucleic acid and DNA are also described in this review.

3.2.4.3 Distribution

Chromium transported by blood is distributed to tissues and organs which have a different retention capacity (11, 13). The highest levels of chromium are found in liver, kidneys, spleen, and lungs. The data on exposed humans almost exclusively refer to chromium accumulation in the lungs following inhalation exposure (26).

In a man, accidentally exposed dermally to chromium (III) sulphate, the highest concentrations were in the kidney, while no chromium was detected in the brain (27). Studying the distribution and kinetics of intravenous trivalent radioactive chromium in six human subjects, Lim et al. (28) found some accumulation in the liver, spleen, soft tissues and bones. The data fitted to a model consisting of a plasma pool in equilibrium with fast (half-time = 0.5–12 hours), medium (1–14 days) and slow (3–12 months) compartments (28).

The oxidation state of chromium is the determining factor in its mode of distribution in the blood stream.

3.2.4.4 Elimination

Chromium after oral and intravenous uptake is principally excreted in the urine, with some excretion through the bile and faeces; minor routes of excretion include milk, sweat, hair, and nails (11, 12, 29). However, when chromium is administered by inhalation or intratracheal instillation, appreciable excretion can occur in faeces. Following intravenous administration, 40% of the injected dose of chromium (III) was excreted in the urine and 5% in the faeces, and 40% of the injected dose of chromium (VI) was excreted equally in urine and faeces over a 4-day period. In oral administration studies, as much as 80% of the chromium (VI) dose was recovered in urine in 4 days (11, 12).

3.2.5 Summary of toxic effects

The health effects of chromium and different chromium compounds have been reviewed (1, 4, 5, 10, 13).

Metallic chromium does not seem to have harmful health effects. Chromium (VI) compounds may cause adverse effects to the skin, the respiratory tract and to a lesser degree, the kidneys in humans, while chromium (III) is less toxic.

Hexavalent chromium compounds seem to be more potent skin allergens, but trivalent compounds may also cause eczema.

According to IARC (1) there is sufficient evidence in humans for the carcinogenicity of chromium (VI) compounds as encountered in the chromate production, chromate pigment production and chromium plating industries. There is inadequate evidence in humans for the carcinogenicity of metallic chromium and of chromium (III) compounds.

3.2.6 Biological monitoring indices

There are a few recent reviews on biological monitoring of chromium exposure (9, 10, 30). The indicators proposed for the biological monitoring of workers exposed to hexavalent chromium are chromium concentrations in urine and in erythrocytes. At present the determination of the concentration of chromium in urine seems to be the most practical for biological monitoring purposes. A differentiation between hexavalent and trivalent chromium based on biological specimens can be performed by the determination of chromium in erythrocytes and serum. Available biological monitoring indices are shown in table 3.2.2.

Table 3.2.2. Available biological monitoring indices

Indicator	Suitability for use	Ref
Chromium in urine	Practical, non-invasive	31
Chromium in erythrocytes	Measurement of Cr in erythrocytes and plasma allows differentiation between hexavalent and trivalent chromium exposures.	32
Chromium in hair	A possible index of exposure to trivalent chromium, but the likelihood of contamination limits its use. Difficult to interpret the results.	33

3.2.7 Chromium in urine index

3.2.7.1 Toxicokinetics

While trivalent chromium ions are rapidly cleared from the blood, hexavalent ions are retained much longer owing to internalization by red blood cells. Following clearance from the blood, chromium is excreted principally in the urine (34). The elimination is triphasic as observed in stainless steel welders (35–39). A single compartment model with a half-time of 15 to 41 hours has been proposed (40).

Detailed kinetic studies, however, suggest the existence of three compartments (10, 35, 38). The half-times are about 7 hours, 15 to 30 days, and 3 to 5 years. The best estimates for the size of the different compartments are 40%, 50% and 10% (10). Lindberg and Vesterberg (41) suggested a two compartment model for chromeplaters. Estimated from the median values, an initial phase with an assumed half-time of 2–3 days is followed by a half-time of approximately a month.

3.2.7.2 Sampling

(a) Sampling time and specimen

Timing of urine collection for pre-shift specimens is not critical. Urine specimens collected at the end of the shift should represent a collection period in the bladder of at least 2 hours to be representative of exposure during the entire shift.

(b) Contamination possibilities

Specimens should be collected after the worker has changed clothes and, if possible, showered to minimize contamination of the samples.

(c) Sampling device and container

Urine should be collected in acid-washed, (rinsed once with 1 mol/L nitric acid, rinsed two times with ultra pure water and dried) polyethylene or polypropylene bottles.

(d) Preservatives, shipment and stability

The urine samples should be processed for analysis as soon as possible after their collection. Acidifying of the samples with nitric acid or acetic acid is recommended. Preservatives used to stabilize the urine must be checked for chromium content before use. If the analysis is within 14 days, samples may be refrigerated, otherwise they should be deep frozen.

3.2.7.3 Recommended analytical method

Chromium in urine is usually analysed by atomic absorption using the graphite furnace technique (GF-AAS) with background correction. Samples may be assayed using a standard addition method, or by a method using matrix-matched standard calibration (42–47). Using the matrix-matched standard calibration technique the sample capacity can be doubled (50 samples per day in duplicate) in comparison to the standard addition technique (44). However, Paschal and Baily (45) consider that the matrix effect is not significant enough to influence the final result and prefer calibration using aqueous standards. The urine specimen is diluted with 2% nitric acid and 0.001% Triton X-100 and absorbance measurements are made with Zeeman-effect GFAAS; the detection limit is about 0.5 µg/L (9.6 nmol/L).

A proposed selected method for chromium in urine has been published (31).

The analytical determination of chromium in urine described here is an outline of the method by Fleischer (46), which has been recommended by the German Research Foundation.

(a) Principle of the method

Chromium is determined in urine by means of graphite furnace atomic absorption spectrometry. The diluted urine sample is analysed directly without further treatment. Interference from the biological matrix may be largely eliminated by using background correction (with quartz halogen lamp or Zeeman compensation) and the standard addition procedure.

(b) Reagents required

Only the purest analytical grade of reagents should be used. Aqueous Triton^R X-100 solution, 0.1%; nitric acid, 0.05 mol/L; calibration standards: 8, 16 and 24 µg Cr/L in 0.05 mol/L nitric acid.

(c) Equipment required

Atomic absorption spectrometer with background correction (Zeeman or quartz halogen lamp); graphite furnace; monoelement chromium hollow cathode lamp; graphite tube: pyrolytically coated.

(d) Procedure

The sample preparation and standard additions are performed as shown in table 3.2.3.

Table 3.2.3. Sample preparation and standard additions

No.	Urine μL	0.1% Triton solution μL	0.05 M Nitric acid μL	Standard			Added chromium in terms of urine volume assayed μg/L
				A 8μg/L μL	B 16μg/L μL	C 24μg/L μL	
a	-	100	900	-	-	-	- (blank)
b	400	100	500	-	-	-	-
c	400	100	400	100	-	-	2
d	400	100	400	-	100	-	4
e	400	100	400	-	-	100	6

Atomic absorption spectrometer
Wavelength   357.9 nm
Background correction   Zeeman compensation or quartz halogen lamp
Analytical determination   Determination of peak heights
Graphite furnace
Inert gas   Argon
Injected volume   20 μL

The temperature-time programme for the graphite furnace depends on the type of instrument. The data given below may serve as a guide. However, an optimization must be carried out for each individual instrument.

Table 3.2.4. The temperature-time programme for the graphite furnace

Analytical step	Step duration		Temperature (°C)
	Ramp time (s)	Hold time (s)	
Drying	10	25	110
Charring I	20	20	1200
Charring II	1	2	1200 Gas stop
Atomization	0	4	2300 Gas stop
Heating	1	7	2650
Cooling	1	9	20

(e) Criteria of analytical reliability

i) Trueness
Trueness based on recovery was 96%.

ii) Precision
The within-series precision: 1.6–2.2% relative standard deviation at 2.6 and 0.7 μg Cr/L (50 and 13 nmol/L).

The between-day precision was 2.0–5.5% relative standard deviation at a range from 3.2 to 59 µg/L (61–1130 nmol/L).

iii) Detectability
The limit of detection was 0.1 µg/L (2 nmol/L).

(f) Quality assurance

Due to the low concentration of chromium in a complex matrix it is important to employ a quality control programme, both internally and externally (48–51).

For **internal quality control** commercially available control material specimens from e.g. Bio Rad Lab., Anaheim, California, USA – Lyphocheck[R] Urine Metals Control and Nycomed AS, Oslo, Norway – Seronorm[TM] Trace Elements Urine can be used.

For **external quality control** international intercomparison programmes are available from e.g. Centre de Toxicologie du Québec, Canada (48), the German Society of Occupational and Environmental Medicine, Erlangen, Germany (51) and the Japan Federation of Occupational Health Organizations (49).

(g) Sources of possible errors

i) Pre-analytical
Attention has been drawn to the danger of exogenous contamination (52). For this reason the recommended cleaning procedure must be strictly observed for all containers, tubes and glassware used.

ii) Analytical
The chemicals used must be of highest purity; reagent blanks must be measured in each run of analysis. The blank samples of pure water must be stored in specimen containers of the same lot as used for the field specimens.

The three most important variables in this method are the background correction capability of the instrument, the charring or "ashing" temperature, and the use of the method of standard additions. An inadequate background correction capability of the instrument will manifest itself in abnormally high and erratic values. Use of Zeeman compensation or a quartz halogen lamp is preferred. Normal deuterium background correction is inadequate at chromium wavelength. Related to this problem is the charring temperature. If the samples are charred at temperatures below 1200°C, not enough matrix may be removed from the samples to allow the background correction system to function properly during the atomization step. At temperatures above 1300°C, chromium is quickly volatilized (53).

Use of the method of standard additions is essential in this determination, because urine samples differ widely in composition, the slope of the analytical curve usually differs from that of the aqueous standard, and even from that of other urine specimens.

(h) Reference to the most comprehensive description of the method

Fleischer M. Chromium in urine. In: Analyses of Hazardous Substances in Biological Materials. Vol. 1. Angerer J, Schaller KH. (Eds.) VCH Verlag Weinheim 1985:97–115.

(i) Evaluation of the method

The method has been proven valid in several intercomparison programmes (46, 47).

3.2.7.4 Other analytical methods

Published methodology for determining chromium in biological samples has relied on the use of a wide variety of analytical techniques, e.g. neutron activation analysis (NAA), chemiluminescense, isotope dilution mass spectrometry, gas chromatography (54). But these techniques are, however, only for exceptional cases appropriate in the concentration ranges relevant in occupational medicine.

The National Institute for Occupational Safety and Health (NIOSH) (54) has published a Inductively Coupled Plasma-Atomic Emmission Spectroscopy method for the determination of total chromium in urine. The sample preparation, however, (binding on poly-dithiocarbamate resin, filtration, masking) is very time-consuming and cumbersome.

3.2.7.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

Chromium concentrations in urine of non-occupationally exposed persons measured by GFAAS are less than 1 µg/L (19 nmol/L) (9). Current reference values for median urinary chromium levels of the general population are 0.4 µg/L (8 nmol/L) urine in the range between 0.24 to 1.8 µg/L [4.6–35 nmol/L] (1, 55). The older literature reports higher values (up to 5 µg/L [100 nmol/L]) (9).

The validity of the unexposed reference values for total chromium in urine reported in the literature over the last decade were evaluated as part of the TRACY project (56). In 41 selected papers the arithmetic mean values ranged between 2–10 nmol/L or 0.2–1 µmol/mol creatinine.

(b) Published biological action levels

The American Conference of Governmental Industrial Hygienists (ACGIH) (57) recommends a Biological Exposure Index (BEI) of 10 µg/g creatinine (22 µmol/mol creatinine) for the increase in urinary total chromium concentrations during the workshift as the best indicator of exposure during that workday in workers with a history of long-term chromium (VI) exposure. Two urine samples are required, one before the shift and one after the shift ends. The net increase in chromium concentration is expected to be much lower (5 µg/g creatinine [11 µmol/mol creatinine]) for newly exposed workers.

The Committee recommends a BEI of 30 µg/g creatinine (65 µmol/mol creatinine) for urine samples collected near the end of the workweek at the end of the shift. This BEI is

consistent with exposure of a welder to 0.05 mg/m^3 of soluble Cr(VI) fumes for an 8-hour workshift and applies only to workers with a history of chronic chromium exposure.

The concentration of chromium found in a single urine specimen, collected at the end of the shift, is predictive of exposure. However, the test does not distinguish between intensity of exposure on the day of urine sampling and exposure at high concentrations on previous days. The concentrations of chromium in the urine of workers with recent exposure (newly exposed workers or workers returning to work after an extended absence) are expected to be much lower (7 µg/g creatinine [15 µmol/mol creatinine) (57).

The German Research Foundation (58) does not establish biological action levels for substances which, by their own action or by that of their reactive intermediates or metabolites, are known to cause malignant growths in man or for which there is good evidence of a human cancer risk because it is not possible to specify safe levels of such substances in biological materials. For this reason they investigate the relationship between the concentration of the carcinogen in the workplace air and that of the substance or its metabolites in biological material (Exposure equivalents for carcinogenic substances, EKA). From these relationships, the internal exposure which results from uptake of the substance exclusively by inhalation can be determined. The following correlation could be established for exposure to Cr(VI):

Table 3.2.5. Relationship between CrO$_3$ concentrations in workplace air and excretion of Cr in urine

Air CrO$_3$ mg/m^3	Cr-urine (µg/L)
	Sampling: end of exposure
0.03	12
0.05	20
0.08	30
0.10	40

This correlation is also applicable for an exposure to welding fumes.

(c) Non-analytical interferences

Welders who smoke have increased concentrations of urinary chromium, compared with non-smoking welders (57). This increase may be due to contaminated cigarettes or to adsorption of chromium fumes or smoke particles.

The solubility, valence, and physical form (mist, dust, fumes) of chromium affects the absorption, distribution, and elimination of chromium in humans. Thus, workers in a wide variety of occupations may be exposed to similar concentrations of a particular form of chromium but, because of the factors noted above, will excrete different concentrations of chromium (9, 57).

The distribution and elimination kinetics of accumulated chromium are affected by the duration of exposure during the work-day as well as by years of previous exposure.

Welders with chronic exposure to Cr(VI) show increased renal clearance of chromium, compared with new welders without previous occupational exposure to chromium (9, 57).

The variability of background concentrations of chromium in the normal environment is relatively small and has no significant effect on measurements of chromium in occupationally exposed populations.

Contamination of the sample during collection or analysis can lead to a significant problem in interpreting the data. Certain factors might be associated with an increased urinary chromium level, e.g. exercise, past employment in a chromium-exposed occupation, drinking beer, diabetic status (59–61).

(d) Sampling representative of recent or long-term exposure

Determination of the concentration of chromium in urine at the end of the work-day seems to be a good indicator of exposure to soluble chromium (VI) compounds (62, 63); however, trivalent chromium ions probably contribute, at least to some extent, to these levels. The end-of-shift urinary chromium level reflects mainly recent exposure, but is also influenced by the body burden. The difference between chromium levels in spot samples collected before and after the shift can reflect the exposure during the day (current exposure) (see 3.2.7.5b above).

3.2.8 Chromium in erythrocytes index

3.2.8.1 Toxicokinetics

Toxicological investigations have shown that chromium is capable of penetrating cell membranes only in its hexavalent oxidation state. This applies especially to erythrocyte membranes (64, 65). The chromium in erythrocytes (irreversibly bound to haemoglobin) might specifically reflect the internal dose of hexavalent compounds (66, 67).

3.2.8.2 Sampling

(a) Sampling time and specimen

Blood specimens should be collected at the end of an exposure interval.

(b) Contamination possibilities

Exogenous contamination of the specimen must be carefully avoided. In the literature, contamination of the blood from the commonly used stainless steel syringe needles is discussed.

(c) Sampling device and container

Blood specimens should be drawn from the arm vein using disposable syringes containing K-EDTA as an anticoagulant, e.g. Monovette[R].

(d) Preservatives, shipment and stability

The separation of the erythrocytes from the plasma must be performed within eight hours of the specimen collection. Haemolysis of the erythrocytes must be prevented.
Erythrocytes which have been isolated, washed and dissolved in ultrapure water can be stored at room temperature for three days. It is possible to keep the samples in a deep freezer (at -20°C) for three months.

3.2.8.3 Recommended analytical method

The preferred method for the determination of chromium in erythrocytes is graphite furnace atomic absorption spectrometry (GFAAS) (65, 68). The procedure described below is an outline of the method of Lewalter and coworkers (32).

(a) Principle

The erythrocytes are isolated from whole blood by centrifugation and washed three times with isotonic saline solution. For the atomic absorption spectrometric determination the biological specimens (whole blood, plasma, erythrocytes) are diluted with Triton X-100 and immediately placed in a pyrolytically coated graphite tube. There the samples are dried, charred and atomized. The absorption at 357.9 nm gives a measure of the chromium concentration of the investigated sample. The standard addition procedure is used for calibration because of various matrix effects. Background interference is largely eliminated by Zeeman compensation.

(b) Reagents required

Triton[R] X-100, physiological saline (154.0 mmol/L), calibration standards: 2, 5, 10 µg Cr/L in water.

(c) Equipment required

Atomic absorption spectrometer with Zeeman compensation or quartz halogen lamp background correction; graphite furnace; chromium hollow cathode lamp; graphite tube pyrolytically coated; centrifuge; haematocrit centrifuge.

(d) Procedure

Before separation of the erythrocytes from the whole blood the haematocrit of the intact sample is determined. For the determination of chromium in erythrocytes, 2.5 mL whole blood is diluted with 1 mL isotonic saline solution in 5 mL centrifuge tubes (graduated at 2.5 mL), carefully mixed and centrifuged for 5 min at 1200g. The supernatant is carefully drawn off with a pipette and transferred to another 5 mL centrifuge tube. About 2.3 mL plasma and approximately 1.2 mL erythrocytes are obtained. The isolated plasma sample can be used for determination when the chromium level is requested. The erythrocytes must be thoroughly washed by mixing with 2.5 mL of isotonic saline followed by centrifugation and discarding of the supernatant, at least five times. Finally, 2.5 mL of isotonic saline are added to the erythrocytes and the haematocrit of the suspension measured. The suspension is then centrifuged, the supernatant discarded and 2.5 mL of ultrapure

water added. The chromium level in this prepared solution is then analysed by standard addition according to the following scheme:

Table 3.2.6. Sample preparation and standard additions

No.	Standard				Sample erythrocyte	Equivalent chromium in the whole blood sample (100 µL)
	I	II	III	IV		
	0µg/L	2µg/L	5µg/L	10µg/L		
	µL	µL	µL	µL	µL	µg/L
1	500	-	-	-	100	0
2	-	500	-	-	100	10
3	-	-	500	-	100	25
4	-	-	-	500	100	50

A reagent blank containing 100 µL ultrapure water instead of the biological sample is included in each test series. As an alternate matrix matched standards can be used.

Atomic absorption spectrometer:
Wavelength   357.9 nm
Background correction   Zeeman compensation or quartz halogen
Spectral slit width   0.7 nm
Graphite tube   Pyrolytically coated
Analytical determination Determination of peak heights or peak areas of the signal during the atomization step

The following temperature-time programme serves only as a guide. Optimization of the parameters must be carried out for each individual instrument.

Table 3.2.7. The temperature-time programme as a guide

| Analytical step | Step duration | | Temperature (°C) |
| | Ramp time | Hold time | |
	s	s	
Drying I	15	5	90
Drying II	25	10	150
Charring I	15	15	350
Charring II	10	15	500
Charring III	5	15	1200
Atomization	0	4	2400 Gas stop
Heating	1	5	2700

Inert gas: Argon
Injected volume: 20 µL

(e) Analytical reliability

i) Trueness
Trueness, based on recovery studies was 107–113% (at 12.5 to 50 µg/L).

ii) Precision

The within-series precision was 1.4–3.2% relative standard deviation at 12.5 to 50 µg Cr/L (240–960 nmol/L). The precision of duplicate analyses was 10.3% relative standard deviation at 1.0 to 39 µg Cr/L (19–750 nmol/L).

(f) Detectability

The limit of detection was 0.5 µg/L (10 nmol/L) for whole blood (32).

(g) Quality assurance

No control specimens are available for the determination of chromium in erythrocytes. In-house erythrocyte controls, consisting of pooled, spiked packed cells must be used. Whole blood controls for the chromium determination are provided by Nycomed AS, Oslo, Norway-Seronorm[TM] – Whole Blood Metals.

Intercomparison programmes including chromium in erythrocytes are not available. The German Society of Occupational and Environmental Medicine, Erlangen, Germany, offers a 'round robin' scheme for the determination of chromium in whole blood (51).

(h) Sources of possible errors

Contamination in the preanalytical phase and during isolation and washing of the erythrocytes must be strictly avoided.

Chromium contamination can be caused by the coloured stoppers of sampling devices which are used to identify the various anticoagulants. Haemolysis during isolation and washing of the erythrocytes must be avoided. Fresh blood specimens must be processed within 8 h of their collection. Interferences or losses caused by the time-temperature programme are identical with the urine method (see also 3.2.7.3).

3.2.8.4 Other analytical methods

Neutron activation analysis has been used for the determination of chromium in serum (69); however, this technique should only be considered as a reference method due to cost and availability.

3.2.8.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

In the review of Iyengar and Wottiez (55) the chromium concentration in blood from non-occupationally exposed subjects is below 0.5 µg/L (10 nmol/L). The corresponding chromium levels in the erythrocytes are also less than 0.5 µg/L. Chromium is undetectable in the erythrocytes of unexposed persons using the described analytical method (32).

(b) Published biological action levels

As described in section 3.2.7.5b above, the German Research Foundation (58) had de-

fined exposure equivalents for chromium in erythrocytes (EKAs). The following correlation has been reported:

Table 3.2.8. Relationship between chromium concentrations in air and chromium in erythrocytes

Air CrO_3 mg/m^3	Erythrocytes µg Cr/L of whole blood
	Sampling: long-term exposure after several shifts
0.03	9
0.05	17
0.08	25
0.10	35

This correlation is not applicable for an exposure to welding fumes.

(c) Sampling representative of recent or long-term exposure, or biological effect

There can be large variations in chromium levels in erythrocytes after the same occupational exposure. The differences can be explained by variation of the individual reducing ability of erythrocytes on chromium (VI) (fast and slow reducers) (58, 66, 68). The chromium concentration in erythrocytes might be an indicator of hexavalent chromium during the lifetime of the red blood cells.

3.2.9 Research needs

Workers are occupationally exposed to different types of chromium compounds. These chromium compounds vary greatly in their toxic and carcinogenic effects. For this reason a speciation of exposure is desirable. More data should be collected to differentiate between the internal exposure to chromium (III) and chromium (VI). Internal-external exposure relationships should be assessed by simultaneous biological and ambient monitoring in workers exposed to different types of chromium-containing aerosols. Especially for chromium (VI) exposure only limited data are available to evaluate biological action levels. Reference levels for chromium in erythrocytes in persons without occupational exposure should be established.

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3.3 Inorganic lead

3.3.1 Introduction

Because of its physical properties, malleability, and resistance to corrosion, as well as its abundance, lead is one of the most useful metals known to man. Lead is the most widely used non-ferrous metal. The major modern uses are for electric storage batteries, paint pigments, petrol additives, various metal products, and as cable sheathing. This document reviews inorganic lead as related to occupational exposure and the possibilities of the biological monitoring of exposure. The literature regarding the biological monitoring of exposure to lead is abundant. In this review, only data of immediate relevance to the possibilities for the biological monitoring of lead exposure will be surveyed.

3.3.2 Physical-chemical properties

Lead (Pb; CAS 7439-92-1) is a heavy, ductile, bluish-white, metallic element in Group IV of the Periodic table. Chemical and physical properties include:

Atomic mass: 207.2
Valences: In its inorganic compounds lead usually has the oxidation state +II, but +IV also occurs.
Solubility: Metallic lead is very insoluble but will dissolve in nitric acid and concentrated sulphuric acid. Only a few lead compounds (e.g. lead nitrate) are appreciably soluble in water; many are dissolved by acids (e.g. lead sulphide, lead oxides) and most are sufficiently soluble in body fluids to be toxic, especially when inhaled as fumes or in finely divided form.

Further information on physical and chemical properties may be obtained in e.g. CRC Handbook of Chemistry and Physics (1).

Conversion factors:
1 μg/l = 4.83 nmol/l
1 μmol/l = 207 μg/l

3.3.3 Possible occupational and non-occupational exposures

(a) Occupational exposures

The hazards from lead exist in a wide range of industrial settings, and between 100 and 200 different occupations and job titles are considered to be associated with a potential risk of exposure (2, 3). High-risk occupations include blasting or scrapping of lead-painted metal, brass foundry work, flame welding and cutting of lead-painted metal, indoor shooting, storage battery manufacture and lead smelting. Moderate to low-risk oc-

cupations include antique restoration, car repairing, lead mining, lead soldering, and porcelain manufacture. A comprehensive list of work tasks that may lead to lead exposure is given in reference 4.

It should be emphasized that most solid metallic lead products are considered relatively safe in normal use and are seldom thought of as posing a significant health hazard. However, when various modes of treatments, such as heating, grinding, spraying, or burning, are applied to the metal surface the danger becomes greater. When heat is applied to the surface, lead fumes are generated which can readily be inhaled and absorbed (5). Considerable exposure is associated, therefore, with primary and secondary lead smelting and refining of lead and with flame cutting of lead-painted steel structures (6–8).

(b) Non-occupational exposures

Man has always been exposed to lead as it is ubiquitous in the environment; sources include air, dust, food and water (4, 9). Although atmospheric lead originates from a number of industrial sources, leaded petrol appears to be a principal source of general environmental lead pollution (10). It is estimated that 90% of the atmospheric lead comes from automobile exhausts (11–14). Industrial emissions are also important sources in specific localities (14–16). The magnitude of lead pollution in the general environment of industrialized countries is generally higher than in areas remote from industries and traffic (17, 18).

In many countries there has been a major reduction of lead in petrol, which has resulted in a reduction of air lead levels in urban areas and is probably the most important contributing factor for lowering the lead exposure. In most studies a decline in blood lead levels following a reduction of lead in petrol have been shown (19–21).

Other sources of non-occupational lead exposure include: tobacco smoking (22–26), contaminated snuff (27), hobbies (14, 28), soil and street dust (29, 30), drinking water (23, 31), foods (including contamination from cooking pots) (30, 32–37), alcoholic beverages (38–40), lead-based house paints (30, 41, 42), lead shot in food (43, 44), gunshot bullets (45–47), some cosmetics (48) and some herbal medicines (49).

3.3.4 Summary of toxicokinetics

There are several recent reviews on the toxicokinetics of inorganic lead in man (4, 9, 50).

3.3.4.1 Absorption

(a) Inhalation

Pulmonary deposition of lead particles suspended in air varies as a function of particle size distribution and respiratory rate. Particles with an aerodynamic diameter above 5μm are mainly deposited in the upper airways, cleared by the mucociliary mechanism,

and swallowed. Some of this lead is then absorbed from the gastrointestinal tract. Particles with a diameter below 1µm are deposited to a large extent directly in the alveolar region of the lung. From radioactive and stable isotope studies it can be concluded that lead deposited in the deep lung is completely absorbed. This appears to be true for all forms of lead (50, 51). Barry (52) found that lead does not accumulate in lungs of workers.

(b) Dermal

Percutaneous absorption was studied in eight volunteers with topical application of radio-labelled lead acetate in a cosmetic preparation (53). Absorption of 0% to 0.3% was observed. Lead nitrate solution placed on the arm can be absorbed through the skin and rapidly distributed throughout the body. The lead transport occurs in plasma and may be rapidly concentrated into the extracellular fluid pool of sweat and saliva without significant uptake by erythrocytes (54). Probably, the absorption of lead soaps (lead naphtenate and lead stearate) is higher.

(c) Gastrointestinal

From studies of the gastrointestinal absorption of stable lead in adults an average absorption of about 10% may be estimated (52, 55–58). Rabinowitz et al. (57) and Heard and Chamberlain (59) found that in the fasting state adults absorb lead at rates up to 63%. In adults there seems to be a considerable inter-individual variation of lead uptake from the intestine.

3.3.4.2 Metabolic pathways and biochemical interactions

Inorganic lead does not undergo any metabolic transformation or digestion in the intestines or detoxification in the liver.

Lead is an electropositive metal with high affinity to the negatively charged sulphhydryl groups. This is manifested by the inhibition of sulphhydryl-dependent enzymes in several organs. Changes in haem synthesis are the first critical effects of increased lead burden. Inhibition of the activity of delta-aminolevulinic acid (ALA) dehydratase, increase in the urinary excretion of delta-ALA and coproporphyrinogen and increase in erythrocyte protoporphyrin are characteristics which distinguish excessive lead exposure from other disorders of pyrrole metabolism in man. Accumulation of erythrocyte protoporphyrin is induced by the inhibition of mitochondrial enzymes which regulate the incorporation of iron in the porphyrin molecule.

Moreover, divalent lead is similar in many aspects to calcium and acts competitively with this element in several biological systems, such as mitochondrial respiration and various neurological functions. The similarities between calcium and lead partially explain why both elements appear interchangeable in bone and why more than 90% of the total body burden of lead is stored in the skeleton.

3.3.4.3 Distribution

The total burden of lead is distributed throughout various parts of the organism, and the biological activity of lead varies between these segments as well. The rapidly exchangeable portion of lead in plasma is the most biologically active part of the total body burden, but it must be emphasized that this pool constitutes only about 0.1% of this total. Several mathematical models of the pharmacokinetic characteristics of lead have been proposed (60, 61).

From a practical point of view, a two-compartment model describes the metabolism sufficiently. There is a rapid compartment (reflecting soft tissues) with a half-time of about one month, and a slow one (reflecting the bone pool) with a half-life of approximately one decade.

The distribution of lead between various tissues within the body and the rates at which lead transfers from one compartment to another are important to the design of medical surveillance programmes, to decisions concerning exposure control and to diagnosis and treatment.

3.3.4.4 Elimination

Lead is eliminated from the body mainly in urine and faeces, but there are also minor routes of elimination via sweat and hair. The percent eliminated by each route depends on the route of absorption, age of the individual, dietary constituents, and other variables. Ninety percent of the ingested lead is excreted unabsorbed in the faeces. About 76% of absorbed lead is excreted in urine, 16% in gastrointestinal secretions and less than 8% in hair, nails and sweat.

3.3.5 Summary of toxic effects

A large number of reviews have been published (9, 62–69). Particularly comprehensive is the review by the US Environmental Protection Agency (9). Lead poisoning is in most cases a chronic disease. The toxic effects of lead for humans can be viewed as a broad spectrum of laboratory and clinical manifestations, ranging from subtle "subclinical" biochemical abnormalities to severe clinical emergencies. In the beginning, inhibition of enzymes and other biochemical effects occur (see paragraph 3.3.4.2). At the intermediate stage, the effects of various enzyme inhibitions can be measured, such as inhibitions of enzymes in the biosynthetic pathway of haem or accumulation of enzyme substrates.

Lead has also been shown to inhibit erythrocytic pyrimidine-5'-nucleotidase (P5N), in both children and adults exposed to lead, which results in the accumulation of nucleotides in erythrocytes affecting the stability of the cell membrane. This and other interactions with cell membranes, such as interference with Na^+K^- ATPase activity, the Na^+/K^+ pump, and Na^+/K^+ co-transport system have been suggested as the biochemical basis for a variety of lead-related effects, including shortened erythrocyte survival time

and haemolysis, renal toxicity and hypertension.

Lead toxicity disturbs the haem synthesis, erythrocyte survival, both the peripheral and central nervous system, the kidneys and the gastrointestinal tract. Lead also affects reproduction, and possibly also causes cardiovascular and mutagenic effects. The immune system is also a target for sub-clinical lead-related toxicity (70).

In the 1987 review, the International Agency for Research on Cancer, IARC considered the evidence for carcinogenicity of lead and lead compounds in humans to be inadequate (71).

3.3.6 Biological monitoring indices

There are several recent reviews on biological monitoring of inorganic lead exposure (4, 68, 72, 73).

Several laboratory tests are available for evaluating the degree of lead absorption and related health effects. The biological tests that have been used can be classified into two groups: those directly reflecting the exposure and/or the amount stored in soft tissues (lead in blood, in urine, in teeth and in hair, and lead excretion after the administration of a chelating agent) and those indicating the biological effects of lead related to the intensity of exposure (coproporphyrin in urine, delta-aminolevulinic acid in urine, porphobilinogen in urine, free erythrocyte protoporphyrin [FEP] or zinc protoporphyrin [ZPP], delta-aminolevulinic acid dehydratase [ALA-D] and pyrimidine-5'-nucleotidase in red blood cells). In health surveillance programmes also heamoglobin and heamatocrit can be measured.

The significance, advantages and limitations of the principal biological tests are discussed extensively by Skerfving (4, 68), Alessio and Foa (72), and Lauwerys and Hoet (73). The currently available biological indicators are listed in table 3.3.1.

Table 3.3.1. Available biological monitoring dose and effect indices

Indicator	Suitability for use
Lead in blood	In a steady-state situation, lead in blood is the best indicator of the concentration of lead in soft tissues and of recent exposure
Lead in urine	Reflects the amount of lead recently absorbed
Lead in faeces	Index of dietary lead intake; not practicable, infrequent monitoring
Lead in hair	Difficulties in sampling, sample preparation and interpretation; limited interest in occupational monitoring
Chelatable lead in urine	Gives information on chelatable lead, which probably reflects the metabolically active lead pool; does not supply more information than Pb in blood; not practical in frequent monitoring
Coproporphyrin in urine	Unspecific, not recommended for biological monitoring
ALA in urine	Biological test used for monitoring of occupational lead-exposed subjects (> 400 µg Pb/L blood)
ALA-D in erythrocytes	High degree of sensitivity, but instability limits its usefulness for the monitoring of exposed persons
Free protoporphyrin (FEP) and zinc protoporphyrin (ZPP) in red blood cells	Biological response test useful in assessing lead-related biological effects, recommended for screening purposes in occupational and environmental medicine, biological indicator of equal interest to individual and collective monitoring
Pyrimidine-5'-nucleotidase in red blood cells	No practical experiences with this test in occupational medicine
Lead in bone	Not useful for monitoring current exposure, but may be useful as part of a clinical evaluation of long-term exposure; in vivo XRF techniques are available.

At present, the blood lead concentration is the best available indicator of current lead absorption or dose and health risk, and blood lead measurement is the mainstay of biological monitoring world-wide. The biological response tests, such as ZPP, FEP and ALA-D are useful in assessing lead-related biological effects. For occupationally exposed workers and community groups whose exposures are not well-characterized or in countries where the availability of blood lead determination is limited, measurements of ZPP and FEP are recommended for screening purposes. The ZPP test can be performed at the examination site and has much practical value. Elevated values of ZPP must be verified by measurement of blood lead concentration, which is more specific to the current degree of lead absorption.

ZPP provides a complementary test which, although there is no correlation between ZPP and blood lead at low exposures, gives an estimate of exposure over the previous three

months.

In conclusion, for biological monitoring of occupational lead exposure the determination of the lead concentration in blood is the primary means. ALA in urine and ZPP in blood are further relevant parameters.

3.3.7 Lead in blood

3.3.7.1 Toxicokinetics

Lead concentration in blood rises immediately after the first inhalation exposure, increases gradually, usually to reach a steady state after weeks to months. However, after a heavy exposure, the level may rise within a few hours (62).

After cessation of exposure there is an initial rather rapid decrease, later on the decrease is slower. In adults, the average decline rate is compatible with an initial phase with a half-time of about one month and represents mainly the washout from soft tissues and red blood cells. The slower phase has two components, one with a half-time of about one year, the longer phase of about one or two decades mainly represents the washout from the skeleton (4, 62). Data on the elimination of lead from blood after cessation of exposure indicate an inter-individual variation.

In subjects without occupational exposure the blood lead levels may be remarkably stable over time.

3.3.7.2 Biological sampling

(a) Sampling time and specimen

Blood lead levels are usually determined from analysis of venous blood. Blood may be collected at any time before, during or after the shift. It usually takes a month of occupational exposure for blood lead levels to reach a steady state.

(b) Contamination possibilities

Meticulous attention has to be paid to avoid contamination from work clothes and the work environment when taking the sample.

(c) Sampling device and container

Blood should be collected using disposable sampling devices and containers. Random analyses should be used to check these supplies for contamination. Anticoagulant must be lead-free. K_2-EDTA has proved to be particularly suitable. Heparin-stabilized blood appears to be unstable, when stored over longer periods. Monovettes[R] or Vacutainers[R], which are supplied already containing the anticoagulant, are particularly suitable. The blood specimens can be stored and transported in these devices.

(d) Preservation, shipment and stability

It suffices to store the specimens in the refrigerator (at +4°C) for a period of up to three weeks. If, however, a longer storage period is necessary, the specimens should be stored in the deep freeze (-18 to -20°C).

3.3.7.3 Recommended analytical methods

Atomic absorption spectrometry (AAS) is the most widely used analytical technique for determination of lead in blood. In the 1980s standardized methods using flame-AAS were recommended by NIOSH (74) and the German Research Foundation (75). In these methods the lead in the blood was chelated using ammonium pyrrolidine dithiocarbamidate (APDC), extracted with methyl isobutyl ketone and determined quantitatively with a flame atomic absorption spectrometer. The precision of these methods was around 5% with detection limits between 15 and 50 µg/L (70–240 nmol/L).

Nowadays, the majority of AAS methods employ the graphite furnace atomic absorption spectrometry (GF AAS) technique, using either Zeeman background correction or deuterium background correction for the determination of lead in blood. Automated sample injection is important to ensure reproducible results. The analysis may cause considerable errors due to incomplete atomization, volatility of lead compounds, spectral interferences and build-up of carbonaceous residue reducing sensitivity and precision. These analytical problems can be eliminated by optimum sample preparation, e.g. dilution, matrix modification, optimized time-temperature programmes, background correction and calibration with the standard addition technique (76). Application of the "Stabilized Temperature Platform Furnace" (STPE) technology together with the use of sophisticated data handling systems have the potential to make the graphite furnace an interference-free and reliable technique for the determination of lead in blood.

Typically, Triton X-l00 is added to whole blood to eliminate dispensing problems by reducing the viscosity of the sample, improve contact between sample and furnace wall and homogenize the sample by lysis of the erythrocytes. Other commonly used matrix modifiers are $NH_4H_2PO_4$ or $(NH_4)_2HPO_4$ which remove NaCl interference by forming volatile NH_4Cl at high temperatures, while the phosphate ions react with Pb(II) to form the relatively thermally stable $Pb_3(PO_4)_2$. The modifier $Mg(NO_3)$ probably acts by embedding lead in a matrix of MgO, thereby delaying volatilization. A few methods use direct determination of lead without the use of matrix modifiers.

Examples of recent GF AAS methods for lead determinations in blood are well documented (77–84). A critical review of the determination of lead in blood by graphite furnace atomic absorption spectrometry has been published by Subramanian (76).

Observations from external quality control schemes for lead in blood have shown that the quality of results was more dependent on the skill of the analyst, experiences of the laboratories than on any one particular method. There were many methods that gave results and performed well in external quality control schemes. As important as the choice of the method is suitable training and attention to detail of the analyst, as well as rigor-

ous internal quality assurance procedures (85, 86).

The analytical determination of lead in blood described here is an outline of the method by Miller et al. (79). It uses "older", more available equipment and is reasonably well documented regarding performance characteristics. A minor modification of this method, using modern instruments (Longitudinal Zeeman background correction with a transversely heated graphite atomizer) has been published recently (84).

(a) Principle of the method

Lead is determined in blood by means of graphite furnace atomic absorption spectrometry and background correction. The sample is diluted 1+9 with a matrix modifier and analysed directly without further treatment. The matrix modifier stabilizes lead so that the majority of the blood matrix may be removed during the charring step. The calibration is performed with matrix-matched calibration curves.

(b) Reagents required

Matrix modifier: 0.5 % V/V Triton X-100, 0.2% V/V 16 M nitric acid and 0.2% W/V $NH_4H_2PO_4$.
Standards: 50, 100, 250, 500, 750 and 1000 µg Pb/L in the matrix modifier solution.

(c) Equipment required

Atomic absorption spetrophotometer with background correction (deuterium lamp or Zeeman); graphite furnace with pyrolytic platforms; autosampler; mono-element lead hollow-cathode lamp.

(d) Procedure and calibration

Standards are prepared from a sample of whole blood which should be well mixed with a vortex mixer. The whole blood chosen should have a low blood lead content and be collected with the same anticoagulant as the specimens.

Calibration is accomplished most easily using an automated diluter. 100 µL of blood is pipetted with a 400 µL portion of matrix modifier for dilution. Seven dilutions are prepared in a series of pre-rinsed 2-mL autosampler cups. Then, after the sampling delivery tip had been rinsed 6–8 times to remove all traces of blood, 100 µL of blank matrix modifier or each of the standards in matrix modifier are added. Each of the additions is accompanied with a rinse of 400 µL of matrix modifier into each cup. This calibration procedure results in a standard additions curve with a matrix modifier-blood mix of 9+1.

20 µL aliquots of the diluted blood, standards and control blood pools are dispensed onto the platform and the integrated absorbances are measured. Analyses should be performed in duplicate.

Atomic absorption spectrometer
Wavelength   283.3 nm, dif 0.7 (low)
Background correction   Deuterium lamp or Zeeman compensation
Analytical determination Peak area

The temperature-time programme for the graphite furnace depends on the type of instrument, an optimization must be carried out for each individual instrument. The following temperature programme is given as an example.

Table 3.3.2. Temperature-time programme for the graphite furnace

Step	Temperature °C	Ramp time/s	Hold time/s	Argon flow rate ml/min
Dry*	180	10	25	300
Char	750	5	25	300
Atomise+	2400	1	4	20
Cool	20	1	4	200

* Temperatures used for drying vary slightly between lots of pyrolytic furnaces and platforms; each lot should be evaluated for smooth drying of the diluted specimen without splattering.
+ Base-line function set at -7 s; read at 0; recorder at -1.

With the use of area integration, the linearity of the calibration graph has been demonstrated up to about 830 µg/L (4.0 µmol/L).

(e) Criteria of analytical reliability

i) Trueness
The accuracy of the method has been rigorously evaluated with the determination of lead in selected whole blood pools from National Institute for Standards and Technology (NIST) and from the New York and the Pennsylvania State Health Department Lead Proficiency Programs. Comparability has been established with the previous blood lead method used in other surveys via the analysis of 435 specimens by both the previous (modified Delves cup) and proposed methods. The equation of the resulting line is: [GFAAS]= 1.0007 [Delves] - 0.051, r=0.924.

ii) Precision
The overall precision was 2–5 % relative standard deviation at a range from 50 to 500 µg/L (240 to 2415 nmol/L).

iii) Detectability
The detection limit was 14 µg/L (68 nmol/L).

(f) Quality assurance

Intercomparison programmes showed, that there are still considerable problems with the determination of lead in blood (87–90). It is important to employ a rigid quality control programme both internally and externally (91–94). Precision must be controlled within

each run, accuracy after every fourth run. Control charting allows the laboratory to monitor performance trends over time, and should be set up with control limits.

For **internal quality control** commercially available control blood can be used, which shows low variability and high long-term stability (93, 95, 96). A number of sources exist for matrix based control specimens. Many of these materials represent spiked samples. Some sources are: Lyphocheck^R – Whole blood control, BioRad Lab., Anaheim, California, USA; and spiked human whole blood, Referensmaterial AB, Ulrichehamn, Sweden.

Certified reference materials are available from e.g. the National Institute for Standards and Technology (NIST), Gaithersbury, Maryland, USA (NIST SRM 955a-1 till 955a-5); from the Community Bureau of Reference (BCR), Commission of the European Communities, Brussels, Belgium (CECBCR 194, 195, 196); and US Centers for Disease Control, Nutritional Biochemistry Branch, Atlanta, GA, USA (CDC BLLRS Base, 190, 290, 390, 590, 690, 890).

For **external quality control** (inter)national intercomparison programmes are running frequently (87–89, 96, 97) for the determination of lead in blood. Existing quality control programmes are available from: Centre de Toxicologie du Québec, Canada; Danish National Institute of Occupational Health, AMI, Copenhagen, Denmark; German Society of Occupational and Environmental Medicine, Erlangen, Germany; Japan Federation of Occupational Health Organizations, Japan; UKEQAS programme, Birmingham, UK; UK Robens Institute Programme, UK.

3.3.7.4 Other analytical methods

Anodic stripping voltammetry (ASV) can be used as a reference method to the AAS method in especially experienced laboratories. In comparison to AAS this technique is usually more troublesome, more time consuming and the risk of contamination is higher. A method is recommended by the German Research Foundation (98). The detection limit is 10 µg/L (48 nmol/L) blood, the in-series imprecision 3.7% (at 86 µg/L blood) and the between-day imprecision 9% (at 375 µg/L blood).

Potentiometric stripping analysis (PSA) in contrast to ASV is not subject to background interferences from organic electroactive constituents. Therefore, it is sufficient to dilute the blood sample with an appropriate supporting electrolyte (0.5 mol/L HCL). For 1 mL of blood and a 1-min deposition time, the detection limit is 1 µg/L (5 nmol/L) (99).

Analytical methods for lead in blood involving inductively coupled plasma methods with atomic emission spectrometry (ICP-AES) or combined with mass spectrometry (ICP-MS) have been described (100, 101). A detection limit for blood lead determination of 15 µg/L (72.5 nmol/L) has been established and an excellent agreement with AAS observed. Isotope dilution mass spectrometry (MS) and thermal ionisation-MS can be considered as definitive methods.

3.3.7.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

Since the presence of lead in the environment is ubiquitous, the blood lead levels of persons not occupationally exposed are dependent on the environmental exposure conditions (dietary and drinking habits, residence, etc.). The lead concentrations in blood vary in different areas of the world. The blood lead levels of subjects living in industrialized areas are generally higher than in subjects from areas remote from industries and traffic. Blood lead concentrations vary with age (children, especially those aged 1–3 years, have higher levels than adults), sex (males have higher levels than females), drinking and smoking habits (drinkers and smokers have higher levels) and can be influenced by season (maximum in winter and a summer minimum) (4).

The blood lead levels in the general population have decreased over time in many countries, probably as a result of actions against such sources as lead in petrol (21, 102–106). In most countries the average blood lead concentrations are below 100 µg/L (480 nmol/L). In Sweden the average level is about 80 µg/L (386 nmol/L) in males and 60 µg/L (289 nmol/L) in females. Similar levels have been recorded in other parts of Scandinavia (4). In other areas, the levels may be considerably higher, up to an average of 200 µg/L (996 nmol/L) (102). In some population strata almost one fifth have levels above 250 µg/L (1200 nmol/L) (85).

(b) Published biological action levels

The current biological action levels for the lead concentration in blood in various countries have been summarized by Skerfving (4). In 1980 the WHO recommended a health-based maximum individual biological action level of 400 µg Pb/L (1932 nmol/L) blood (PbB) for male workers and a PbB of 300 µg/L (1450 nmol/L) for female workers of reproductive age, primarily to protect individual female workers and their offspring (107).

In 1982 the EEC issued a directive for the protection of workers exposed to lead. The maximum individual PbB was set at 700 µg Pb/L (3380 nmol/L) for all workers; a PbB of 800 µg Pb/L (3860 nmol/L) is accepted if, at the same time, the ALAU < 20 mg/g creatinine or the ZPP < 20 µg/g haemoglobin, or the ALA-D > 6 European Units.

The American Conference of Governmental Industrial Hygienists (ACGIH) has adopted a Biological Exposure Index (BEI) of 500 µg/L (2420 nmol/L) for lead in blood. This BEI corresponds to the current Threshold Limit Value (TLV) for lead of 0.15 mg/m^3 (time-weighted average). In 1994, the ACGIH published a notification of the intended change of the TLV to a time-weighted average concentration of 0.05 mg/m^3 with a corresponding BEI of 300 µg/L (1450 nmol/L) for lead in blood (108). The BEI was adopted in 1995.

In the USA, the standard of the Occupational Safety and Health Administration (OSHA) addressing occupational lead exposures requires monitoring of blood lead and zinc pro-

toporphyrin levels of potentially exposed workers every 6 months. Monitoring every 2 months is required, if a worker's blood lead levels reach 400 μg/L (1930 nmol/L) whole blood. If a worker's blood lead levels reach 600 μg/L (2900 nmol/L) or average 500 μg/L (2420 nmol/L) over three consecutive tests, that worker must be removed from work until the blood lead levels return to less than 400 μg/L (1930 nmol/L), based on monthly monitoring. (Code of Federal Regulations, Title 29, Part 1910.1025 Lead, US Government Printing Office, Washington, DC 20402-9328).

In Germany the Biological Tolerance Value in Biological Materials (BAT) is 700 μg/L (3380 nmol/L) for lead in blood for males and 300 μg/L (1450 nmol/L) for females below 45 years (109). In the United Kingdom, a similar limit is applied for men, while for women in childbearing age the limit is 400 μg/L (1930 nmol/L) (110).

In Sweden the employer has to investigate the cause of lead absorption, and take measures to decrease exposure, if the blood lead level is above 410 μg/L (2000 nmol/L). A worker who displays a blood lead level of more than 520 μg/L (2500 nmol/L) [310 μg/L = 1500 nmol/L for women less than 50 years of age] may not be employed in lead-exposed work until he/she has been examined medically and the blood lead level has decreased to <410 μg (2000 nmol)/L [250 μg = 1200 nmol/L for women less than 50 years of age]. The Labour Inspectorate may grant exemptions from the rules to exclude a worker from the work in case of a large body burden of lead, caused by previous long-term exposure (111).

In Finland, workforces in which any worker has Pb-B of 400 μg/L (1930 nmol/L) or more must be carefully monitored for potential health effects. A worker displaying a Pb-B of 500 μg/L (2420 nmol/L) or more cannot be employed for assignments involving lead exposure (112).

In Denmark and Norway the transfer level is 600 μg/L (2900 nmol/L) with no special regulation for women (4).

In France, workers with Pb-B above 400 μg/L (1930 nmol/L) have to be medically monitored, those above 600 μg/L (2900 nmol/L) are removed from exposure (113).

In Australia, the worker is removed from exposure at a Pb-B of 700 μg/L (3380 nmol/L) until work has been recommended by a physician (114). Further, there is a recommendation, that pregnant women should not be employed in work which exposes them to a risk of lead absorption. Also, "maternal B-Pb" should be maintained below 400 μg/L (1930 nmol/L). These are state-based limits.

In the United States the Centers for Disease Control (CDC) recently lowered the concentration of blood lead, considered harmful to young children, from 250 to 100 μg/L (from 1210 to 480 nmol/L) (115).

(c) Non-analytical interferences

The contribution of dietary and environmental intake of lead have been discussed above in section 3.3.7.5a.

(d) Sampling representative of recent or long-term exposure or biological effect

Table 3.3.2 summarizes the relationships between blood lead levels and lead-induced abnormalities (5).

Table 3.3.2. Summary of Lowest-Observed-Effect Levels for key lead-induced health effects in adults (5)

Lowest-observed-effect level (PbB)* µg/l	Haeme synthesis and haematological effects	Neurological effects	Effects on the kidney	Reproductive function effects	Cardiovascular effects
1,000–1,200		Encephalopathic signs and symptoms	Chronic nephropathy		
800	Frank anemia				
600		– – – – – – ↑		Female reproductive effects	
500	Reduced hemoglobin production	Overt subencephalopathic neurological svmptoms ↓		Altered testicular function ↓	
400	Increased urinary ALA and elevated copdporphyrins	Peripheral nerve dysfunction (slowed nerve conduction)	– – – – –	– – – – –	
300		– – – ↓ – – –			Elevated blood pressure (white males aged 40–59)
250–300	Erythrocyte protoporphyrin (EP) elevation in males				
150–200	Erythrocyte protoporphyrin (EP) elevation in females				
<100	ALA-D inhibition				?

3.3.8 Research needs

In spite of the fact that lead is the most extensively studied metal from the point of view of industrial toxicology, further research is still necessary. Further clarification of the effects of low long-term exposures to lead on neurobehavioural and immune system, as well as developmental disabilities in new-borns and young children are needed. The relevance of lead in serum measurements for biological monitoring should be studied. Many laboratories still experience difficulties with the lead in blood determination either because they use methods that are not fully optimized or because elements of their quality assurance programmes are compromised to accelerate sample throughput, which results in loss of data quality. Optimized methods and quality control programmes must be provided.

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3.4 Inorganic mercury

3.4.1. Introduction

Mercury occurs in a number of physical and chemical forms in three oxidation states: Hg^O (elemental or metallic mercury, Hg_2^{2+} (mercurous or monovalent mercury) and Hg^{2+} (mercuric or divalent mercury). Mercuric mercury also forms a number of organo-metallic compounds.

Exposure to mercury vapour is the most common form of occupational exposure to mercury, occurring in various industries. The main route of absorption is via the respiratory tract, the main effects are on the central nervous system (1).

Mixed exposure to aerosols of organic or inorganic mercury also occurs. Chlorine in combination with mercury vapour produced in chloralkali industries forms mercuric chloride aerosols. Exposure to aerosols of methyl- and ethylmercuric compounds has been described in connection with manufacture and use of mercuric salts in the chemical industry and in seed treatment. Disinfectant manufacturers, fungicide manufacturers, seed handlers and wood preservers may be exposed to organic mercury compounds. This chapter considers only occupational exposure to mercury vapours and inorganic mercury.

3.4.2 Physical-chemical properties

Mercury (Hg: CAS 7439-97-6, elemental mercury) is a silvery-white, odourless metal, volatile liquid at room temperature. Chemical and physical properties include:

Atomic mass	200.59
Specific gravity	13.5939 at 20°C
Melting point	-38.87°C
Boiling point	356.58°C
Vapour pressure	0.24 Pa at 25°C

Solubility: Mercurials differ greatly in their solubilities. Mercury vapours are considered to be nearly insoluble in water (60 µg/L at 30°C), more soluble in plasma and whole blood. Water soluble mercury compounds include chlorides, nitrates, chlorates, cyanides, fluorides and bromides. Water insoluble forms include oxides and sulphides. In general monovalent salts are less soluble than bivalent salts (e.g. mercurous chloride, 2 mg/L water, mercuric chloride, 69 g/L water).

Further information on physical and chemical properties may be obtained in e.g. CRC Handbook of Chemistry and Physics (2) and in the IARC Monographs, Volume 58 (3).

Conversion factors:
1 µg/L = 4.99 nmol/L
1 µmol/L = 200.6 µg/L
1 µmol/mol creatinine = 1.75 µg/g creatinine
1 µg/g creatinine = 0.57 µmol/mol creatinine

3.4.3 Possible occupational and non-occupational exposures

(a) Occupational exposure

Exposure to mercury vapour is the most common form of occupational exposure to mercury in various industries, e.g. mining, smelting and refining mercury and the use of liquid mercury. The manufacture of dry batteries and that of chlorine by brine electrolysis (chloralkali industry, where brine is electrolysed in mercury cells in which the cathode is a flowing sheet of liquid mercury) are potential occupational exposures. Liquid metallic mercury, used in the extraction of gold from ore concentrates or from recycled gold articles in uncontrolled processes, may result in high levels of exposure to workers and may have serious impacts on the environment (1). Exposure can also occur in the manufacture and repair of rectifiers, thermometers, vacuum pumps, transformers, mercury vapour lamps, barometers, manometers and mercurial thermostats in ovens. Broken instruments containing mercury can result in accidental exposure to high concentrations of mercury vapour. Dental personnel are exposed to mercury vapours through the use of dental amalgam.

IARC Monograph Volume 58 (3) summarizes data on exposure to elemental and inorganic mercury in air and the results of biological monitoring in various industries and occupations.

It should be noted that air concentrations of mercury detectable in the general work environment are generally lower than exposure levels of an individual worker measured with personal air sampling. This is due to the fact that mercury can accumulate on the clothes, hair and skin of workers, creating a situation which has been called "micro-environmental exposure".

b) Non-occupational exposures

Mercury is ubiquitous in the environment. People in the general environment are exposed to mercury via food, drinking water and air. Food products usually contain mercury below 20 µg/kg fresh weight (3). A large part of the mercury in food, at least in animal and fish products, is likely to be in the form of methylmercuric compounds. In areas of polluted water, levels of methylmercury in fish flesh may exceed 10 mg/kg (4). The intake from fish consumption (contaminated water) can rise to toxic levels, as occurred in Minamata and Niigata in 1953–1966. Mercury intake from non-fish sources is negligible. Intake from air and drinking is generally low.

Other sources of non-occupational exposures are medical and domestic use of mercury and its compounds. Inorganic and organic mercury are used as local disinfectants (5).

Mercury-containing cream and soap used for skin-lightening can lead to a significant internal exposure to mercury (1, 6). Mercury metal from broken thermometers, barometers and old mirrors are the most frequent sources of domestic mercury vapour exposure.

The release of mercury from dental amalgams provides a significant contribution to human exposure to inorganic mercury, including mercury vapour, in the general population (1, 7–11).

3.4.4 Summary of toxicokinetics

There are several reviews on the toxicokinetics of inorganic mercury in man (1, 5, 12–14).

3.4.4.1 Absorption

(a) Inhalation

Inhalation represents the main route of uptake of metallic mercury. About 80% of inhaled metallic mercury vapour is absorbed by the body and about 20% is retained (15–17). Absorption of inorganic mercury salts has not yet been systematically studied. In general, solubility enhances the absorption rate while size of the particles inversely relates to the absorption rate.

(b) Dermal

Elemental mercury is moderately absorbed through the skin (18). At exposures to 0.05 mg/m^3, the dermal penetration rate of mercury vapours is about 72 μg/cm^2/hr and the dermal uptake at whole-body exposure accounts for 2.2% of pulmonary uptake. Thus, the dermal uptake of vapours is not likely to affect the biological levels significantly. However, dermal contact with liquid mercury can significantly increase the biological levels.

There are no data to quantify percutaneous absorption of inorganic compounds of mercury. Its importance is limited in comparison to the pulmonary route.

(c) Gastrointestinal

Gastrointestinal uptake of metallic mercury is negligible. Less than 0.01% of ingested elemental mercury is absorbed (19). Absorption of inorganic (II) compounds from food is about 7% of the ingested dose, depending on the solubility in the stomach (19, 20). This intake is usually accidental or intentional.

3.4.4.2 Metabolic pathway and biochemical interaction

Absorption in the lungs is facilitated by two processes: 1) it is absorbed and circulated as elemental mercury and 2) it is oxidized in the lung tissues by hydrogen peroxide-catalase and enters the circulation as mercuric iron (13). In other tissues of the body, elemental mercury is also oxidized to ionic bivalent mercury. The oxidation is mediated mainly by

catalases and peroxidases (21). Deficiency of these enzymes may alter the distribution of mercury in the body. Studies by Ogata et al. (22, 23) showing that mercury vapour and mercuric chloride can both be transformed back and forth from oxidized mercury to elemental mercury illustrate the complexity of the biotransformation of mercury compounds. Mercury cations have a high affinity for reversible binding to sulphhydryl groups, it also binds to metallothionein (24).

3.4.4.3 Distribution

Following absorption, red cell uptake and physical dissolving of mercury vapour, mercury is rapidly transported to all parts of the body (25). Mercury vapour penetrates the brain and can cross the placenta (25, 26). In contrast, little mercuric mercury crosses the blood-brain barrier or placenta.

The principal sites of deposition of mercury are the kidneys and the brain after exposure to mercury vapour; the majority of mercuric mercury ends up in the kidney.

The biological half-time of mercury is in the order of two months in the kidneys, but is much longer in the central nervous system (1).

The distribution of mercury between erythrocytes and plasma depends on the form of the mercury. The erythrocyte to plasma ratio is approximately 1 for inorganic mercury (compared to approximately 10 for methylmercury).

3.4.4.4 Elimination

Elemental mercury is eliminated in the faeces, urine, expired air, sweat, saliva and milk. In long-term high exposure the kidney is the major pathway exceeding the faecal route. The smaller the exposure, the smaller the fraction excreted in urine and the larger the fraction excreted in faeces (27).

Large individual fluctuations in daily mercury excretion are common even when exposure conditions are the same. Urinary excretion indicates an elimination half-time of about 40 days (the range of individual values being 35–90 days) (15). In urine also a very small percentage of mercury (<0.1%) is excreted as elemental mercury (28).

The small amounts eliminated in breath show half-times of about 1 min and 18 hours (15, 16). In the case of perspiration the amount of mercury eliminated in sweat may be important (29).

There are differences in the elimination rate of mercury from individual organs (15, 30). The concentration of mercury in the brain of an individual was high even after the cessation of exposure, suggesting that the brain does not follow the same kinetics of elimination as the whole body (19).

3.4.5 Summary of toxic effects

A large number of reviews have been published (1, 4, 13, 14).

Following intense exposure to elemental mercury vapour, lung damage (bronchial irritation, erosive bronchitis, diffusive interstitial pneumonitis) occurs, as do gastrointestinal and renal tubular necrosis after ingestion of mercuric mercury.

Long-term exposure to elemental mercury can affect the central nervous system and kidney, chronic exposure to mercuric mercury causes renal tubular damage. Immunologically-based glomerulonephritis can occur. The characteristic tremor may begin in the fingers, eyelids, lips, or tongue. Slight tremor has been considered as an early sign of metallic mercury vapour exposure (31). The tremor is usually accompanied by severe behavioural and personality changes, memory loss, increased excitability, and in severe cases, delirium and hallucinations. This constellation of symptoms is called mercurial erethism.

Renal manifestations of mercury exposure range from proteinuria at low exposures through the nephrotic syndrome (32–34). Subclinical effects on the kidney have been detected through the measurement of urinary excretion of low and high molecular weight proteins and renal tubular enzymes (N-acetyl-β-D-glucosaminidase (NAG), β-galactosidase (GAL)) (34–38). The significance of these two enzymes contained in intracellular lysosomes to current and future health of the worker is uncertain (37).

A series of studies has attempted to correlate exposure measurements, such as blood and urine mercury concentrations with measurement of tremors, psychological and renal function (21, 39).

The IARC considered the evidence for carcinogenicity for metallic mercury and inorganic mercury compounds to humans to be inadequate (3).

3.4.6 Biological monitoring indices

There are several recent reviews on biological monitoring of occupational exposure to mercury (12, 13, 21, 40). Available biological monitoring indices are shown in table 3.4.1.

Table 3.4.1. Available biological monitoring indices

Indicator	Suitability for use	Reference
Mercury in blood (HgB)	Invasive, indicator of recent exposure	12, 13, 21, 40, 41
Mercury in urine (HgU)	Non-invasive, tends to reflect cumulative exposure over previous 2–4 months	12, 13, 21, 40, 41
Mercury in saliva	Good correlation with HgB and HgU. Lack of a standardized sampling technique and contamination from amalgam fillings limits its use	42, 43
Mercury in exhaled air	Non-invasive but further technical developments are needed; possibility of contamination from dental amalgams	_
Mercury in hair and nails	Unreliable, no standardized technique available	_

For the biological monitoring of mercury exposure at the workplace, mercury concentrations in whole blood and urine are the most important parameters (12, 13, 21, 40, 41).

To estimate the body burden and the toxicologically relevant pool of mercury the urinary excretion of mercury after the administration of a chelating agent can be determined. Mercury may be complexed by sodium 2,3-dimercaptopropane-1-sulphonate (DMPS) (44, 45) or by meso-2,3-dimercaptosuccinic acid (DMSA) (46, 47). Data on workers seem to confirm that, after a few days of cessation of occupational exposure to mercury vapour, the difference between the concentration of mercury in urine before and after the administration of DMSA mainly reflects the amount of mercury stored in the kidney (47). Molin's data (44) indicate that mercury mobilized after a single DMPS dose relates closely to exposure and is mainly an index of recent exposure. However, the chelating-agent provoked excretion of mercury cannot be recommended as a routine test for the biological monitoring of occupationally exposed workers.

3.4.7 Mercury in urine index

3.4.7.1 Toxicokinetics

The kinetics of urinary mercury is confounded by the effects of high individual variation in response to the same exposure when samples are collected at different times from the same individual and by day-to-day fluctuations of mercury excretion in urine. It was, however, found that the intra-individual variations can be reduced by collecting the urine sample always at the same time (e.g. in the morning) (48). Aitio et al. (49) have also shown that there is a diurnal pattern of excretion but that the daily rate of excretion is quite constant.

Furthermore, it is reported that correcting the mercury concentration for specific gravity or osmolality significantly reduced the variance in spot urine samples (49, 50). Barber

and Wallis (51) noted that correction for creatinine was more effective in reducing this variability. On the other hand, Araki et al. (52) clearly showed that mercury excretion rate is unaffected by urinary flow; whereas correction for creatinine excretion introduced increased variability, as creatinine excretion is affected by the urine flow rate and is highly variable itself due to the influence of diet.

Urine mercury measurements can only be reliable when the subjects have been exposed for a sufficiently long time to reach a steady state, and not have been off work for several weeks (53). There is a latency period in urinary excretion of mercury under occupational exposure conditions. Peak mercury in urine was not seen until 2–3 weeks after three days of high exposure to metallic mercury vapour (54). After long-term exposure the plateau is not reached until the kidneys have accumulated a certain amount of mercury, which can take 10 days for high exposure and six months for low exposure (55).

After removal of subjects from exposure, two phases of urinary excretion of mercury have been identified: the first phase lasts on average two days and accounts for not more than 20–30% of excretion in a steady state, the second phase has a half-time of 70 days (48). This confirms the experimental data obtained in human subjects with radio-labelled mercury, showing that mercury has a half-time of 64 days in the kidney (30).

According to Nakaaki et al. (55) and Barregård (54), the half-time of urinary excretion is about 40 to 50 days. Mercury concentrations in the urine of workers with mercury poisoning remain elevated for 100 days after the end of exposure (56).

3.4.7.2 Biological sampling

(a) Sampling time and specimen

As discussed in section 3.4.7.1 on toxicokinetics, urine samples from each worker should be collected at the same time of the day. Diurnal variation in mercury excretion appears to be unaffected by shift work (57). Sampling prior to the shift is recommended to reduce the opportunity of contamination.

(b) Contamination possibilities

It is important to avoid contamination of urine samples. Special cleaning procedures (cleaning with 1 mol/L nitric acid, rinsing three times with ultrapure water and drying at room temperature) and the use of disposable plastic containers (polyethylene, polypropylene) have been recommended.

(c) Sampling device and container

To avoid mercury absorption by the container wall the urine specimens, Pyrex[R] glass, polycarbonate and Teflon[R] containers should be used (58). Acidification with nitric acid (1 mol/L) or acetic acid (1 mL to 100 mL urine) can further reduce adsorption on container walls.

(d) Preservation, shipment and stability

Urine samples taken into plain untreated polycarbonate or polystyrene bottles are stable without loss of mercury up to one week when stored under refrigeration and for many weeks deep frozen at -20°C. Some authors have recommended adding 1-cysteine to complex the mercury and aid stability (59). It is important to avoid bacterial growth in urine, as bacteria may reduce some mercury compounds to elemental mercury, which might give rise to significant losses of mercury by volatilization.

The sampling and storage of urine have been discussed in detail by Clarkson et al. 1988 (13).

3.4.7.3 Recommended analytical methods

Cold vapour atomic absorption (CV-AAS) is the most popular and reliable technique. Inorganic mercury, or both inorganic and organic mercury in biological or environmental specimens, is converted by reducing agents to elemental mercury and released as mercury vapour (hydride system), which is either directly pumped through the quartz cell of the atomic absorption spectrophotometer or analysed after amalgamation on a Au/Pt gauze. The total mercury content is determined using $NaBH_4$ or $CdCl_2$-$SnCl_2$ as reducing agents, while only the inorganic mercury content of the sample is released by reduction with $SnCl_2$ alone. The organic mercury content of the sample is given by the difference between total and inorganic mercury (60, 61).

The amalgamation technique results in an increase in the sensitivity of the method, as well as the associated reduction of background interferences. The hydride system with amalgamation device is commercially available.

For routine analysis of urine samples, the total mercury content is determined using sodium borohydride as the reducing agent. A time-consuming digestion of the samples is thus unnecessary. A method based on this principle is selected for recommendation (62).

(a) Principle of the method

The determination of mercury in urine is carried out directly by atomic absorption spectrometry with the so-called cold vapour method. A commercially available hydride system with amalgamation device (gold/platinum gauze) is used for the analysis after reduction of the sample with sodium borohydride. The method determines the total mercury content of the processed material. The quantitative evaluation is carried out by means of aqueous standards dissolved in water.

(b) Reagents required

All chemicals must be analytical grade or purer. Some suppliers deliver reagents specially prepared for mercury analysis. Sodium borohydride, sodium hydroxide, monohydrate; 95–97 % sulphuric acid with guaranteed maximum mercury content of 0.0000005%; 65% nitric acid; potassium permanganate with guaranteed maximum mercury content of 0.000005%; 1-Octanol, pure silicon anti-foaming agent (e.g. Antifoam

110 A from Dow Corning).

The following reagents should be prepared:
Acid solution (nitric acid/sulphuric acid): equal volumes of nitric (15 g/L) and sulphuric acid (15 g/L) are mixed.
Anti-foaming agent: the silicon anti-foaming agent is diluted 100 times with ultrapure water.
5% Aqueous potassium permanganate solution
3% Sodium borohydride in 1% sodium hydroxide solution. This solution must be freshly prepared at least every second day. Unused sodium borohydride solution must be decomposed in a fume cupboard by drop-wise addition of dilute sulphuric acid.

Standards: Calibration standards containing 0.5, 2.0, 10.0, 40.0 and 100 µg Hg/L in acid solution (nitric acid/sulphuric acid) are prepared. The standards must be freshly prepared each day.

(c) Equipment required

Atomic absorption spectrometer with background correction at 253.7 nm
Hydride system with amalgamation device, interchangeable cartridge and chart recorder
Mercury hollow cathode lamp, preferably ED lamp with power supply

(d) Procedure and calibration

As a rule, a 1 mL sample of the acidified urine specimen is analysed. If the mercury level does not lie within the linear range of the method the specimens must be diluted appropriately.

The reaction vessel of the hydride system is rinsed with ultrapure water before use. The urine sample is pipetted directly into the reaction vessel and 100 µL saturated potassium permanganate solution together with 10 mL acid solution is added. After adding 50 µL 1-octanol or the anti-foaming agent the reaction vessel is connected to the hydride system. A reagent blank is included in each analytical series. The aqueous calibration standards are treated and analysed as for the urine samples.

Atomic absorption spectrometer
Wave length	253.7 nm
Background correction	Deuterium lamp or Zeeman
Spectral slit width	0.7 nm
Lamp current	According to manufacturer's instructions

Hydride system: The settings of the hydride system vary from one type of instrument to another. The optimum time settings must be ascertained by each operator on his own instrument. The following time programmes have proved reliable and are intended as a guide.

Table 3.4.2. Time-programmes intended as a guide

Analytical step	Step duration (sec)	Procedure
Reaction	15	Addition of sodium borohydride solution
Purge I	40	Release of the free metallic mercury and its transfer into the amalgamation device
Manual heat	15	Heating the gold/platinum gauze filter and vaporization of the mercury
Purge II	40	Transfer of the mercury vapour into the quartz cuvette
Cooling	50	

(e) Criteria of analytical reliability

i) Trueness
The trueness based on recovery studies was 99%.

ii) Precision
The within-series precision was 1.9–6.2 % relative standard deviation at concentrations from 6.4 to 270 µg/L (32–1350 nmol/L).

The between-day precision was 3.1–4.9 % relative standard deviation at concentrations of about 30 µg/L (150 nmol/L).

iii) Detectability
The limit of detection was 0.3–0.4 µg/L (1.5–2 nmol/L).

(f) Quality assurance

Results of intercomparison programmes have shown that there are still considerable problems with the determination of mercury in urine (63, 64). It is important to employ a rigid quality control programme both internally and externally.

Dahl and coworkers 1990 (65) demonstrated that no detectable concentration changes occurred with lyophilized control material stored at -20°C during a 5-year period. However, the assigned mercury concentrations are valid only for the first day after reconstitution. The addition of gold to the solution minimizes mercury loss and increases the usable time for the solution to up to 8 days.

For **internal quality control** commercially available control material from e.g. Bio Rad Lab., Anaheim, California, USA – Lyphocheck[R] Urine Metals Control and Nycomed AS, Oslo, Norway – Seronorm[TM] Trace Elements Urine can be used.

For **external quality control** (inter)national intercomparison programmes are available from e.g. Centre de Toxicologie du Québec, Canada (63), and the German Society of Occupational and Environmental Medicine, Erlangen, Germany (64) and the United Kingdom National External Quality Assessment Scheme (UKNEQAS), Sheffield, UK.

(g) Source of possible errors

i) Pre-analytical

Special precautions are necessary to avoid sample contamination from the workplace, from work clothing and from storage containers. Losses by volatilization and absorption may also occur and care should be taken to minimize these (see section 3.4.7.2 on biological sampling).

Urine samples that have been stored at low temperatures (1–4 °C) or deep frozen contain precipitates which can adsorb mercury. Nitric or hydrochloric acid should be added to the thawed contents to elute any mercury from the container or from the surface of any precipitate.

ii) Analytical

Only reagents of the purest grade commercially available should be used. Reagent blanks must be measured in each run of analysis. Urine foams considerably on addition of the sodium borohydride solution. A large amount of foam production results in poor precision of the method, reduction of peak height and the possibility of contamination of the whole apparatus. Tests of various anti-foaming agents have demonstrated that 1-octanol and silicon anti-foaming agents are equally effective.

Unusually large blank values can be observed when cold vapour AAS analysis for total mercury follows analysis for inorganic mercury (66).

(h) Reference to the most comprehensive description of the method

Angerer J, Schaller KH. (Eds). Analyses of Hazardous Substances in Biological Materials. Methods for Biological Monitoring, Vol. 2., VCH Verlagsgesellschaft mbH, Weinheim, Germany, 1988.

(i) Evaluation of the method

The comparison of the cold vapour AAS method with a polarographic method showed a good agreement (r= 0.982, slope of the linear correlation curve was 1.06).

The accuracy of the urine analyses was also demonstrated by comparison with a standard reference urine from NIST. A value of 49 µg/L (245 nmol/L) was obtained for a given urine concentration of 49.8±4.2 µg/L (249±21 nmol/L), and an average value of 93 µg/L (460 nmol/L) was found for an expected value of 105±8 µg/L (520±40 nmol/L). The accuracy of the method can be improved by a vapour-injection calibration method (67).

3.4.7.4 Other analytical methods

For the determination of mercury in urine many modifications of the AAS method exist (68–71). Estimation can follow acid digestion which also converts organic mercury to inorganic mercury. Oxygen bomb combustion or pyrolysis, e.g. in a graphite furnace, can be substituted for acid digestion (72–74). An automatic method for quantification of mercury in urine, blood, and plasma based on digestion overnight with nitric acid and perchloric acid and the use of a mercury monitor was reported by Vesterberg (75). Recently, Welz et al. 1992 (76) developed a complete automatic on-line system for the determination of total mercury in urine with microwave sample pretreatment and flow injection CV-AAS.

The method described by Magos and Cernik (60) allows the inorganic and organic mercury in biological fluids to be quantified separately. While only inorganic mercury is released from the sample by tin (II) chloride ($SnCl_2$) total mercury (inorganic and organic-bound) is released by a tin (II) chloride-cadmium chloride reagent ($SnCl_2$-$CdCl_2$). Therefore in two different analyses with CV-AAS (one with $SnCl_2$ and the other with $SnCl_2$-$CdCl_2$) the concentrations of inorganic mercury and total mercury can be determined. The concentration of organomercury can be calculated as the difference. Other methods are neutron activation analysis, X-ray spectrometry, colorimetry (dithizone method), and anodic stripping voltammetry (1, 77). Recently, atomic fluorescence spectrometry has been applied to the determination of mercury in biological materials (78).

3.4.7.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

The measured background value depends on the analytical procedure and on the living environment. It is generally agreed that in persons non-occupationally exposed to mercury the excretion is less than 5 µg/L or 5 µg/g creatinine (2.9 µmol/mol creatinine) (56, 79). In 380 Italian subjects not occupationally exposed to mercury, the mean urinary level was 3.5 µg/L (range: 0.1–6.9 µg/L) [17 nmol/L, range: 0.5--34 nmol/L] (80).

(b) Published biological action levels

WHO (19) recommended for long-term exposure a health-based occupational exposure limit of 50 µg/g creatinine (29 µmol/mol creatinine) in order to prevent symptoms of central nervous system dysfunction. This value has been proposed also by Buchet et al. 1980 (46) as a biological threshold limit value.

The BEI-committee of the American Conference of Governmental Industrial Hygienists recommends a urine concentration of 35 µg/g creatinine (20 µmol/mol creatinine) for mercury in urine as a BEI for urine specimens collected preferably in the morning, prior to the shift at the end of a workweek (39). Because of the cumulative effect associated with the deposition of mercury in the kidneys, the BEI should be applied cautiously if the occupational exposure to mercury is shorter than 6 months. Owing to the large daily variability of urine levels, monitoring must be systematic and occasional high levels

should not be the cause for alarm. Correction for creatinine is recommended. Methods for determining only inorganic (elemental and ionic) mercury must be used if comparison with the BEI is to be made.

In Germany the Biological Tolerance Value in Biological Materials (BAT) for mercury in urine is 200 µg/L (1 µmol/L), measured as total mercury (41).

A study by Soleo et al. (81) looked at the effects of low exposure to inorganic mercury on psychological performance. They have suggested that the TLV-TWA for mercury should be lowered to 0.025 mg/m^3 and that the biological urinary exposure indicator for biological monitoring should be 25 µg/L (125 nmol/L).

Yamamura (56) considers inorganic mercury concentrations in urine above 50 µg/L (250 nmol/L) as a sign of increased absorption and above 100 µg/L (500 nmol/L) as a warning level. Lauwerys (82) estimates that a urinary concentration above 50 µg/L (250 nmol/L) is a warning sign and a value above 100 µg/L (500 nmol/L) justifies removal from exposure.

(c) Non-analytical interferences

i) Exposure to other chemicals (the same metabolite), coexposure, ethanol intake, medication
D-penicillamine which is the main biodegradation product of penicillin type antibiotics, enhances urinary excretion of mercury (21, 83).

ii) Diet and environment
Sources of non-occupational exposure have been described in section 3.4.3. Dental restorations (amalgam fillings) and the use of local disinfectants containing mercury will transiently increase the urinary excretion of mercury (84–86).

Since mercury in fish is mainly methylmercury, which is not excreted through the kidney, the dietary habits do not influence the concentration of mercury in urine.

(d) Sampling representative of recent or long-term exposure

Urinary mercury can be used as an indicator of internal dose. Several studies have demonstrated that there is a correlation between the intensity of recent exposure and urine mercury in workers who have previously been exposed for 6–12 months. However, urine mercury tends to reflect cumulative exposure over the previous 2–4 months. High intra-individual variation requires the average of several mercury determinations in urine. Monitoring of mercury in urine is useful for assessing the risk of adverse effects and the need for preventive measures (87). Under steady-state exposure conditions there is also a relationship between urinary and blood concentration of mercury. However, urinary mercury cannot be used to assess the dose in the critical organ, i.e. in the brain, because the average life of mercury deposited in the brain is much longer than in other organs and therefore mercury can remain in the brain after cessation of exposure (12).

(e) Ethnic differences (enzyme deficiency, environment, diet)

In persons with catalase deficiency, a rare occurrence in Caucasians, biological monitoring may give spurious results (88).

3.4.8 Mercury in blood index

3.4.8.1 Toxicokinetics

Absorbed elemental mercury is dissolved in the blood. In volunteers for a single exposure to mercury vapour the maximum concentrations in erythrocytes was reached immediately after the end of exposure and then declined with a half-time of 74 hours. On the other hand, plasma concentrations rose very slowly, reaching maximal values 20 hours after exposure, and then declined with a half-time of 85 hours (30). After 3 days of high exposure (>100 $\mu g/m^3$) to metallic mercury vapour, Barregård (54) observed a first phase of blood mercury decrease with a half-time of 3.1 days and a slower decrease with a half-time of 18 days.

After long-term exposure to mercury vapour, the decrease in blood mercury can be described by two half-times: one of 2–4 days, accounting for about 90% of the absorbed mercury, and another of 15–30 days, accounting for most of the remainder. The existence of a third compartment with a still longer half-time, however, cannot be excluded (13).

After protracted occupational poisoning with mercury, the decline in the mercury concentration in blood was triphasic with half-times in plasma of 10.28 and 105 days and in erythrocytes of 7.27 and 170 days (89).

Duration and intensity of exposure to metallic mercury seem to influence the biological half-times in blood.

Adequate data for the toxicokinetics of inorganic mercury compounds in blood are not available.

3.4.8.2 Biological sampling

(a) Sampling time and specimen

Blood may be collected at any time during the workday.

(b) Contamination possibilities

Care should be taken to clean the skin adequately when taking blood samples to avoid contamination. The arm of the worker must not be disinfected with agents containing mercury.

(c) Sampling device and container

Blood samples are best collected in disposable sampling devices, i.e., Vacutainer[R] or

Monovette[R]. EDTA-K is preferred as an anticoagulant. The container and the anticoagulant used must be free of mercury contamination. Random analyses should be used to check these supplies for contamination.

(d) Preservation, shipment and stability

The blood samples can be stored for a few days in the refrigerator, for longer periods storage in the deepfreezer is recommended.

3.4.8.3 Recommended analytical procedure

Suitable analytical methods for the determination of mercury in blood are based on cold vapour atomic absorption spectrometry (60–62, 75, 90). The determination is based on the same principle as the methods for urine analyses. The total mercury content in blood is determined using sodium borohydride, or a tin(II) chloride-cadmium chloride mixture as reducing agents (61, 62). While only inorganic mercury is released from the blood samples by tin (II) chloride (60), the organic mercury content of the sample is given by the difference between total and inorganic mercury.

A method, based on sodium borohydride reduction as described for urine analysis is selected for recommendation (62).

(a) Principle of the method

The determination of mercury in blood is carried out directly by cold vapour atomic absorption spectrometry. A commercially available hydride system with amalgamation device (gold/platinum gauze) is used for the analyses after reduction of the sample sodium borohydride. The method determines the total mercury content of the blood sample. The quantitative evaluation is carried out by means of standards dissolved in blood.

(b) Reagents required

See section 3.4.7.3 b

(c) Equipment required

See section 3.4.7.3 c

(d) Procedure and calibration

For the mercury determination 1 mL whole blood is pipetted into the reaction vessel of the hydride system. The reaction vessel must have previously been rinsed well with ultrapure water. 10 mL acid solution, 200 µL 1-octanol and 100 µL saturated potassium permanganate solution are then added and the reaction vessel is connected to the hydride system. For blood mercury levels which lie outside the linear range of the method, appropriately smaller sample volumes (500 or 200 µL) are employed. The sample volume is made up to 1 mL with ultrapure water. A reagent blank is included in each analytical series.

Calibration curves established with aqueous standards have a different slope from curves plotted for standards made up with whole blood. Therefore matrix-matched standards are required for calibration. It is sufficient to prepare a calibration curve based on pooled normal human blood since the curves obtained with a variety of blood samples are strictly parallel to the curve obtained with pooled blood.

For each point on the calibration curve, 1 mL of pooled blood is mixed with 1 mL of an aqueous calibration standard, 9 mL acid solution and 200 µL 1-octanol. The mixture is analysed as described above. In parallel, the blood without the addition of the mercury-containing calibration standard is analysed.

Atomic absorption spectrometer
Wave length   253.7 nm
Background correction   Deuterium lamp or Zeeman
Spectral slit width   0.7 nm
Lamp current   According to manufacturer's instructions

Hydride system: The settings of the hydride system vary from one type of instrument to another. The optimum time settings must be ascertained by each operator on his own instrument. The following time programmes have proved reliable and are intended as a guide.

Table 3.4.3. Time-programmes intended as a guide

Analytical step	Step duration (sec)	Procedure
Reaction	20	Addition of sodium borohydride solution
Purge I	40	Release of the free metallic mercury and its transfer into the amalgamation device
Manual heat	15	Heating the gold/platinum gauze filter and vaporization of the mercury
Purge II	40	Transfer of the mercury vapour into the quartz cuvette
Cooling	50	

(e) Criteria of analytical reliability

i) Trueness
Trueness, as based on recovery studies, was 98%.

ii) Precision
The within-series precision was 1.7–3.2% relative standard deviation at concentrations from 3.0 to 98.0 µg/L (15–490 nmol/L). The between-day precision was 3.5% relative standard deviation at concentrations from 40.5 to 77.3 µg/L (202–390 nmol/L).

(f) Quality assurance

It is important to employ a rigid quality control programme, both internally and externally. For **internal quality control** blood specimens in three concentration adjustments are available from Nycomed AS, Oslo, Norway (Seronom™ TE Whole Blood).

For **external quality control** (inter)national intercomparison programmes are initiated by e.g. Centre de Toxicologie du Québec, Canada (63) and the German Society of Occupational and Environmental Medicine, Erlangen, Germany (64).

(g) Sources of possible errors

Contamination is of major concern in the pre-analytical phase, as well as in the analytical phase. The sources of errors are identical with the problems mentioned for the mercury urine index. In addition, mixing each sample before an aliquot is taken for analysis is critical because of unequal distribution of mercury between erythrocytes and plasma.

(h) Reference to the most comprehensive description of the method

Angerer J, Schaller KH. (Eds). Analyses of Hazardous Substances in Biological Materials. Methods for Biological Monitoring, Vol. 2. VCH Verlagsgesellschaft mbH, Weinheim, Germany, 1988.

(i) Evaluation of the method

The comparison of this method with another cold vapour method using a digestion of the blood with sulphuric acid and potassium permanganate showed a good agreement ($r= 0.996$).

3.4.8.4 Other analytical methods

For the speciation of mercury in blood (inorganic and organic) the method by Magos is recommended (60, 61). Field experiences of Magos' method has led to several modifications and improvements (90).

Automated methods for the determination of mercury in blood have been reported by several authors (75, 91, 92). Other analytical techniques for the routine determination of mercury in blood have not gained any importance.

3.4.8.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

The baseline levels for mercury in blood specimens from the general population should usually be established for each laboratory. Levels of mercury in blood for those not occupationally exposed are currently given as less than 5 µg/L (25 nmol/L) (79). Minoia et al. (80) observed mean blood values of 5.3 µg/L, range: 1.7–9.9 µg/L (27 nmol/L, range: 8.5–50 nmol/L) in 368 Italian subjects not occupationally exposed to mercury.

To establish reference values for mercury concentrations in whole blood, blood cells and plasma, 97 publications in the international scientific literature have been critically reviewed by Brune et al. (93). They categorized subjects according to their level of fish consumption and found 95th percentile levels for blood mercury of up to 83 µg/L (415 nmol/L) in groups of people with the highest fish consumption. The mean level in low fish eaters was around 5 µg/L (25 nmol/L) .

(b) Published biological action levels

The BEI Committee of the American Conference of Governmental Industrial Hygienists (1994–1995) has adopted an inorganic mercury concentration of 15 µg/L (75 nmol/L) for blood specimens collected at the end of the shift at the end of the workweek (39). Methods determining only inorganic (elemental and ionic) mercury must be used if comparison with the BEI is to be made. The BEI carries a sufficient margin of safety for protecting workers against signs of any known effect of mercury.

In Germany the Biological Tolerance Value in Biological Materials (BAT) is 50 µg/L (250 nmol/L) in blood, measured as total mercury (41).

According to Roels et al. (31) a blood mercury concentration of 18 µg/L (90 nmol/L) corresponds to the biological action level of 50 µg/g creatinine (29 µmol/mol creatinine) proposed by WHO 1980. End of shift blood samples and urine from the following morning are the basis for this correlation.

(c) Non-analytical interferences

Diet and environmental and non-occupational exposure can affect the measurements. Sources of non-occupational exposure have been described in section 3.4.3. The most important are attributed to fresh dental amalgams or the use of disinfectants containing organomercury which can be biodegraded to inorganic mercury.

As described above, a significant amount of mercury can be ingested in food, especially with fish consumption, and dietary habits may well influence the concentration of mercury in blood. Yamamura (56) measured total mercury concentrations in blood samples from Japanese subjects and found a mean of 54±22 µg/L blood, (270±110 nmol/L) of which 30% is inorganic mercury and 70% is organic mercury.

Probably due to competition for the reaction site on catalase there is the possibility of an apparent decrease in blood mercury in the presence of ethanol. Absence of catalase activity would also result in a lower concentration of mercury in blood (89, 94, 95).

(d) Sampling representative of recent or long-term exposure

According to the BEI Committee (21) mercury in blood is an indicator of recent exposure. Blood concentrations of mercury are mainly influenced by exposure to mercury during recent days (40). A good correlation between intensity of the recent exposure and mercury in blood has been demonstrated (31). Under long-term exposure conditions (more than 6 months) there is also a relationship between blood or urinary concentration

of mercury in workers not consuming fish regularly (28, 40, 93). Weaker correlations between mercury in blood and urine can be partly explained by diurnal fluctuations of urinary mercury excretion and exposure periods less than 6 months (21).

For exposure to organic mercury compounds, the only test generally accepted as valid for biological monitoring is mercury in blood, which is an indicator of exposure that can integrate the quantity of mercury absorbed by inhalation with the quantity absorbed through skin (12).

3.4.9 Research needs

There are a lot of good studies, with both occupational and laboratory exposures, relating the mercury levels in blood and urine to airborne mercury exposures. However, there are few studies which relate low internal exposure to early health effects. Effects of low level long-term exposures to metallic and inorganic mercury on neurobehavioural and renal systems needs further clarification. Controlled studies need to be performed to study the behaviour of mercury in blood and urine in relationship to duration of exposure and evaluation of the relationship between the indicators of internal dose and integrated exposure (dose x time). An adequate compartment model for disposition of inorganic mercury in man should be developed.

3.4.10 References

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Chapter 4. Selected solvents

4.1 Carbon disulfide

4.1.1 Introduction

Carbon disulfide is a widely used industrial solvent. Its principal use is in the production of rayon and cellulose. It is used as an intermediate in the production of carbon tetrachloride and some pesticides and as a solvent in the rubber industry. Exposure occurs primarily by inhalation, although significant skin absorption is possible. Carbon disulfide is a neurotoxin, and has been associated with cardiovascular effects primarily due to its effects on lipids. There are reviews on the toxicology of carbon disulfide (1–4).

Published biological action limits are based on the relationship between airborne exposure and the level of the urinary carbon disulfide metabolite, 2-thiothiazolidine-4-carboxylic acid (TTCA). The BAT value is based on the relationship between TTCA in urine and health effects.

4.1.2 Physical-chemical properties

Carbon disulfide (CAS 75-15-0) is a liquid at room temperature.
Molecular formula CS_2
Molecular weight   76.14
Specific gravity   1.263 at 20°C
Vapour pressure   The saturated vapour pressure is 40 kPa at 20°C.
Solubility: It is highly soluble in blood and fat and moderately soluble in urine and water (2.9 g/L at 20°C) (1). The blood-gas partition coefficient is 2.2 (5). The log of the octanol/water partition coefficient is 2 (2).

Conversion factors:
CS_2 1 mg/L = 0.0131 mmol/L; 1 mmol/L = 76.14 mg/L.
          1 mg/m³ = 0.321 ppm; 1 ppm = 3.11 mg/m³
TTCA   1 mg/g creatinine = 0.7 mmol/mol creatinine
          1 mmol/mol creatinine = 1.4 mg/g creatinine
          1 mg/L = 0.006 mmol/L
          1 mmol/L = 163 mg/L

4.1.3 Possible occupational and non-occupational exposures

Occupational exposure to carbon disulfide occurs during the manufacture of rayon fibres, carbon tetrachloride, pesticides, vulcanizers and other chemicals. Exposure is usually by the inhalation route although significant exposure can occur through the skin (6).

Non-occupational exposure to carbon disulfide is unlikely. However, exposure to substances that can be metabolized to carbon disulfide, such as the pesticide Captan (7–9), the anti-alcoholic drug disulfiram (Antabuse, tetraethylthiuram disulfide), some rubber accelerators, and dithiocarbamates, e.g. Thiram (tetramethylthiuram disulfide), may result in the appearance of carbon disulfide and its metabolites in the body and excreta (10).

4.1.4 Summary of toxicokinetics

4.1.4.1 Absorption

(a) Inhalation

Carbon disulfide is absorbed via the lungs and skin. Pulmonary absorption is the most significant. Pulmonary retention at the start of exposure is about 80% and declines steadily, reaching a plateau within 2 hours of about 40% of the inhaled concentration (1, 5).

(b) Dermal

Extensive dermal absorption of vapour (11) and liquid (12) carbon disulfide was observed in experimental animals. The physical and chemical properties also predict a significant dermal absorption (penetration rate = 0.89 mg/cm^2/hr [0.012 mmol/cm^2/hr]). The penetration rate measured in volunteers immersing a hand in an aqueous solution of carbon disulfide varied between 0.23 and 0.79 mg/cm^2/hr [0.002–0.010 mmol/cm^2/hr] (13). Higher absorption was indicated in earlier papers (14). Available evidence suggests that percutaneous absorption may add to the adverse effect induced by occupational exposure (15).

(c) Gastrointestinal

Gastrointestinal absorption has been reported in case reports (2).

4.1.4.2 Metabolic pathways and biochemical interaction

Carbon disulfide is extensively metabolized. Three metabolites were identified in urine of exposed workers: 2-mercapto-2-thiazolinone-5 (16), thiocarbamide (17), and 2-thiothiazolidine-4-carboxylic acid (TTCA) (18, 19). The latter metabolite has been shown to have activity in the iodine-azide test (19) and has been used to evaluate high exposures to carbon disulfide (18, 20–22). TTCA has been shown to represent about 2–6% of the carbon disulfide absorbed during occupational exposure (20, 23). Figure 4.1.1 shows the metabolic pathways of carbon disulfide in humans.

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\begin{array}{c}
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\parallel \\
H_2N-C-NH_2
\end{array}
$$

THIOCARBAMIDE

EXPIRED AIR ◄——————— CS₂ ——————►

H₂C————CH—COOH
| |
S N–H
‖
S

2–THIOTHIAZOLIDINE–
4–CARBOXYLIC ACID
(TTCA)

2–MERCAPTO–2–THIAZOLINONE–5
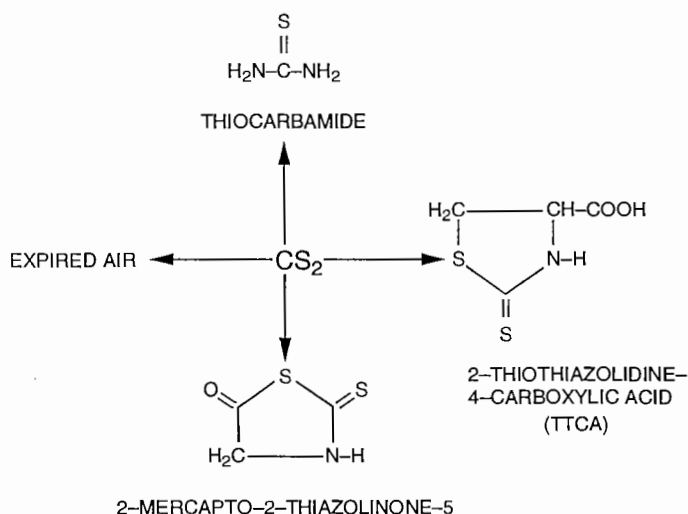
Figure 4.1.1 Metabolic pathways of carbon disulfide in humans

4.1.4.3 Distribution

Carbon disulfide is widely distributed through the blood to all tissues of the body. The greatest concentrations are found in lipid-rich tissues, such as brain and liver. There is evidence of carbon disulfide binding to albumin and amino acids in erythrocytes (1).

4.1.4.4 Elimination

The main excretory pathway of carbon disulfide is the kidney. Less than 1% of the absorbed dose is excreted unchanged in urine (5). Following an inhalation exposure, 5% to 30% of the absorbed dose is exhaled (1). About 3% of the amount absorbed via skin was found in breath of volunteers exposing their hands to aqueous solutions of carbon disulfide (13). One study in volunteers showed that the pulmonary elimination of carbon disulfide is rapid with a half-life of about 10 minutes (24). Another study indicates biphasic elimination with half-lives of 1 minute and 110 minutes (25). However, carbon disulfide was found in the breath of workers 16 hours after the end of high exposure (20), which indicates a triphasic elimination.

Carbon disulfide is present in blood in both a free and bound form (1). Studies in rats show that a free carbon disulfide in blood is eliminated within 1 day, while a bound carbon disulfide, released upon acidification of the blood is eliminated over 2 to 3 days (26, 27). Bound carbon disulfide was also found in blood of workers in a viscose rayon plant (20). The slow elimination phase of carbon disulfide and moderate accumulation over the workweek is probably associated with bound carbon disulfide.

4.1.5 Summary of toxic effects

Carbon disulfide is a potent neurotoxin. Peripheral neuropathies, cranial nerve dysfunction and neuropsychiatric changes characterize the neurologic illness associated with

excessive carbon disulfide exposures. These neuropsychiatric symptoms may be irreversible. Chronic long-term exposure may result in central nervous system involvements and peripheral neuropathies, decreased glucose tolerance, reduced serum thyroxine levels, ocular injury, central and peripheral neuropathies, increased triglycerides and low density lipoproteins, and parkinsonism. Increases in incidence of atherosclerosis, coronary artery disease, suicide rates, personality changes and hypertensive disease have been reported from epidemiological studies (1, 2, 6, 28–30).

4.1.6 Biological monitoring indices

Table 4.1.1 lists biological monitoring indices that have been suggested for monitoring occupational exposure to carbon disulfide.

Table 4.1.1. Biological monitoring indices for carbon disulfide

Indicator	Suitability for use	Ref
TTCA in urine	Best indicator of exposure	31–35
Iodine-azide test in urine	Insensitive below TWA exposures of 30 mg/m^3	6, 21, 34, 36
Carbon disulfide in urine	Poor, limited data	37
Carbon disulfide in blood	Limited data on utility	5
Bound carbon disulfide in blood liberated after acidification of blood	Poor, limited data in humans	20
Carbon disulfide in expired air	Poor, limited occupational exposure data	20
Antabuse test (measure of dithiocarbamates in urine following administration of Disulfiram)	Poor, invasive test not recommended for routine screening	38

TTCA in urine is the most widely used biological indicator of exposure to carbon disulfide. The American Conference of Governmental Industrial Hygienists (ACGIH) Biological Exposure Indices (BEI) committee have established a BEI for TTCA (31–32). The German Science Foundation has also established a Biological Tolerance Value for Working Materials (BAT) value for TTCA (33).

The iodine-azide test, first described by Vasak et al. (39) was used extensively in the past (6, 21, 34, 35, 36, 38, 40). However, the test is not responsive at currently accepted exposure limits (1, 20), and the iodine-azide tests is not recommended as an index of exposure to carbon disulfide.

The measurement of carbon disulfide in urine and blood collected immediately after exposure has been suggested (5, 37). Acid-labile carbon disulfide in blood may also be useful in assessing carbon disulfide exposure (20). These indices are not recommended

because of the lack of reliable data in humans. Breath analysis is not suitable for exposure monitoring (20).

Djuric (38) has recommended the administration of disulfiram to workers and measurement of diethylthiocarbamates in urine as an exposure test for carbon disulfide. This test is not recommended since it involves dosing the worker with a drug in order to assess exposure.

4.1.7 TTCA in urine index

4.1.7.1 Toxicokinetics

A study in six volunteers, exposed to carbon disulfide concentrations of 31.1 mg/m^3 or 62.2 mg/m^3, indicates that TTCA appears in urine shortly after the start of exposure and the concentration peaks at the end of exposure. The concentration then declines, with a half-life of about 2 hours (41). A similar excretion was observed in workers with occupational exposure to carbon disulfide (18, 20, 22). Following high exposures, TTCA was found in urine collected from workers 16 and 64 hours after the last workweek exposure (22); this suggests that the excretion is at least biphasic. Recent pharmacokinetic studies have confirmed the biphasic excretion with half-lives of 6 hours and 68 hours respectively (23).

4.1.7.2 Biological sampling

(a) Sampling time and specimen

Urine samples should be collected at the end of the workshift near the end of the workweek following routine exposures during the past week.

(b) Contamination possibilities

Sample contamination from the workplace is not likely.

(c) Sampling device and container

Urine can be collected in any clean container.

(d) Preservatives, shipment and stability

Stability studies have shown that TTCA is stable for at least three days at room temperature, for at least 1 week at 10°C and for at least 3 months at -20°C (8, 9, 20, 42, 43).

4.1.7.3 Recommended analytical method

High-performance liquid chromatography (HPLC) with ultraviolet detection at 273 nm is the method of choice for the determination of TTCA in urine (18). The determination of creatinine is suggested as many reference values are quoted as creatinine corrected data (32). In practice, the use of creatinine is best used to assess the dilution of the urine.

If urine samples have creatinine levels below 0.3 g/L (2.9 mmol/L), an additional urine sample should be collected.

(a) Principle of the method

Urine is acidified and TTCA extracted with diethyl ether. A methanol solution of the residue from the ether extract is analysed by HPLC using a reverse phase column and UV detector. TTCA is eluted from the HPLC column using a mixture of two solvents. UV detection is at 273 nm. Standards can be prepared in urine and carried through the procedure or in methanol or water and injected directly.

(b) Reagents required

TTCA (commercially available), peroxide-free diethyl ether, methanol, glacial acetic acid, and sodium chloride are the only reagents required.

(c) Equipment required

An HPLC equipped with gradient solvent programming and a variable wavelength UV detector is required. A 150 mm reverse phase column (C-18) is used for separation. In addition, extraction tubes, a tube shaker, water bath and centrifuge are required for sample preparation.

(d) Procedures including calibration

i) Calibration
Since no certified standards are available, the purity of purchased or synthesized standards must be verified. Standards are diluted in methanol for calibration of the HPLC over the range of 0.002 mol/L to 0.3 mol/L. Ogata and Taugchi (44) have also shown that standards can be prepared in blank urine or in water with quantitative recovery.

ii) Procedure
Two-mL of urine is extracted with 8.0 mL of diethyl ether in a tube containing sodium chloride and 0.1 mL of 5 mol/L HCl. After shaking for 15 min, the tubes are centrifuged, and the ether layer removed and evaporated to dryness on a water bath. The residue is taken up with 0.2 mL methanol and 0.05 mL injected onto the HPLC. A solvent switching programme is required and utilizes 5 min of solvent A (95% water, 4% methanol and 1% glacial acetic acid), 5 min solvent B (99% methanol, 1% glacial acetic acid) followed by 5 min of solvent A. TTCA is detected with a UV detector set at 273 nm. **Caution:** Diethyl ether should be handled in a fume hood, with precautions taken to avoid the formation of peroxides.

(e) Criteria of analytical reliability

i) Trueness
Trueness, as assessed by recovery studies done with blank urine spiked with TTCA was reported by the author to be 52±3% for 12 determinations (range not stated) (18). Recoveries reported by most other authors ranged from 80–90% (8, 20, 23).

ii) Precision

Precision has been reported by others to be 12% relative standard deviation for day-to-day precision and between 2 and 5% relative standard deviation for within-day precision (20, 23).

iii) Detectability

The estimated detection limit was 5×10^{-7} mol/L (0.082 mg/L), quite adequate for detection of occupational exposures below 0.1 ppm (0.31 mg/m^3) (18, 20, 45).

(f) Quality assurance

i) Special precautions

Quality control must be performed within the laboratory, using portions of pooled urine collected from exposed workers, as no external proficiency testing programmes are currently available.

ii) Interferences

Interferences have not been reported using this method.

(g) Sources of possible errors

i) Pre-analytical

Urine samples must be collected at the end of the shift, since the initial elimination half-life of TTCA is rapid. Contamination of urine samples during collection and processing is unlikely.

ii) Analytical

Ogata and Taguchi (44) reported that traces of peroxides in diethyl ether destroy TTCA so ethyl ether must be checked to ensure that it is peroxide-free.

(h) Reference to most comprehensive description of the method

Van Doorn R, Delbressine LPC, Leijdekkers ChM, et al. Identification and determination of 2-thiothiazolidine-4-carboxylic acid in urine of workers exposed to carbon disulfide. *Archives of Toxicology* 1981;47:51–8 (18).

(i) Evaluation of the method

No comprehensive method evaluation was found, although the method and its many modifications are in wide use.

4.1.7.4 Other analytical methods

TTCA has also been determined by automated HPLC with UV detection (44); by HPLC with electrochemical detection (46); by HPLC with UV detection following separation by affinity chromatography (47); and by gas chromatography with flame photometric detection (9). Several reports of minor modifications of the method of van Doorn (18) have also been reported (8, 20, 23).

4.1.7.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

TTCA has not been found in the urine of workers without occupational exposure to carbon disulfide (18).

(b) Published biological action levels

The American Conference of Governmental Industrial Hygienists recommend a Biological Exposure Index (BEI) of 5 mg TTCA/g creatinine (3.5 mmol/mol creatinine) for urine samples collected at the end of a workshift. The BEI corresponds to an 8-hour time-weighted average exposure at 10 ppm (31 mg/m^3), the current Threshold Limit Value (TLV) (32).

The German Science Foundation Biological Tolerance Value for Working Materials (BAT) recommends a BAT of 8 mg/L (0.049 mmol/L) for a urine sample collected at the end of the workshift. This value corresponds to a MAK of 10 ppm (31.1 mg/m^3), however, BATs are based on health effects, not exposure (33).

Individual authors have also reported on the relationship between TTCA in urine and carbon disulfide in air. Lauwerys and Hoet report a Tentative Maximum Permissible Concentration of 5 mg/g creatinine (3.5 mmol/mole creatinine) (35); Campbell et al. report a TTCA concentration of 4 mmol TTCA/mol creatinine (5.7 mg TTCA/g creatinine) corresponds to an 8-hour TWA exposure to 10 ppm (31 mg/m^3) (20); Riihimäki et al. report a TTCA concentration of 4.5 mmol/mol creatinine (6.4 mg/g creatinine) corresponds to an 8-hour TWA exposure to 31 mg/m^3 in samples collected near the end of the workweek (23).

Note that all of these biological action levels, with the exception of the BAT value, are based on the relationship between exposure and level of TTCA in the urine. Dermal exposure to liquid carbon disulfide, as well as workload (breathing rate) will alter the relationship between concentrations of carbon disulfide in air and TTCA in urine (45).

Recent reports have shown neurotoxic sequelae at long-term exposures less than 31 mg/m^3. These reports generally indicate no adverse effects at carbon disulfide levels below 12 mg/m^3 (4 ppm) (48, 49). Existing biological action levels, which are based on 31 mg/m^3 (10 ppm) TWA exposures may not be protective.

(c) Non-analytical interference

i) Exposure to other chemicals (the same metabolite), co-exposure, ethanol intake, medication

Workers undergoing anti-alcoholic treatment with the drug, disulfiram should not participate in biological monitoring of occupational exposure to carbon disulfide. Disulfiram has been shown to be metabolized to carbon disulfide and TTCA. Humans treated with 250 mg of disulfiram excrete up to 0.49 mg TTCA/g creatinine (0.34 mmol/mol creatinine) 10 hours after dosing (10). Exposures to other dithiocarbamates, such as

Thiram and rubber accelerators metabolized to carbon disulfide, may also result in the excretion of TTCA. However, the experimental evidence has not been reported. TTCA has been identified as a metabolic product of Captan (7–9).

ii) Diet and environment
No information was located in the literature regarding effects of diet and environment on TTCA excretion.

(d) Sampling representative of recent or long-term exposure or biological effect

The measurement of TTCA in urine collected at the end of the shift is suitable for monitoring same day exposure to carbon disulfide. There is an indication of build-up of TTCA during the workweek (50). Therefore, the measurement may reflect not only the exposure during the day of sampling but may also reflect the overexposure on previous days.

(e) Ethnic differences (enzyme deficiency, environment, diet)

No information was located regarding ethnic differences in metabolism of carbon disulfide.

4.1.8 Research needs

There are good studies, with both occupational and laboratory exposures, relating the concentrations of TTCA in urine to airborne carbon disulfide exposures. There are also good studies, again from both occupational and laboratory exposure, relating carbon disulfide exposures to neurotoxic effects. However, there are few studies which relate the concentration of TTCA in urine to neurotoxic effects.

Carbon disulfide exposures can occur through dermal contact with liquid solvent. Controlled laboratory studies need to be performed to determine the extent of exposure from dermal exposure and the influence of dermal exposure on urinary TTCA concentrations.

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4.2 N,N-Dimethylformamide

4.2.1 Introduction

N,N-Dimethylformamide (DMF) is extensively used as a solvent in artificial leather production (1). This chemical is also present as a polar solvent in some solvent preparations (2–3). Comprehensive reviews on toxicology and biological monitoring are available (1, 4–6). As it is shown below, N-Monomethylformamide (MMF) is the major metabolite used in biological monitoring.

4.2.2 Physical-chemical properties

N,N-Dimethylformamide (DMF; CAS 68-12-2) is a liquid at room temperature.

Molecular formula $HCON(CH_3)_2$
Molecular weight   73.09
Specific gravity   0.949 at 20°C
Vapour pressure   The saturated vapour pressure is 0.36 kPa at 20°C.
Solubility   The logarithm of the octanol/water partition coefficient is -0.87.

Conversion factors:
DMF   1 mg/m^3 = 0.333 ppm; 1ppm = 3.0 mg/m^3 at 25°C.
N-monomethyl-   1 mg/L = 0.017 mmol/L; 1 mmol/L = 59.07 mg/L
formamide (MMF)

4.2.3 Possible occupational and non-occupational exposure

Exposure of workers in factories, especially in association with artificial leather production is well documented (7–10). General population exposure to this solvent should, however, be quite unlikely.

4.2.4 Summary of toxicokinetics

4.2.4.1 Absorption

(a) Inhalation

The major route of exposure to DMF is via inhalation, with a retention in the lungs of 72% (11).

(b) Dermal

In addition, this solvent can be readily absorbed through intact skin (7, 12). Experiments on volunteers suggest that penetration of liquid DMF through the skin usually contributes substantially more to the total intake than percutaneous penetration of DMF vapours (13).

4.2.4.2 Metabolic pathways and biochemical interaction

When absorbed, DMF is oxidized in the body to N-(hydroxymethyl)-N-methylfor-mamide (DMF-OH), which is the major metabolite in urine (figure 4.2.1). DMF-OH, when analyzed by gas chromatography, is heat-decomposed to be detected as N-monomethylformamide (MMF) (7, 14; figure 4.2.1). A small amount of MMF formed *in vivo* is also excreted in urine (15).

$(CH_3)_2NCHO$
N,N-Dimethylformamide (DMF)
$\downarrow$
CH_2OHCH_3NCHO
N-Hydroxymethyl-N-methylformamide
$\downarrow$
CH_3NHCHO
N-Monomethylformamide (MMF)
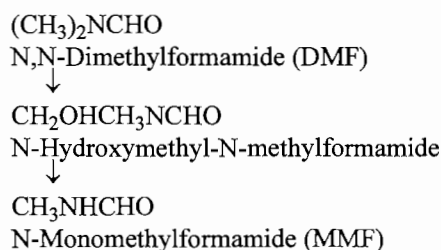
Figure 4.2.1. Schematic metabolism of DMF (simplified from ref. 7 and 14).

4.2.5 Summary of toxic effects

The primary toxic effect of DMF, after repeated occupational exposure, is on the liver, resulting in elevation of, e.g. aspartate and alanine aminotransferases (ASAT and ALAT) (16, 17) coupled with histopathological changes (18). Such liver damage may be reflected in the reduced alcohol tolerance among heavy drinkers (7, 19).

4.2.6 Biological monitoring indices

Available biological monitoring indices are given in table 4.2.1. Only MMF in urine is recommended for biological monitoring.

Table 4.2.1. Available biological monitoring index

Indicator	Suitability for use	Ref
MMF in urine	Non-invasive and sensitive	(10)

4.2.7 MMF in urine

4.2.7.1 Toxicokinetics

This is summarized in section 4.2.4.

4.2.7.2 Biological sampling

(a) Sampling time and specimen

Urine samples should be collected at the end of a workshift, but on any work day of a week, because DMF has a short biological half-time (20).

(b) Contamination possibilities

As the analyte is a metabolite of DMF, there is little possibility of contamination and no special precautions are necessary when collecting the sample.

(c) Sampling device and container

Urine samples should be collected into clean containers.

(d) Preservative, shipment and stability

No preservatives are needed. Samples should be refrigerated if they cannot be analysed on the day of collection.

4.2.7.3 Recommended analytical method

(a) Principle and calibration

Urine samples containing DMF-OH and MMF are introduced to a gas chromatograph (GC) equipped with a thermionic detector. DMF-OH is decomposed by heat in the injection port and detected as MMF. Methods have been described by various authors (7, 10, 13, 21). The following is one example (10).

(b) Reagents required

Monomethylformamide as a standard and methanol are the only reagents required.

(c) Equipment required

A GC equipped with a thermionic detector and, if available, a data processor are required. An automatic liquid sampler is useful, if available. Helium is required as carrier gas and hydrogen as fuel gas. A clinical centrifuge is also needed.

(d) Procedure

A capillary column [ULBON HR-20M (30 m in length, 0.53 mm internal diameter, and 1 μm in film thickness)] is used. Both the injection port and the detectors are heated at 250°C. Helium as a carrier gas is allowed to flow at 25 mL/min, and the supply of H_2 and air to the detector is at 60 kPa (0.6 kg/cm^2) and 50 kPa (0.5 kg/cm^2), respectively. For analysis, 1 mL of the urine sample is mixed with 1 mL of methanol, and 1 μL of the supernatant fraction after centrifugation (e.g. at 600 x g for 10 minutes with a clinical centrifuge) is introduced to the GC. The GC oven temperature is kept at 90°C for 1 minute, increased at a rate of 5°C/min to 150°C, then 20°C/min to 200°C, where it is kept for 5 minutes, followed by cooling down to the initial temperature of 90°C.

Standards are prepared by adding monomethylformamide to blank urine samples which are then taken through the analytical procedure with the samples.

(e) Criteria for analytical reliability

i) Trueness
Trueness, based on recovery studies, was 98%.

ii) Precision
The day-to-day precision of the analysis at 0.17 mmol/L (10 mg/L) was 6% relative standard deviation.

iii) Detectability
The estimated detection limit of MMF in urine was 0.0017 mmol/L (0.1 mg/L).

(f) Quality assurance

i) Special precautions
No external proficiency testing programmes are available. Internal quality control is necessary to ensure quality results.

(g) Sources of possible errors

The use of a thermionic detector (sensitive to detect nitrogen) allows higher sensitivity, whereas a flame ionization detector (FID) is not sensitive enough. The injection port temperature is another important factor for better sensitivity. One experience (10) showed that the heat-decomposition at the port reached a plateau when the port was kept at 250°C (which is unusually high for GC analysis). The amount of MMF detected when the port temperature was set at 200°C was estimated to be a quarter of that at 250° C.

(h) Reference to the most comprehensive description of the method

Kawai T, Yasugi T, Mizunuma K, Watanabe T, Cai S-X, Huang M-Y, Xi L-Q, Yao B-Z, Ikeda M. Occupational dimethylformamide exposure. 2. Monomethylformamide excretion in urine after occupational dimethylformamide exposure. International Archives of Occupational and Environmental Health 1992;63:455–60.

(i) Evaluation of the selected method

The method is specific to DMF exposure and sensitive enough to detect occupational levels of DMF exposure.

4.2.7.4 Other analytical methods

Other procedures for GC analysis are available (7, 13, 21).

4.2.7.5 Guide to interpretation

Occupational health surveys have shown that MMF in end-of-shift urine samples corre-
late significantly with time-weighted average DMF exposure (7–10).

(a) Measured values in groups without o ccupational exposure

MMF levels without occupational exposure are very low [e.g. 0.003 mmol/L (0.2 mg/L
urine) (10)].

(b) Published biological action levels

The American Conference of Governmental Industrial Hygienists (22) has adopted a
value of 40 mg/g creatinine (77 mmol/mol creatinine) for MMF urine samples collected
at the end of a work-shift as a 'Biological Exposure Index (BEI)'. This value is set taking
both inhalation and skin absorption into consideration. It is proposed to reduce the value
to 20 mg/g creatinine (38 mmol/mol creatinine) (23).

Deutsche Forschungsgemeinschaft (24) has adopted a value of 15 mg/L (0.26 mmol/L)
for MMF in urine samples collected at the end of exposure, or at the end of several
workshifts, as a 'Biological Tolerance Value' (BAT-value).

(c) Non-analytical interference

i) Exposure to other chemicals (the same metabolite), co-exposure, ethanol intake,
   medication
In a human volunteer experiment (25), it was observed that the intake of 19 g ethanol
(50 mL of 38% gin) reduced *in vivo* biotransformation of DMF. The observation sug-
gests that alcohol intake may be a confounding factor of DMF biological monitoring.

ii) Diet and environment
No reported interferences.

4.2.8 Research needs

Effects of ethanol intake on the metabolism and toxicity of DMF need to be confirmed
in occupational health studies.

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4.3 2-Ethoxyethanol (EGEE) and 2-Ethoxyethyl acetate (EGEEA)[1]

4.3.1 Introduction

EGEE and EGEEA are common solvents used in the semiconductor photoresist process. They are also used as solvents for epoxy paints and dyes used in the leather industry. Non-occupational exposure is not common, although some may occur in countries where EGEE and EGEEA use is not restricted. These two solvents are teratogens in animals and are implicated as hematological and reproductive toxins in humans from epidemiological investigations. There are reviews on the toxicology of EGEE and EGEEA (1, 2).

There are limited human data on the relationship between human exposure and biological monitoring indices. The biological action levels reported are based on human data. The source of the biological action level determines the basis of that number. The Biological Exposure Index (BEI) is based on the relationship between human exposure and levels of urinary metabolites. The BAT is based on a safety factor approach of the reported health effects and levels of urinary metabolites. Methods reported are well documented and published in peer-reviewed publications.

4.3.2 Physical-chemical properties (2, 3)

2-ethoxyethanol (EGEE, CAS 110-80-5) and 2-ethoxyethyl acetate (EGEEA, CAS 111-15-9) are present in the air of the workplace as vapours.

Molecular weights   EGEE 90.1; EGEEA 132.2

Molecular formulas   EGEE: $CH_3CH_2\text{-}O\text{-}CH_2CH_2OH$

     EGEEA: $CH_3CH_2\text{-}O\text{-}CH_2CH_2O\overset{\displaystyle O}{\overset{\|}{\text{-}C}}\text{-}CH_3$

Specific gravities   EGEE 0.926; EGEEA 0.975
Vapour pressures   Saturated vapour pressures are EGEE 0.49 kPa; EGEEA 0.3 kPa at room temperature.

Solubility: EGEE and EGEEA are miscible with water. They are soluble in non-polar solvents. Partition coefficients for EGEE at body temperature are: blood/gas 22,093; water/gas 23,069; oil/gas 962; oil/water 0.042 (3). EGEEA is more soluble in oil and less soluble in water with corresponding partition coefficients of: oil/gas 4860,

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[1]These two glycol ethers are considered together because they have the same toxicity, and because EGEEA is metabolized rapidly to EGEE. The metabolite responsible for the reproductive toxicity, 2-ethoxyacetic acid is a common metabolite of both EGEE and EGEEA.

water/gas 3822, and oil/water 1.271. The blood/gas partition coefficient for EGEEA cannot be determined because EGEEA is immediately hydrolyzed to EGEE (3).

Conversion factors:
2-Ethoxyacetic Acid (EAA):

    1 mg/g creatinine = 1.09 mmol/mol creatinine
    1 mmol/g creatinine = 0.917 mg/g creatinine
    1 mg/L = 0.0096 mmol/L
    1 mmol/L = 104 mg/L

EGEE 1 ppm = 3.69 mg/m^3; 1 mg/m^3 = 0.27 ppm
EGEEA 1 ppm = 5.41 mg/m^3; 1 mg/m^3 = 0.19 ppm

4.3.3 Possible occupational and non-occupational exposures

Occupational exposure to EGEE and EGEEA is declining due to concern for its possible reproductive effects in humans. Exposures are common in the manufacture of semiconductors. These chemicals are also commonly used as solvents because of their unique solubility in water and in oils. EGEE and EGEEA are common ingredients of surface coatings particularly those based on epoxy resins, and as solvents used for cleaning printing ink formulations (4).

Non-occupational exposure to EGEE and EGEEA is unlikely. Use of these solvents in household products has been largely discontinued.

4.3.4 Summary of toxicokinetics

4.3.4.1 Absorption

(a) Inhalation

EGEE and EGEEA are absorbed via the dermal and respiratory routes, dermal being the major route of absorption in the workplace. Measurements of EGEE concentrations in mixed-exhaled air of volunteers exposed to 18.5 mg/m^3 (5 ppm) EGEE indicate that pulmonary retention 30 minutes after the start of inhalation exposure accounts for about 64% of the inhaled amount, and remained at 64% at the end of a 4-hour exposure (5). Retention for volunteers exposed for 30 minutes to 18.5 mg/m^3 (5 ppm) EGEEA was 64%, declining to 57% at the end of a 4-hr exposure (5).

(b) Dermal

The high solubility in water and lipids indicates a significant dermal absorption of liquid EGEE and EGEEA. The predicted penetration rate for EGEE is 1.20 mg/cm^2/hr (log P = -0.54); penetration rate for EGEEA is unpredictable due to its immediate hydrolysis to EGEE. The dermal penetration rate of EGEE and EGEEA measured experimentally *in vitro* in human abdominal skin were essentially the same (0.796 mg/cm^2/hr, and 0.800 mg/cm^2/hr respectively (6). It can be predicted, that dermal contact with liquid or concentrated solutions raises the biological levels of 2-ethoxyacetic acid significantly above

the levels reached during inhalation exposure at 18.5 mg/m^3 (5 ppm) (1). Dermal absorption of dilute aqueous solutions of EGEE and EGEEA were reported to be higher than for pure EGEE and EGEEA (1). Low saturated vapour pressure, high water solubility, and high boiling point indicate that vapours of both compounds can condense on body surfaces and thus facilitate dermal absorption of vapours (1).

(c) Gastrointestinal

Gastrointestinal absorption has been reported only for accidental or intentional ingestion of glycol ethers. A case study reported extensive absorption of 2-butoxyethanol following ingestion of 250 mL of window cleaner (7).

4.3.4.2 Metabolic pathways and biochemical interaction

The metabolic pathways of EGEE and EGEEA in humans are shown in figure 4.3.1. The major metabolite in man is 2-ethoxyacetic acid (EAA), which is considered to be the cause of testicular effects (8) and embryotoxicity in rats (9) at doses equivalent to effects produced by EGEE and EGEEA. Hematological effects, also related to metabolism of EGEE and EGEEA were reported in animals and possibly in humans (2, 10). At exposures permissible in the workplace, metabolism is a first order process, and the relationship between pulmonary uptake and biological levels of metabolites is linear, assuming no dermal absorption (11, 12).

```
                          EGEEA
                            |
                            | 100%
         0.4%               |
EXPIRED AIR <---------- EGEE
                            |
                            | 35%
                            |
                        EAA in urine
                            |
                            | (in rats)
                            |
        (CO2  + OXALIC ACID)
```
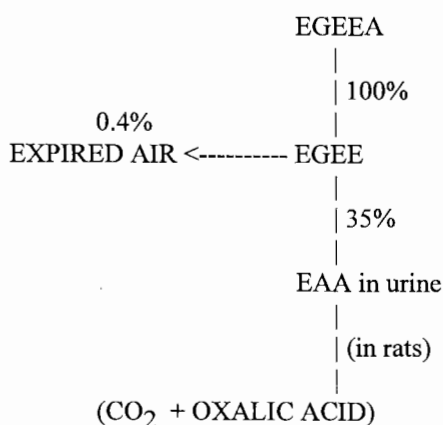
Figure 4.3.1. Metabolic pathways of EGEE and EGEEA in man. Oxalic acid and carbon dioxide, shown in parentheses were only found in animals dosed with radiolabeled glycol ethers. The percentage indicates the fraction of the absorbed amount excreted by the indicated pathway during and following a four-hour exposure of humans to 18.5 mg/m^3 (5 ppm) of EGEE or EGEEA (11, 12).

4.3.4.3 Distribution

EGEE and EGEEA are rapidly metabolized and do not accumulate in fatty body tissues. EGEE is present in low quantities in circulating blood immediately following exposure, but is eliminated via the kidney appearing in the urine as metabolites. Any EGEEA entering the blood stream is immediately hydrolyzed by esterases to EGEE.

4.3.4.4 Elimination

EGEE is eliminated unchanged in exhaled air; EGEEA is eliminated as EGEE after hydrolysis. The pulmonary elimination which represents a small fraction of uptake (<0.5% for 4-hr exposures) is biphasic (5, 13). Metabolism is the major elimination pathway for both glycol ethers. The total amount excreted in urine as metabolites accounts for about 35% of the amount of EGEE and EGEEA absorbed during a 4-hr exposure for a urine collection period of 40 hours. This would correspond to a total elimination of more than 70% when the collection period was extrapolated to infinity.

4.3.5 Summary of toxic effects

EGEE and its acetate have been shown to be reproductive toxins producing reduced sperm counts in several animal species, and are considered by NIOSH and other organizations to be probable human reproductive toxins (10, 14–15). Hematological effects in humans have been reported following chronic exposure to EGEE. In addition, high chronic exposures may cause liver, kidney and lung damage (2).

4.3.6 Biological monitoring indices

Table 4.3.1 lists biological monitoring indices found for EGEE and EGEEA reported for assessment of human exposure. No other biological monitoring indices have been recommended.

Table 4.3.1. Biological monitoring indices for EGEE and EGEEA

Indicator	Suitability for use	Ref
2-Ethoxyacetic acid in urine	Best indicator of exposure	2, 16–18

4.3.7 2-Ethoxyacetic acid (EAA) in urine index

4.3.7.1 Toxicokinetics

EAA appears in the urine shortly after the start of exposure and its concentration steadily rises during exposure. The concentration usually peaks 4–8 hours after the end of exposure and remains measurable for several days (11, 12, 19, 20). The decline of EAA concentration was reported as monophasic with an apparent elimination half-life of 42 hrs, measured in human volunteers (1, 19) or 48 hrs, measured in workers (19, 20). The elimination is probably multiphasic with a longer delayed half-life since workers have

measurable EAA in their urine after returning from a two-week holiday (20). The metabolites accumulate over the workweek as shown by steadily increasing concentrations in urine specimens collected during a full workweek of occupational exposure (20).

4.3.7.2 Biological sampling

(a) Sampling time and specimen

The elimination kinetics indicate that urine samples should be collected after the last workshift at the end of the week as a quantitative estimation of weekly exposure. Where exposures are intermittent, urine samples should be collected at the end of the shift or after exposure.

(b) Contamination possibilities

Since EAA is not present in the workplace, no special precautions are necessary to avoid contamination of the sample.

(c) Sampling device and container

Urine specimens should be collected in clean containers.

(d) Preservatives, shipment and stability

Decomposition of EAA during storage or transportation is possible and should be prevented by using hydrochloric acid or thymol as a urine preservative. Samples should be stored at 4°C and analyzed within two weeks after collection. Samples are stable for at least eight months if kept frozen at -20°C (21).

4.3.7.3 Recommended analytical methods

Gas chromatography with flame ionization detection is the method of choice. The method utilizes lyophilization of urine, pentafluorobenzylbromide (PFBB) derivitization and internal standards (22). The determination of creatinine is suggested as some reference values are quoted as creatinine corrected data (16). In practice, creatinine is best used to assess the dilution of the urine. If urine samples have creatinine levels below 0.3 g/L (2.65 mmol/L), an additional urine sample should be collected.

Caution should be exercised in the handling of EGEE and EGEEA standards as these have been shown to be reproductive toxins to animals and are suspect reproductive toxins to humans. PFBB is a lacrimator and should be handled in a fume hood.

(a) Principles of the method

Urine is neutralized with potassium hydroxide, frozen and lyophilized to remove water. 2-EAA is extracted with methanol and derivitized with pentafluorobenzyl bromide. The derivative is extracted with dichloromethane and chromatographed on a GC-FID using a fused silica capillary column. Standards are prepared in urine and carried through the procedure. 3-Chloro-propionic acid is used as an internal standard.

(b) Reagents required

2-ethoxyacetic acid, 3-chloro-propionic acid, pentafluorobenzylbromide (PFBB), di-chloromethane, methanol, and 5 mol/L KOH are the only reagents required.

(c) Equipment required

A gas chromatograph equipped with a flame ionization detector and capillary split/splitless injection system is required. In addition to hydrogen and air for the detector, helium is required as a carrier gas. The column is a 25 m CP Sil 5 fused silica capillary column. A freezer and a lyophilizer are required for sample preparation. Heating blocks and derivitization vials are required, along with pH paper for pH adjustment.

(d) Procedure and calibration

i) Calibration

Standards are prepared in urine over the concentration range of 0.1–200 μg/L. Fifty μg of 3-chloro-propionic acid internal standard is added, in 50 μL water, to one mL of standard in urine. Standards are carried through the entire procedure. The ratio of standard to internal standard peak areas are plotted against standard concentration. Results are read from the linear curve.

ii) Procedure

Urine is adjusted to pH 7 with 5 mol/L potassium hydroxide. One mL of urine and 50 μg of internal standard in 50 μL of water are added to a 1.5 mL reaction vial. Samples are frozen and lyophilized overnight. The dry residue is dissolved in 350 μL of methanol containing 5% (v/v) PFBB, the vials capped and placed in a heating block at 90°C for 3 hours. After cooling, 350 μL of distilled water is added and the mixture extracted with 350 μL of dichloromethane. Five μL of the organic layer are injected into the gas chromatograph with the split ratio set at 5:1. Gas chromatographic conditions are: injector and detector, 230°C; oven programmed from 85–160°C at 5°C/min with a post run hold of 7 minutes at 265°C. The helium carrier gas flow rate is 1 mL/min.

(e) Criteria of analytical reliability

i) Trueness

Recoveries averaged 94.8% over the range of 15–150 mg/L (0.14–1.4 mmol/L).

ii) Precision

Overall average precision was ±3.5% relative standard deviation (RSD) over 0.1–200 mg/L (0.00096–1.92 mmol/L) with a range of 1.1% RSD at 25 mg/L (0.24 mmol/L) to 20% RSD at 0.1 mg/L (0.00096 mmol/L).

iii) Detectability

The limit of detectability was 30 μg/L (0.29 μmol/L) at a signal to noise ratio of 5.

(f) Quality assurance

i) Special precautions
No external proficiency testing programmes are available. Internal quality control using split samples, pooled urine and blanks is necessary to insure quality results.

ii) Interferences
None reported.

(g) Sources of possible errors

i) Pre-analytical
The recovery of derivitized 2-ethoxyacetic acid was over 100% because of the partial solubility of the dichloromethane solutions in 50:50 methanol:urine.

ii) Analytical
None specified by the authors.

(h) Reference to the most comprehensive description of the method

Groeseneken D, et al. An improved method for the determination in urine of alkoxyace-tic acids. International Archives of Occupational and Environmental Health 1989;61: 249–54.

(i) Evaluation of the method

The method was optimized for pH, reaction time, derivitization reagent concentration, derivitization yield, and extraction efficiency. The method was also compared with the methanol extraction method (23) and found to have a correlation coefficient (r) of 0.973 and $P < 0.001$.

4.3.7.4 Other analytical methods

Two other methods are equally acceptable. They both use extraction, derivitization with PFBB, an internal standard, and electron capture detection (24, 25). Characteristics of these two methods are compared with the recommended method in table 4.3.2. The de-tectability limits for these methods are less than 1 mg/L (0.0096 mmol/L). Other gas chromatographic methods for the determination of EAA in urine, using derivitization, and FID detection (21, 23) have also been reported.

Table 4.3.2. Other recommended analytical methods *

	Groseneken, 1989 (23)	Johanson, 1989 (25)	Bergerow, 1990 (24)
Principle of the method	Lyophilize, derivatize with PFBB, analysis by GC-FID	Extract, derivitize with PFBB, analysis with CP GC-ECD	Extract, derivitize with PFBB, analysis with CP GC-ECD
Reagents required	EAA, PFBB, dichloro-meth-ane, methanol, KOH	EAA, PFBB, DCM, tetrabutyl-ammo-nium,	EAA, PFBB, H_2SO_4, DCM
Equipment required	Lyophilizer, GC-FID, CP Sil 5 fused silica capillary column, 25 m.	GC-ECD, Fused silica, CP Sil-5, 25 m.	GC-ECD, Fused silica DB-1 CP column, 60 m.
Calibration/ internal standard	Peak area ratios to 3-chloro-propionic acid (internal stan-dard)	Peak area ratios to 2-pentoxyacetic acid (internal standard)	Peak area ratios to isopropoxy acetic acid (internal standard)
Procedure outline	Alkalinize urine, freeze, ly-ophilize, extract with metha-nol, derivatize with PFBB, extract with methylene chlo-ride, analyze	Buffer urine, extract with dichlorometh-ane, derivatize, ana-lyze.	Acidify urine, extract with dichloromethane, dry, derivatize, ana-lyze.
Analytical reliability			
Trueness	Recovery: 95%	Not stated	Recovery: 65%
Precision	Average: ±3.5% over 0.1–200 mg/L (0.00096–1.92 mmol/L)	8.4% using 2-pen-toxyacetic acid as internal standard	3.3–11.5% at 29, 58 and 116 mg/L(0.28, 0.56, and 1.11 mmol/L)
Limit of detectability	Less than 100 µg/L (0.96 µmol/L)	230 µg/L (2.2 µmol/L)	100 µg/L (0.96 µmol/L)
Quality Assurance special precautions	None: EAA not present in workplace or in normal diet	None: EAA not pre-sent in workplace or in normal diet	None: EAA not pre-sent in workplace or in normal diet
Interferences	None reported, high blanks possible	None for EAA	None reported
Sources of errors			
Pre-analytical	None specified	None specified	None specified
Analytical	None specified	None specified	None specified
Evaluation	Compared with methanol ex-traction method (17)	None reported	None reported

* Abbreviations used: gas chromatography, GC; Flame ionization detector, FID; Electron capture detec-tor, ECD; Pentafluorobenzylbromide, PFBB; Capillary, CP, Dichloromethane, DCM

4.3.7.5 Guide to Interpretation

(a) Measured values in groups without occupational exposure

EAA is not a product of endogenic metabolism, a component of the diet, or present in the environment, therefore none is expected in urine from individuals with no occupational exposure.

(b) Published biological action levels

The American Conference of Governmental Industrial Hygienists recommend a Biological Exposure Index (BEI) of 100 mg EEA/g creatinine (109 mmol/mol creatinine) for urine samples collected at the end of the workshift at the end of the week. The BEI is based on the 8-hour time-weighted average exposure at 5 ppm (18.5 mg/m^3), the current Threshold Limit Value (TLV) (16).

The German Science Foundation Biological Tolerance Value for Working Materials (BAT) recommends a BAT of 50 mg/L (0.48 mmol/L) for a urine sample collected at the end of exposure. This value corresponds to a MAK of 75 mg/m^3 (20 ppm), however, BATs are based on health effects, not exposure (17).

Lauwerys and Hoet report a Tentative Maximum Permissible Concentration of 150 mg/g creatinine (163 mmol/mol creatinine) based on an 8-hour TWA of 18.5 mg/m^3 (5 ppm) (18).

Dermal exposure to liquid EGEE or EGEEA as well as workload (breathing rate) will alter the relationship between concentrations of EGEE and EGEEA in air and EEA in urine.

(c) Non-analytical interference

i) Exposure to other chemicals (the same metabolite), co-exposure, ethanol intake, medication

No other chemicals are known to produce EAA in urine; EAA in urine is thus a specific indicator of exposure to EGEE and EGEEA. Environmental measurements are necessary to distinguish the source of EAA. Dermal exposure and the activity of the worker can affect the biological leveis of EAA.

Ethanol affects the metabolism of EGEE and EGEEA in rats, and probably in humans, due to competitive inhibition of alcohol dehydrogenase, an enzyme involved with the metabolism of EGEE, EGEEA and ethanol (1). Consumption of ethanol on the day of urine sample collection should be avoided, as ethanol can result in lower excretion of EAA, and lead to underestimation of uptake (1).

ii) Diet and environment

EAA, a specific metabolite of EGEE and EGEEA, is not expected in the diet or from the environment.

(d) Sampling representative of recent, or long-term exposure, or biological effect

Urine sampling represents exposure over the last week or weeks due to the estimated biological half-life in excess of 70 hours. Samples collected immediately after exposure at the end of the shift are not reflective of exposure during that shift.

(e) Ethnic differences (enzyme deficiency, environment, diet)

No data were found identifying ethnic differences.

4.3.8 Research needs

Dermal exposure is an unknown contributor to EAA in urine. Most experimental laboratory studies provide lower recommended exposure limits than those studies conducted in the workplace; the difference can be attributed to dermal exposure and perhaps workload. Further definitive research should be conducted to determine the relationship of dermal and inhalation exposure to the concentrations of EAA in urine.

EGEE and EGEEA are documented reproductive and hematological hazards in laboratory animals, and are suspected hazards in humans. Epidemiological studies, where possible, on health effects from exposures to EGEE and EGEEA in the absence of other reproductive or hematological toxins need to be conducted to establish the relationship between exposure, EAA in urine and possible reproductive and/or hematological effects in humans.

4.3.9 References

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L.K. Lowry, see section 4.1

4.4 Hexane

4.4.1 Introduction

Hexane is common in various solvent preparations, such as paints and thinners, and especially in adhesives (1, 2). Hexane is present at high concentrations in low boiling-point petroleum distillates, some of which are home-use products (3). It is also a component in automobile gasoline (4). Comprehensive reviews on toxicology are available (5, 6). 2,5-hexanedione is usually measured in biological monitoring of exposure.

4.4.2 Physical-chemical properties

Hexane (CAS No. 110-54-3) is a liquid at room temperature.

Molecular formula	n-C_6H_{14}
Molecular weight	86.18
Specific gravity	0.659 at 20°C
Vapour pressure	The saturated vapour pressure is 16.0 kPa at 20°C.
Conversion factors:	
Hexane	1 mg/m^3 = 0.284 ppm; 1 ppm = 3.52 mg/m^3 at 25°C
2,5-Hexanedione	1 mg/mL = 0.009 mmol/L; 1 mmol/L = 114 mg/L

4.4.3 Possible occupational and non-occupational exposure

In industrial health, occupational exposure to hexane is well documented (7–9). Hexane is ubiquitous in the general environment at levels up to 0.1 mg/m^3 (10). In addition, general population exposure through the use of hexane-containing consumer products is possible (4).

4.4.4 Summary of toxicokinetics

4.4.4.1 Absorption

(a) Inhalation

Hexane is readily absorbed via the lungs. Retention is 15–20% (11–13).

(b) Dermal

Dermal absorption is ignorable from the viewpoint of biological monitoring.

4.4.4.2 Metabolic pathways and biochemical interaction

Hexane is oxidized stepwise *in vivo* to hydroxy- and keto-derivatives primarily at 2- and 5- positions, and the metabolites identified in urine from hexane-exposed subjects include 2,5-hexanedione (2,5-HD), 2,5-dimethylfuran, γ-valerolactone (14–15: fig. 4.4.1).

2,5-HD is thought to be the ultimate neurotoxic metabolite (16). 4,5-dihydroxy-2-hex-anone is identified as a major metabolite (17) from which 2,5-HD and dimethylfuran may be formed during acid hydrolysis (fig. 4.4.1).
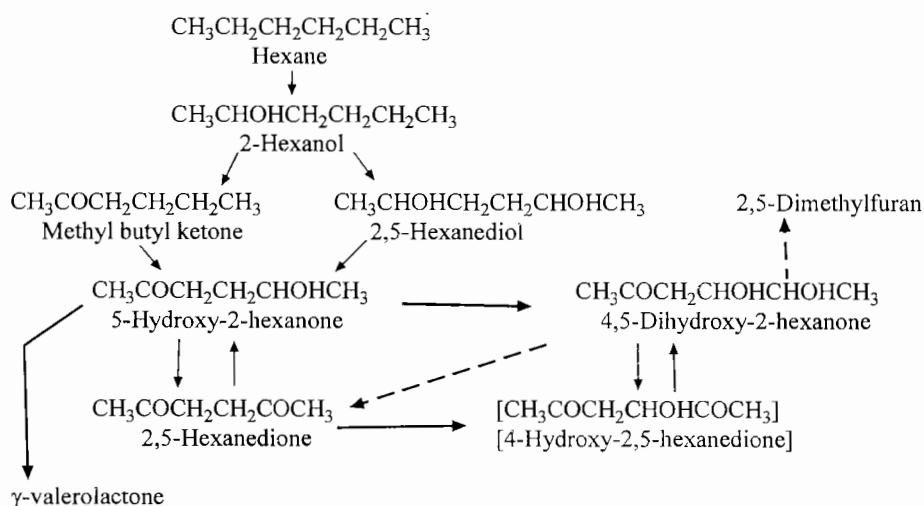
Figure 4.4.1. Schematic metabolism of hexane (modified and simplified from ref. 11 and 14. Broken lines show in vitro conversion during analysis.)

4.4.4.3 Distribution

When absorbed the solvent accumulates in the fat tissue (18).

4.4.4.4 Elimination

Hexane is rapidly eliminated unchanged in exhaled air. After the cessation of exposure, the elimination of hexane from blood of volunteers is rapid and biphasic, with a half-time of 12 min for a rapid phase and 120 min for a slow phase (11). Urinary excretion of the metabolites has been discussed in section 4.4.4.2.

4.4.5 Summary of toxic effects

Hexane is narcotic at high concentrations (19), but it is most well-known as a peripheral neurotoxin after repeated occupational exposures (7–9).

4.4.6 Biological monitoring indices

Available biological monitoring indices are summarized in table 4.4.1.

Table 4.4.1. Available biological monitoring indices

Indicator	Suitability for use	Ref
2,5-hexanedione in urine	Non-invasive and specific when analysed without hydrolysis	(20, 21)
Hexane in blood	Specific but invasive; further validation needed	(20, 22)

4.4.7 2,5-Hexanedione in urine

4.4.7.1 Toxicokinetics

See 4.4.4 Summary of toxicokinetics. 2,5-Hexanedione in urine increases in relation to the intensity of exposure to hexane vapour (for details, see guide to interpretation).

4.4.7.2 Biological sampling

(a) Sampling time and specimen

Because hexane has a short biological half-time, selection of sampling time is of critical importance for exposure assessment. Theoretically, sampling soon after cessation of heavy exposure is recommended. In the case of daily occupational exposure, urine samples collected near the end of a workshift can be employed for biological monitoring.

(b) Contamination possibilities

As the analyte is a metabolite of hexane, there is little possibility of contamination and no special precautions are necessary when collecting the sample.

(c) Sampling device and container

Urine samples should be collected into clean containers.

(d) Preservatives, shipment and stability

Freezing of the samples is recommended, if they cannot be analysed on the day of sampling and have to be kept overnight or longer.

4.4.7.3 Recommended analytical method

Several methods have been established (20–21, 23–25). The following gas chromatographic method is one example (21). When necessary, a cleanup pre-treatment of urine through an octadecyl silylated silica column and hydrolysis is possible (25).

(a) Principle of the method

Urine samples are heated under acidic conditions, and the hydrolyzates are extracted into solvent. The extract is subjected to analysis by gas chromatography (GC) (the acid

hydrolysis method to measure 'total' 2,5-hexanedione). This acid hydrolysis procedure converts 'precursor(s)' to 2,5-HD (see 4.4.4 Summary of toxicokinetics). Alternatively, urine samples may be subjected to solvent extraction without hydrolysis, and the extract is analysed by GC (the direct method). The direct method allows the measurement of 'free' 2,5-HD.

(b) Reagents required

Hydrochloric acid (38%), dichloromethane, 2,5-hexanedione as a standard, and 3-methylcyclohexanone (as an internal standard) are required.

(c) Equipment required

Gas chromatograph equipped with a flame ionisation detector (FID-GC), using nitrogen as a carrier gas and hydrogen as the fuel gas. A boiling water bath and clinical centrifuge are also required.

(d) Procedure and calibration

When the acid hydrolysis method is used, 5 mL urine is mixed in a tube with 250 µL of 38% hydrochloric acid to bring the pH to about 0.3. The mixture in the tube is heated at 100°C in a boiling water for 30 minutes. After cooling to room temperature, 2 mL dichloromethane (spiked with 0.09 to 183 mg 3-methylcyclohexanone/L as an internal standard) is added to the tube, and the tube is shaken vigorously for 1 minute.

In the case of the direct method, 5 mL urine is extracted with 2 mL dichloromethane as described above, without acid addition and heating steps.

After centrifugation for a short time (e.g. 1600 x g for 10 minutes with a clinical centrifuge) to achieve separation, 2-3 µL of the organic layer is subjected to GC analysis. For FID-GC analysis, a non-polar capillary column, such as DB-1 (30 m in length, 0.25 mm in inner diameter and 0.5 µm in film thickness) is used. Supply of hydrogen to a detector, nitrogen to the column, nitrogen (as a make-up gas) to the detector, and air to the detector is set at 60, 100, 75 and 60 kPa respectively (0.6, 1.0, 0.75 and 0.6 kg/cm^2). The oven temperature is kept at 60°C for 2 min after injection, increased at a rate of 10°C/min to 180°C, and kept at 180°C for 2 min, and then brought down to the initial temperature of 60°C.

Standards are prepared by adding 2,5-hexanedione to blank urine samples which are then taken through the analytical procedure with the samples.

(e) Criteria of analytical reliability

i) Trueness
Trueness, based on recovery studies, was 99%.

ii) Precision
The day-to-day precision of the analysis at 0.026 mmol/L (3mg/L) was 2% relative

standard deviation.

iii) Detectability
The estimated detection limit of 2,5-hexanedione in urine was 9×10^{-5} mmol/L (0.01mg/L).

(f) Quality assurance

i) Special precautions
Participation in external proficiency testing programmes is recommended (see Chapter 2 of this volume). Internal quality control is necessary to ensure quality results.

(g) Sources of possible errors

i) Pre-analytical
The timing of urine sample collection is important as discussed above, e.g. pre-shift urine samples will give considerably lower 2,5-hexanedione than post-shift urine samples.

ii) Analytical
pH is important when the acid hydrolysis method is employed. The amount of 2,5-hexanedione is inversely related to the pH at which the sample is heated for hydrolysis. The maximum amount will be obtained when the pH is 0.3 or lower (20, 24, 26). The second point is the choice of the column. Use of a capillary column is essential for better separation. A non-polar one [e.g. DB-1 (23) or DB-1701 (24)] should be utilized when the acid hydrolysis method is employed, because 2-acetylfuran present in the hydrolyzate confounds the analysis (20) when a polar column [e.g. DB-WAX] is employed.

(h) Reference to the most comprehensive description of the method

Kawai T, Yasugi T, Mizunuma K, Horiguchi S, Uchida Y, Iwami O, Ikeda M. 2-Acetylfuran, a confounder in urinalysis for 2,5-hexanedione as an *n*-hexane exposure indicator. International Archives of Occupational and Environmental Health 1991;63:213–9.

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(i) Evaluation of the method

Better separation of the exposed group from the non-exposed group is achieved by the direct method than the acid hydrolysis method (21) because no 2,5-hexanedione is detected in the urine from the non-exposed subjects when the direct method is applied, whereas considerable amount of 2,5-hexanedione is present when the urine is acid-hydrolyzed (20–21, 23, 27). In addition, no need of acid addition and heating makes the analysis simpler. The acid hydrolysis method is, however, effective, especially when the GC instrument does not offer enough sensitivity, because 2,5-hexanedione after acid hydrolysis is several times higher than the amount without hydrolysis (20).

4.4.7.4 Other analytical methods

Other versions of GC analysis have been described (24–25).

4.4.7.5 Guide to interpretation

Using either the direct method or the acid hydrolysis method, it is well established that urinary 2,5-hexanedione levels increase in proportion to the intensity of hexane exposure (23–24, 28–29).

(a) Measured values in groups without occupational exposure

2,5-hexanedione is detectable in the urine of the non-exposed subjects when analysed by the acid hydrolysis method (17). The amount may vary depending on the analytical conditions, such as the pH for hydrolysis and the column employed for GC analysis (20, 22–24, 27). Concentrations of hexane in general air is reported to be less than 5 $\mu g/m^3$, but the use of hexane-containing consumer products (e.g. petroleum benzine) may increase 2,5-hexanedione levels in urine.

(b) Published biological action levels

The American Conference of Governmental Industrial Hygienists (30) has adopted a value for 2,5-hexanedione of 5 mg/g creatinine (5 mmol/mol creatinine) (hydrolyzed at pH <1) in urine collected at the end of a workshift at the end of a workweek as a 'Biological Exposure Index (BEI)'. This value is set in reference to the time-weighted average concentration of occupational exposure to hexane at 180 mg/m^3 (50 ppm).

Deutsche Forschungsgemeinschaft (31) has adopted a value of 5 mg/L (0.044 mmol/L) for 2,5-hexanedione+4,5-dihydroxy-2-hexanone in urine (i.e., 2,5-hexanedione after hydrolysis at pH <0.5) collected at the end of exposure or the end of a workshift as a 'Biological Tolerance Value (BAT-value)'.

(c) Non-analytical interferences

i) Exposure to other chemicals (the same metabolite), co-exposure, ethanol intake, medication

In animal experiments, co-exposure to toluene resulted in reduced excretion of hexane metabolites in urine (32–33). Exposure to methyl-butyl-ketone may give rise to 2,5-hexanedione in urine.

(d) Sampling representative of recent or long-term exposure, or biological effect

Hexane is rapidly eliminated from the body, and since it is not a cumulative chemical, only recent exposure can be evaluated by a biological parameter.

(e) Ethnic differences (enzyme deficiency, environment, diet)

No data were found identifying ethnic differences.

4.4.8 Other indices

Hexane in urine is reported to increase in relation to the intensity of exposure to hexane vapour (34). Hexane in exhaled breath and in blood can be readily measured by direct injection of the air (11, 35) and by head-space GC (22), respectively.

4.4.9 Research needs

The value of measurements of hexane in blood and in exhaled breath requires further study. The effect of co-exposure to other solvents or chemicals on hexane metabolism may vary. Whether correction for co-exposure is necessary and, if so, how it can be done, should be studied through factory surveys. The observation in animal experiments (30–31) on the metabolic interaction of toluene on hexane (as described above) needs re-examination and further occupational health studies.

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4.5 Styrene

4.5.1 Introduction

Most of the styrene produced is used for production of polymer (polystyrene) and co-polymer (ABS, SB rubber, etc.). A minor portion is employed in fibre-reinforced plas-tics production where styrene is used as a solvent for uncured resin and also participates in polymerization during the curing process (1). Comprehensive toxicology reviews on toxicology and biological monitoring are available (1–3). As it is shown below, man-delic acid and phenylglyoxylic acid are metabolites of styrene, and are used for biologi-cal monitoring.

4.5.2 Physical-chemical properties

Styrene (CAS 100-42-5) is a liquid at room temperature.

Molecular formula	$C_6H_5CH=CH_2$
Molecular weight	104.15
Specific gravity	0.906 at 20°C
Vapour pressure	0.60 kPa at 20°C
Conversion factors:	
Styrene	1 mg/m^3 = 0.231 ppm; 1 ppm = 4.33 mg/m^3 at 20°C
	1 mg/L = 0.0096 mmol/L; 1 mmol/L = 104 mg/L
Mandelic acid	1 mg/L = 0.0066 mmol/L; 1 mmol/L = 152 mg/L
Phenylglyoxylic acid	1mg/L = 0.0067 mmol/L; 1mmol/L = 150 mg/L

4.5.3 Possible occupational and non-occupational exposures

Occupational exposures to styrene in monomer/polymer/copolymer production sites are generally low, whereas at other sites, such as fibre-reinforced boat production work-shops, exposures can be more than ten times higher (1). The general population is ex-posed to styrene via atmospheric air and monomer residues in food containers, etc., but the levels of exposure are much lower than in occupational settings (1).

4.5.4 Summary of toxicokinetics

4.5.4.1 Absorption

(a) Inhalation

The major route of entry for styrene is via the lungs.

(b) Dermal

Studies suggest that prolonged contact of the skin with liquid styrene will be associated with significant absorption of styrene via skin (4).

4.5.4.2 Metabolic pathways and biochemical interaction

In vivo, styrene is oxidized at vinyl side chain to styrene-7,8-epoxide, styrene glycol to mandelic acid (MA) and then to phenylglyoxylic acid (PhGA). Both acids are excreted in urine. MA and PhGA are a major and a minor metabolite, respectively (5–6; fig. 4.5.1). Decarboxylation of MA (followed by oxidation and conjugation to form hippuric acid) is not significant in humans (7).

C_6H_5CHCH2
Styrene (vinylbenzene)

↓

(Side chain epoxidation   (Aromatic oxidation
  & hydration)   as a minor pathway)

↓

$C_6H_5CHOHCH_2OH$
Styreneglycol

↓

$C_6H_5CHOHCOOH$
Mandelic acid

↓

$C_6H_5COCOOH$
Phnylglyoxylic acid
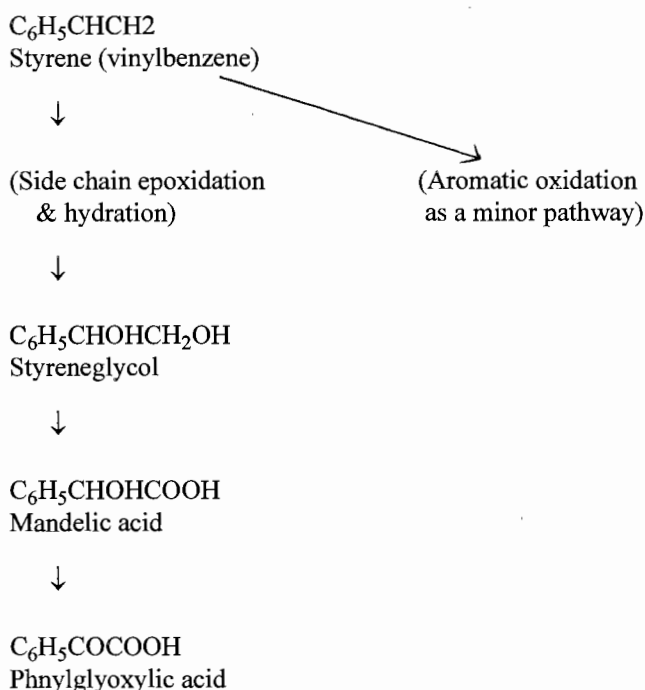
Figure 4.5.1. Schematic metabolism of styrene (simplified from ref. 6 and 7)

4.5.4.3 Distribution and elimination

A small fraction of the absorbed styrene is eliminated unchanged in exhaled breath and an even smaller amount in urine. Both MA and PhGA are excreted in urine with a half-time of about 5–10 hours (7–8).

4.5.5 Summary of toxic effects

Styrene has a disagreeable odour, and is irritating to eyes, nose and throat. The vapour is narcotic at high concentrations (9). Repeated exposure to styrene at high concentrations may inuce cytogenetic effects on the peripheral lymphocytes of exposed workers (10–11). Animal carcinogenicity studies have been reviewed (12–14).

4.5.6 Biological monitoring indices

Available biological monitoring indices are summarized in table 4.5.1.

Table 4.5.1. Available biological monitoring indices

Indicator	Suitablility for use	Reference number
MA in urine	Non-invasive (MA is a major metabolite)	(15)
PhGA	Non-invasive (PhGA is a minor metabolite)	(15)
Styrene in blood	Specific but invasive, further validation necessary	(16)

4.5.7 Mandelic acid (MA) in urine

4.5.7.1 Toxicokinetics

The excretion half-time of MA in urine is about 5–10 hours (7–8).

4.5.7.2 Biological sampling

(a) Sampling time and specimen

Urine samples should be collected at the end of a workshift. Some authors prefer next-day pre-shift urine sampling, arguing that correlation with styrene vapour exposure is better with next-day pre-shift samples than with the end-of-shift samples. The MA concentrations, however, are much lower in the former samples than in the latter ones.

(b) Contamination possibilities

As the analyte is a metabolite of styrene, there is little possibility of contamination.

(c) Sampling device and containers

Urine specimens should be collected into clean containers.

(d) Preservatives, shipment and stability

No preservatives are necessary. Where analysis on the day of collection is not possible, urine samples should be refrigerated or, more preferably, frozen for storage. One report recommends analysis within two weeks at the latest (17).

4.5.7.3 Recommended analytical method

(a) Principle of the method

Most of the methods for hippuric acid determination are also applicable to MA analysis. PhGA can be measured simultaneously. Both gas chromatographic (17–19) and high pressure liquid chromatographic (HPLC) (15, 20) methods are available. HPLC meth-

ods are more popular. The following is one example (15) of an HPLC analytical procedure.

(b) Reagents required

Mandelic acid as a standard, KH_2PO_4, acetonitrile and sodium 1-decanesulfonate are required.

(c) Equipment required

A high pressure liquid chromatograph (HPLC) equipped with a UV detector is required. Data processing facilities and an automated liquid sampler are useful, if available. A clinical centrifuge is also needed.

(d) Procedure and calibration

An aliquot of the urine sample (1 mL) is mixed with 1 mL methanol and the mixture is centrifuged (e.g., at 1600 x g for 10 min in a clinical centrifuge) for removal of the precipitates. The supernatant solution (10 µL/injection) is introduced to a HPLC by means of an automatic liquid sampler. A stainless steel column (4.6 mm in diameter and 150 mm in length) packed with octadecyl silanized silica gel (TSK gel, ODS-80 TM, 5µm) is used for chromatography, and the column is kept at 25°C. The mobile phase is a mixture of 20 mmol/L KH_2PO_4 (pH 3.3; containing 3 mmol/L sodium 1-decanesulfonate) and acetonitrile (85:15), and is allowed to flow at 0.7 mL/min producing a pressure of 10 MPa (100 kg/cm^2). The fractions are monitored at a wavelength of 225 nm.

Standards are prepared by adding mandelic acid to blank urine samples which are then taken through the analytical procedure along with the samples.

(e) Criteria for analytical reliability

i) Trueness
Trueness, based on recovery studies, was 100% (15).

ii) Precision
The day-to-day precision of the analysis of MA at 0.5–2.0 mg/L (0.0033–0.013 mmol/L) was 1% relative standard deviation (15).

iii) Detectability
The estimated detection limit of mandelic acid in urine was 10 mg/L (0.066 mmol/L).

(f) Quality assurance

i) Special precautions
Internal quality control is necessary to ensure quality results. Participation in external quality assessment schemes, where possible, is recommended (see Chapter 2 of this volume).

(g) Sources of possible errors

i) Pre-analytical
Urine samples should be kept frozen when long-term storage is necessary. Any precipitates must be carefully re-dissolved after thawing to avoid loss of the analyte due to co-precipitation.

(h) Reference to the most comprehensive description of the method

Ogata M, Taguchi T. Simultaneous determination of urinary creatinine and metabolites of toluene, xylene, styrene, ethylbenzene and phenol by automated high performance liquid chromatography. International Archives of Occupational and Environmental Health 1988;61:131–40.

(i) Evaluation of the method

The method is sensitive enough to detect occupational exposure.

4.5.7.4 Other analytical methods

Gas chromatographic methods (17–19) and another version of the HPLC method (20) are available.

4.5.7.5 Guide to interpretation

There are a number of studies (21–25) which report a linear correlation between time-weighted average intensity of exposure to styrene vapour and MA concentration in the end-of-shift urine samples.

(a) Measured values in groups without occupational exposure

MA levels in the urine of non-exposed subjects are low, e.g. 0.099 mmol/L (15 mg/L) as a geometric mean (25).

(b) Published biological action levels

The American Conference of Governmental Industrial Hygienists (26) has adopted a value for MA of 800 mg/g creatinine in urine collected at the end of a workshift, and 300 mg/g creatinine in urine collected prior to a shift as a 'Biological Exposure Index (BEI)'. This value is set with reference to the time-weighted average concentration of occupational exposure to styrene at 213 mg/m^3 (50 ppm).

Deutsche Forschungsgemeinschaft (27) has adopted a value for MA of 400 mg MA/L (2.64 mmol/L) in urine collected at the end of exposure or the end of a workshift as a 'Biological Tolerance Value (BAT-value)'.

(c) Non-analytical interference

i) Exposure to other chemicals (the same metabolite), co-exposure, ethanol intake, medications

MA can be derived also from other chemicals, especially ethylbenzene, an intermediate in styrene synthesis. Ethanol is known to suppress styrene metabolism (28–29).

4.5.8 Phenylglyoxylic acid (PhGA) in urine

4.5.8.1 Toxicokinetics

Excretion half-time for PhGA in urine is about 5–10 hours (7, 8).

4.5.8.2 Biological sampling

Statements for MA are applicable also to PhGA. In addition, it should be noted that PhGA is more unstable than MA, and needs greater precautions in storage. Analysis as soon after sampling as possible is recommended (17), although PhGA is stable in urine when the sample is kept frozen.

4.5.8.3 Recommended analytical methods

(a) Principle of the method

The methods used for MA in urine are equally applicable to PhGA. The method described in section 4.5.7.3 can be used to measure PhGA concentrations in urine.

For reagents, equipment required and procedures: see sections 4.5.7.3b, 4.5.7.3c and 4.5.7.3d. PhGA is used to prepare standards in blank urine.

(e) Criteria of analytical reliability

i) Trueness
Trueness, based on recovery studies, was 96–104%.

ii) Precision
The overall precision of the analysis at 0.5 g/L (3.3 mmol/L) was 3% relative standard deviation.

iii) Detectability
The estimated detection limit of PhGA in urine was 2 mg/L (0.013 mmol/L).

(f) Quality assurance

i) Special precautions
No external proficiency testing programmes are available. Internal quality control is necessary to ensure quality results.

(g) Sources of possible error

i) Pre-analytical
It is particularly important to keep samples deep frozen when storing them before analysis. Any precipitates must be carefully re-dissolved after thawing to avoid loss of the analyte due to co-precipitation.

(h) Reference to the most comprehensive description of the method

Ogata M, Taguchi T. Simultaneous determination of urinary creatinine and metabolites of toluene, xylene, styrene, ethylbenzene and phenol by automated high performance liquid chromatography. International Archives of Occupational and Environmental Health 1988;61:131–40.

4.5.8.4 Other analytical methods

Gas chromatographic methods (17–19) and another version of the HPLC method (20) are available.

4.5.8.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

PhGA levels in the urine of non-exposed subjects are essentially nil (25).

(b) Published biological action levels

The American Conference of Governmental Industrial Hygienists (26) has adopted a value for PhGA of 240 mg/g creatinine (181 mmol/mol creatinine) in urine collected at the end of a workshift, and 100 mg/g creatinine in urine (75 mmol/mol creatinine) collected prior to next shift as a 'Biological Exposure Index (BEI)' for styrene with reference to the time-weighted average concentration of occupational exposure to styrene at 213 mg/m^3 (50ppm).

Deutsche Forschungsgemeinschaft (27) has adopted a value of 500 mg/L (approximately 3.32 mmol/L) for MA+PhGA urine collected at the end of exposure or the end of a workshift as a 'Biological Tolerance Value (BAT-value)'.

(c) Non-analytical interference

i) Exposure to other chemicals (the same metabolite), co-exposure, ethanol intake, medication
PhGA can be derived from other chemicals, especially ethylbenzene, an intermediate in styrene synthesis. Ethanol is a known suppressor of styrene metabolism (28–29).

4.5.9 Other indices

Gas chromatography methods for combined determination of MA and PhGA after chemical reduction of PhGA to MA have been developed (18–19). Styrene in blood and

urine can be measured by head-space GC (16) or solvent extraction followed by GC analysis (30). Styrene in blood (24–25, 31) and urine (31–32) collected at the end of a shift correlates significantly with styrene in the air.

The American Conference of Governmental Industrial Hygienists (26) has adopted a value of 0.55 mg/L for styrene in blood collected at the end of a workshift, and 0.02 mg/L in blood collected prior to a shift as a semi-quantitative 'Biological Exposure Index (BEI)' for styrene.

4.5.10 Research needs

The quantitative relationship between styrene in blood and styrene exposure deserves further study because it is a specific test for styrene exposure. Further occupational health studies of styrene in urine as a quantitative indicator of exposure are needed, as it would provide a non-invasive, yet specific test.

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4.6   Toluene

4.6.1 Introduction

Toluene has a number of applications in industry as the most common constituent of various solvent preparations, such as paint, ink, thinner and glue (1–4). It is also present in automobile gasoline, especially unleaded gasoline (5). Comprehensive reviews on toxicology and biological monitoring are available (6–11). Hippuric acid is the major metabolite of toluene and can be used for biological monitoring.

4.6.2 Physical-chemical properties

Toluene (CAS No. 108-88-3) is a liquid at room temperature.

Molecular formula	$C_6H_5CH_3$
Molecular weight	92.14
Specific gravity	0.867 at 20°C
Vapour pressure	The saturated vapour pressure is 2.91 KPa at 20°C.
Solubility	The logarithm of the octanol/water partition coefficient is 2.69.

Conversion factors:

Toluene in air	1 mg/m^3 = 0.267 ppm; 1 ppm = 3.75 mg/m^3 at 25°C
Toluene in blood	1 mg/L = 0.0108 mmol/L; 1 mmol/L = 92 mg/L
Hippuric acid	1 mg/L = 0.0056 mmol/L; 1 mmol/L = 179 mg/L

4.6.3 Possible occupational and non-occupational exposure

Toluene is detectable not only in occupational environments but in the general atmosphere [e.g. at the level of up to 1.31 mg/m^3 (6)]. In addition, toluene is a major component of solvent vapours abused by 'sniffers' who inhale the vapours intentionally.

4.6.4 Summary of toxicokinetics

4.6.4.1 Absorption

(a) Inhalation

Toluene, when inhaled under occupational conditions, can be readily absorbed through the lungs with the pulmonary retention of about 50% (11).

(b) Dermal

Significant absorption through the skin can occur when contact is with liquid toluene, but not through contact with toluene vapour (12).

4.6.4.2 Metabolic pathways and biochemical interaction

A portion of toluene absorbed is exhaled unchanged, whereas the majority (ca. 80%) is oxidized in the liver at the methyl moiety to benzyl alcohol and then to benzoic acid, which will be then conjugated with glycine to form hippuric acid to be excreted into urine. Thus, hippuric acid is a major urinary metabolite of toluene. A small fraction (1% or even less) of toluene will be hydroxylated at the benzene ring to form the three cresol isomers, which will be excreted in urine as conjugates (i.e., sulphates and glucuronides) (10, 13; fig. 4.6.1). Unmetabolized toluene is also detected in urine (14–15).

$C_6H_5CH_3$
Toluene (methylbenzene)
|
(Side chain oxidatiol)   (Aromatic oxidation)
↓   ↓
$C_6H_5CH_2OH$ $CH_3C_6H_4OH$
Benzyl alcohol o-,m-,p-Cresol
↓ |
C_6H_5COOH (Sulphation or glucuronidation)
Benzoic acid   ↓
| Sulphate or glucuronide of cresol
(Glycine conjugation)
↓
$C_6H_5CONHCH_2COOH$
Hippuric acid
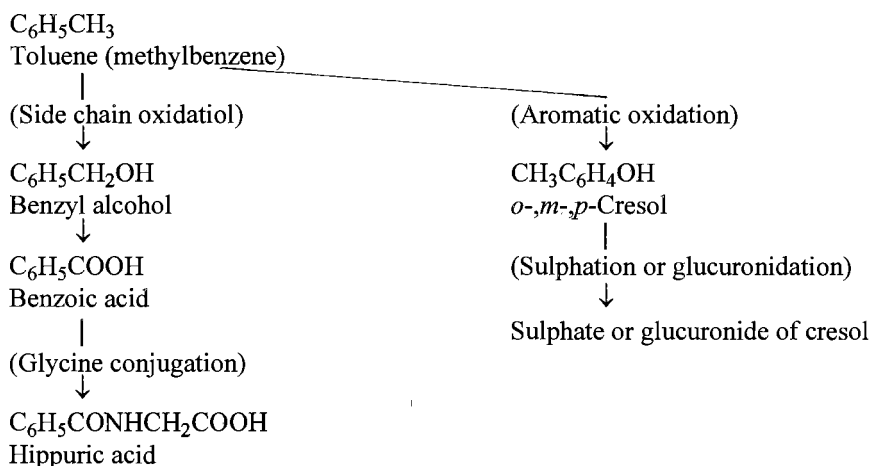
Figure 4.6.1. Schematic metabolism of toluene (simplified from ref. 10 and 13)

4.6.4.3 Distribution and elimination

Elimination kinetics show that both hippuric acid and o-cresol have a biological half-time of 7–8 hours (16). Toluene may accumulate in the body of workers over a workweek (10, 17).

4.6.5 Summary of toxic effects

The most important toxic effect of toluene under occupational conditions is suppression of the function of the central nervous system, as evidenced by increases in various sub-jective symptoms (10, 18).

4.6.6 Biological monitoring indices

Available biological monitoring indices are summarized in table 4.6.1.

Table 4.6.1. Available biological monitoring indices

Indicator	Suitablility for use	Ref.
Hippuric acid in urine	Non-invasive, but less specific	(19–20)
o-cresol in urine	Non-invasive, but with wide variation	(16)
Toluene in blood	Specific but invasive	(15)
Toluene in urine	Specific and non-invasive, but needs further validation	(14)

4.6.7 Hippuric acid in urine

4.6.7.1 Toxicokinetics

Hippuric acid in urine has a half-time of 7 to 8 hours (16).

4.6.7.2 Biological sampling

(a) Sampling and specimen

Because of the short half-time of hippuric acid excretion, the timing of sample collection is of critical importance for exposure assessment. Urine samples should be collected at the end of a workshift, and Monday afternoons should be avoided. As the biological half-time for hippuric acid excretion is 7–8 hours (16), hippuric acid levels on Monday afternoons should be significantly lower than the levels in the afternoon of the other weekdays.

(b) Contamination possibilities

As the analyte is a metabolite of toluene, there is little possibility of contamination.

(c) Sampling device and container

Urine samples should be collected into clean containers.

(d) Preservatives, shipment and stability

Although hippuric acid is not very unstable in urine, spoilage of urine should be prevented either by refrigeration or more preferably by freezing.

4.6.7.3 Recommended analytical methods

Several methods of urinalysis for hippuric acid have been developed, i.e., high performance liquid chromatography (HPLC) methods (19, 21–23), gas chromatography (GC) after derivatization [e.g. methyl esterification (20), or formation of other derivatives (24–25)] of hippuric acid and colorimetry (26). Most of the HPLC methods are applicable also to the determination of urinary metabolites of xylenes, styrene and ethylbenzene. The GC methods can be used for determination of xylene metabolites in urine.

The following gives examples of both HPLC (19) and GC (20) methods.

4.6.7.3.1 High performance liquid chromatography (HPLC)

(a) Principle of the method

After de-salting by addition of methanol, urine samples are analysed by HPLC.

(b) Reagents required

Hippuric acid as a standard, KH_2PO_4, acetonitrile and sodium 1-decanesulphonate.

(c) Equipment required

A high-pressure liquid chromatograph (HPLC) with a UV detector and, if available, a data processor are required. An automatic liquid sampler is a useful addition, if available. A clinical centrifuge is also needed.

(d) Procedure and calibration

An aliquot of the urine sample (1 mL) is mixed with 1 mL methanol and the mixture is centrifuged (e.g. at 1600 x g for 5 minutes with a clinical centrifuge) for removal of the precipitates. The supernatant solution (10 µL/injection) is introduced to a HPLC by means of an automatic liquid sampler. A stainless steel column (4.6 mm in diameter and 150 mm in length) packed with octadecyl silanized silica gel (TSK gel, ODS-80 TM, 5µm) is used for chromatography at 25°C. The mobile phase is a mixture of 20 mmol/L KH_2PO_4 (pH 3.3; containing 3 mmol/L sodium 1-decanesulphonate) and acetonitrile (85:15 by volume), and is allowed to flow at 0.7 mL/min producing a pressure of 10 MPa (100 kg/cm^2). The effluent is monitored at a wavelength of 225 nm.

Standards are prepared by adding hippuric acid to blank urine which are taken through the analytical procedure along with the samples. Care should be taken for the presence of endogenous hippuric acid in urine (See 4.6.7.5a).

(e) Criteria for analytical reliability

i) Trueness
Trueness, based on recovery studies, was 99–100%.

ii) Precision
The day-to-day precision of the analysis at 0.5–2.0 g/L (2.8–11.1 mol/L) was 1% relative standard deviation.

iii) Detectability
The estimated detection limit of hippuric acid in urine was 2 mg/L (0.01 mmol/L).

(f) Quality assurance

i) Special precautions
Internal quality control is necessary to ensure quality results. Participation in external quality assessment schemes is recommended, where possible.

(g) Sources of possible error

Selection of the optimum time for urine sampling in relation to exposure has already been discussed (see section 4.6.7.2a). When samples are stored before analysis, care should be taken to prevent any losses of the analyte by co-precipitation.

(h) The reference to the most comprehensive description of the method

Ogata M, Taguchi T. Simultaneous determination of urinary creatinine, and metabolites of toluene, xylene, styrene, ethylbenzene and phenol by automated high performance liquid chromatography. International Archives of Occupational and Environmental Health 1988;61:131–40.

(i) Evaluation of the method

Analytically, the method is specific and sensitive. In practice, however, high background levels of hippuric acid make it difficult to detect toluene-induced increments when toluene exposure is low (see 4.6.7.5 Guide to interpretation).

4.6.7.3.2 Gas chromatography (GC)

(a) Principle of the method

After solvent extraction, hippuric acid is esterified. The methyl ester of hippuric acid thus formed is analysed by GC.

(b) Reagents required

Hippuric acid as a standard, ethyl acetate, hydrochloric acid, methanol, diazomethane (in ether; prepared from e.g. tolylsulfonyl methylnitrosamide) and heptadecanoic acid as an internal standard.

CAUTION! – The reagent for methyl esterification of hippuric acid needs care for prevention of possible health effects on the analyst. Diazomethane is not only explosive but is carcinogenic in some animal experiments (27).

(c) Equipment required

A gas chromatograph (GC) equipped with a flame ionisation detector (FID-GC) and, if available, a data processor are required. Nitrogen is used as carrier gas and hydrogen as fuel gas. A clinical centrifuge is also needed.

(d) Procedure and calibration

A 0.2 mL portion of the urine sample is mixed stepwise with 0.2 mL of the internal standard solution (1 mg heptadecanoic acid/mL ethanol), 0.1 mL of 0.5 mol/L hydrochloric acid and 3.5 mL of ethyl acetate. After shaking and separation by standing, (or centrifuging at 3000 rpm for 10 minutes with a clinical centrifuge, if necessary) about 3 mL of the ethyl acetate layer is transferred to a tube, and the solvent is removed by evaporation under a nitrogen stream. Hippuric acid in the residue is methylated by the addition of diazomethane solution; the solution should be added until the yellow colour persists. After standing for 10 minutes, the solvent is removed by evaporation (under a nitrogen stream, if necessary) and the residue is dissolved in 0.1–0.2 mL of methanol. The solution is introduced to a FID-GC and analysed on a 1 m-long stainless steel column packed with 3% SE30 on Chromosorb W (AW-DMCS, 80-100 mesh). The GC oven is kept at 160°C, and both the injection port and the FID at 250°C. Nitrogen is employed as a carrier gas and allowed to flow at 40 mL/min. The supply of air and hydrogen gas to the FID is at 300 mL/min and 60 mL/min, respectively.

(e) Criteria of analytical reliability

i) Trueness
Trueness, based on recovery studies, was 98%.

ii) Precision
The day-to-day precision of the analysis at 500 mg/L (2.8 mmol/L) was 5% relative standard deviation.

iii) Detectability
The estimated detection limit of hippuric acid in urine was 10 mg/L (0.06 mmol/L).

(f) Quality assurance

See section 4.6.7.3.1f above.

(g) Sources of possible errors

See section 4.6.7.3.1g above.

(h) Reference to the most comprehensive description of the method

Buchet JP, Lauwerys RR. Measurement of urinary hippuric and m-methylhippuric acids by gas-chromatography. British Journal of Industrial Medicine 1973;30:125–8.

(i) Evaluation of the method

The GC method is specific and sensitive. In general, HPLC and GC give comparable results.

4.6.7.4 Other analytical methods

Colorimetric methods are available, but the reagent is not specific to hippuric acid and

reacts with other endogenous compounds in urine. Thus colorimetry is not recommended, especially when urine samples from workers with low toluene exposure are analysed (28).

4.6.7.5 Guide to interpretation

There are many occupational health studies to show that hippuric acid in the end-of-shift urine samples relates linearly to time-weighted average intensity of toluene exposure (16, 29–33).

(a) Measured values in groups without occupational exposure

Hippuric acid is a normal urinary constituent derived from benzoate which occurs naturally, e.g. in some fruits and vegetables or added as food preservatives, e.g. in some types of soft drinks (33). Urinary hippuric acid levels among subjects with no known occupational toluene exposure may range up to 1.2 g/L (6.7 mmol/L) (15), and the levels may be further elevated by use of toluene-containing home-use products, such as glue and wax. Thus hippuric acid as a toluene exposure indicator is not useful when toluene exposure concentration is low, e.g. 200 mg/m^3 (ca. 50 ppm) or less (34).

(b) Published biological action levels

The American Conference of Governmental Industrial Hygienists (35) previously adopted a value of 2.5 g/g creatinine for hippuric acid in urine collected at the end of a workshift as a 'Biological Exposure Index (BEI)'. This value was set with reference to a time-weighted average concentration of occupational exposure to toluene at 26.5 mg/m^3 (100 ppm). However, this BEI is currently withdrawn based on the reduction of the Threshold Limit Value from 100 ppm to 50 ppm (36).

(c) Non-analytical interference

i) Exposure to other chemicals (the same metabolite), co-exposure, ethanol intake, medication
It is possible that smoking and drinking suppress metabolism of toluene to hippuric acid (37–40). Interaction of toluene metabolism with co-exposed other solvents, such as benzene may take place when the exposure is intense (41).

ii) Diet and environment
It should be noted that benzoic acid (a hippuric acid precursor) has been widely used as a food additive and affects results.

(d) Ethnic differences (enzyme deficiency, environment, diet)

Ethnic differences in toluene metabolism have been described (30, 42).

4.6.8 *o*-Cresol in urine

4.6.8.1 Toxicokinetics

This has been summarized in section 4.6.4 above.

4.6.8.2 Biological sampling

Sampling for *o*-cresol measurements is as for hippuric acid (see section 4.6.7.2).

4.6.8.3 Recommended analytical methods

(a) Principle of the method

Cresols are excreted in urine as conjugates, and therefore a pre-treatment by acid hydrolysis is necessary to make phenolic compounds in free forms before analysis. Because *p*-cresol is endogenous and abundant in urine, separation of *o*-cresol from other cresol isomers is necessary and this is achieved by gas chromatographic (GC) analysis (16, 43). The following is one example of an analytical procedure by a GC method (16).

(b) Reagents required

o-Cresol as standard, 3,5-xylenol as internal standard, hydrochloric acid, carbon disulfide and anhydrous sodium sulphate.

(c) Equipment required

A gas chromatograph equipped with a flame ionisation detector (FID-GC) and, if available, a data processor.

(d) Procedure and calibration

A 1 mL portion of the urine sample is added to 0.5 mL of 15% hydrochloric acid and the mixture is heated at 100°C for 1 hour in a sealed container. The hydrolysate after cooling is added 50 µL 3,5-xylenol solution (25 mg/L water; as an internal standard) and then extracted with 2 mL carbon disulfide. The organic phase is transferred to a tube and dried with sodium sulphate. After mild concentration (to ca. 0.2 mL), 2 µL of the concentrate is introduced to a FID-GC and analysed on a glass column (5 m in length and 3 mm in diameter) packed with KG-02 on Uniport HP (60-80 mesh). The oven and the injection port are kept at 180°C and 210°C, respectively. The carrier gas is nitrogen which is allowed to flow at 30 mL/min, and the supplies of hydrogen and air to FID are at 1 and 2 kg/cm^2, respectively.

Standards are prepared by adding known amounts of *o*-cresol to blank urine samples, which are then taken through the analytical procedure.

(e) Criteria of analytical reliability

i) Trueness
Trueness, based on recovery studies, was 97%.

ii) Precision

The day-to-day precision of the analysis at 10 mg/L (0.093 mmol/L) was 3% relative standard deviation.

iii) Detectability

The estimated detection limit of *o*-cresol in urine was 2 mg/L (0.019 mmol/L).

(f) Quality assurance

i) Special precautions

No external proficiency testing programmes are available. Internal quality control is necessary to ensure quality results.

(g) Sources of possible errors

i) Pre-analytical

Selection of optimum time for urine sampling in relation to exposure is important (see 4.6.7.2a). When samples are stored before analysis, care should be taken to prevent any loss of the analyte by co-precipitation.

(h) Reference to the most comprehensive description of the method

Hasegawa K, Shiojima S, Koizumi A, Ikeda M. Hippuric acid and o-cresol in the urine of workers exposed to toluene. International Archives of Occupational and Environmental Health 1983;52:197–208.

(i) Evaluation of the method

Analytically, the method is specific. In practice, however, a wide variation in *o*-cresol excretion makes it difficult to detect a toluene-induced increment in *o*-cresol when toluene exposure is low.

4.6.8.4 Other analytical methods

Other gas chromatographic methods are available (43).

4.6.8.5 Guide to interpretation

There are many occupational health studies showing that *o*-cresol in the end-of-shift urine samples correlates with time-weighted average toluene exposure (16, 29, 31, 44–45).

(a) Measured values in groups without occupational exposure

o-cresol levels in subjects with no known exposure are very low, e.g. below 0.05 mg/L as a geometric mean (45). The possibilities of non-occupational exposure to toluene (e.g. from use of home products, such as glues), however, should be taken into account when evaluating *o*-cresol levels in urine (see 4.6.7.5).

(b) Published biological action level

No published biological action level is available.

(c) Non-analytical interferences

i) Exposure to other chemicals (the same metabolite), co-exposure, ethanol intake, medication

Interaction of toluene metabolism with other solvents, such as benzene may take place when the exposure is intense (41). In addition, the metabolism of toluene may be affected by co-exposure to xylene, depending on the exposure intensities (46–47). There are conflicting reports of the effects of smoking and drinking habits on the metabolism of toluene to *o*-cresol (37, 48–49).

(d) Ethnic differences (enzyme deficiency, environment, diet)

Ethnic differences in toluene metabolism have been described (42).

4.6.9 Other indices

Toluene in blood and urine can be readily measured by head-space gas chromatography (14–15), and has been proposed as a potential indicator of exposure to toluene (14–15, 44, 50–51). Because toluene in blood or urine is specific and the background levels are essentially nil, the presence when properly analysed can be regarded as the evidence of toluene exposure. In practice, timing is critical, and intense care should be taken to avoid contamination of, or loss from, samples, e.g. absorption of toluene from atmospheric air into blood and leakage of toluene in urine into air.

The American Conference of Governmental Industrial Hygienists (35) had previously adopted a value of 1.0 mg/L for toluene in blood collected at the end of a workshift as a semi-quantitative 'Biological Exposure Index' (BEI). This was set with reference to a time-weighted average concentration for occupational exposure to toluene at 37.5 mg/m^3 (100 ppm). This has been withdrawn, however, based on a reduction in the Threshold Limit Value from 100 ppm to 50 ppm (36).

The American Conference of Governmental Industrial Hygienists (35) and Deutsche Forschungsgemeinschaft (52) have adopted a value of 1 mg/L for toluene in blood (collected at the end of exposure or the end of a workshift) as 'Biological Exposure Index ' (BEI), and 'Biological Tolerance Value' (BAT-value), respectively.

4.6.10 Research needs

Measurements of toluene in blood and urine are highly specific to toluene exposure, but their usefulness in occupational health needs further evaluation. They may prove to be particularly useful in detecting exposure to toluene at the low concentrations observed in modern industries (e.g. below 10 ppm). Effects of co-exposure to other solvents on the hippuric acid and *o*-cresol levels in urine also needs further examination in occupational

settings, because exposure to toluene in combination with other solvents (rather than exposure to toluene alone) is the most common type of exposure in practice.

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4.7 Trichloroethylene

4.7.1 Introduction

Trichloroethylene had been extensively used over many years as a degreasing agent in many industries (1–2). However, its use has been decreasing in some countries due to various concerns, including its potential capacity to pollute the environment. Comprehensive reviews on the toxicology and biological monitoring are available (1–4). As it is shown below, the major metabolites of trichloroethylene are trichloracetic acid and trichloroethanol.

4.7.2 Physical-chemical properties

Trichloroethylene (CAS No. 79-01-6) is a liquid at room temperature.

Molecular formula	$CHCl=CCl_2$
Molecular weight	131.4
Specific gravity	1.464 at 20°C
Vapour pressure	10.3 kPa at 25°C
Solubility	The logarithm of the octanol/water partition coefficient is 2.88.

Conversion factors:

Trichloroethylene	1 mg/m^3 = 0.186 ppm; 1 ppm = 5.38 mg/m^3 at 25°C
Trichloroacetic acid	1 mg/L = 0.0061 mmol/L; 1 mmol/L = 163 mg/L
Trichloroethanol	1 mg/mL = 0.0067 mmol/L; 1 mmol/L = 149 mg/mL

4.7.3 Possible occupational and non-occupational exposures

The presence of trichloroethylene [in the order of mg/m^3 (5)] in the general atmosphere has been well known. Trichloroethylene is also present in ground water and, in some cases, in drinking water (6). Cases of intentional trichloroethylene inhalation (solvent abuse) have also been reported (7–8).

Trichloroethylene had been extensively used in industry for vapour degreasing of metal parts, as well as cold cleaning of other materials. There is also a range of other lesser miscellaneous applications (4).

4.7.4 Summary of toxicokinetics

4.7.4.1 Absorption

(a) Inhalation

The major route of exposure to trichloroethylene in occupational settings is via inhalation with an absorption rate of about 60% (9).

(b) Dermal

Long contact of the skin with liquid trichloroethylene may result in significant dermal absorption.

4.7.4.2 Metabolic pathways and biochemical interaction

The majority of trichloroethylene is metabolized via oxidation at the double bond, translocation of chlorine atom and oxidation to form chloral hydrate, and then further oxidation to trichloroacetic acid (TCA) or reduction to trichloroethanol (TCE) (fig. 4.7.1) (10, 11).

$CHCl_2=CH_2Cl$
Trichloroethylene
|
(Epoxidation and hydration)
↓
$CCl_3CH(OH)_2$
Chloral hydrate
|
(Oxidation)   (Reduction)
↓   ↓
CCl_3CH_2OH CCl_3COOH
Trichloroethanol (TCE)   Trichloroacetic acid
|
(Glucuronidation)
↓
Glucuronide of TCE
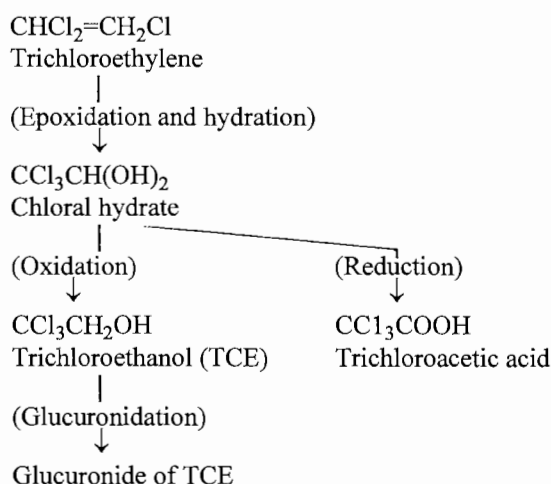
Figure 4.7.1. Schematic metabolism of trichloroethylene (simplified from ref. 9 and 10)

4.7.4.3 Distribution and elimination

Both TCA as it is, and TCE mostly after glucuronidation are excreted into urine. The elimination half-time of total trichloro-compounds (TCA+TCE) is about 40 to 50 hrs (12). The half-time for TCE and TCA individually is about 20 and 55 hrs, respectively (13). Thus the TCA:TCE ratio varies depending on the time of urine sampling after the end of exposure. The ratio for the end-of-shift sampling for TCA:TCE is about 1:2.

4.7.5 Summary of toxic effects

The predominant effect of trichloroethylene after acute intensive exposure is the suppression of the central nervous system function. The liver and the kidney may also be affected after repeated exposure to high concentration (2). In animal carcinogenicity studies, repeated administration of trichloroethylene to mice induced hepatocellular carcinoma and lung tumours in both sexes (14), but evaluation of carcinogenicity in humans is controversial (14–17).

4.7.6 Biological monitoring indices

Available biological monitoring indices are summarized in table 4.7.1.

Table 4.7.1. Available biological monitoring indices

Indicator	Suitability for use	Ref.
TCA in urine	Non-invasive and fairly specific	(18,19)
Total trichloro-compounds (TCA + TCE)	Non-invasive and fairly specific	(18,19)
TCE in blood	Invasive but specific, needs further validation	(20)
Trichloroethylene in exhaled air	Non-invasive and specific, needs further validation	(21)
Trichloroethylene in blood	Invasive and specific, needs further validation	(22)

4.7.7 TCA in urine and Total Trichloro-Compounds (TCA+TCE) in urine

4.7.7.1 Toxicokinetics

This is summarized in section 4.7.4.

4.7.7.2 Biological sampling

(a) Sampling time and specimen

Because the half-time for the excretion of trichloroethylene metabolites in urine is long, choosing the optimum time for urine sampling is important for quantitative evaluation of exposure intensity. There is a significant increase in TCA and total trichloro-compound levels in urine towards the end of a workweek, in addition to the increase towards the shift-end within a working day, when the exposure is reasonably constant throughout the workweek.

(b) Contamination possibilities

As the analytes are metabolite of trichloroethylene, there is little possibility of contamination.

(c) Sampling device and container

Urine samples should be collected into clean containers.

(d) Preservation, shipment and stability

Both TCA and TCE are stable in urine. Nevertheless, it is recommended to keep the urine samples either refrigerated or frozen to prevent the spoilage, if analysis cannot be performed on the day of collection.

4.7.7.3 Recommended analytical methods

Colorimetric methods have been widely used and are still in use. Gas chromatographic (GC) methods, especially when combined with a head-space technique, are convenient and practical. A colorimetric method and a GC method are described below.

4.7.7.3.1 Colorimetric method

(a) Principle of the method

TCA can be measured colorimetrically using the Fujiwara reaction in alkaline pyridine.

For total trichloro-compound measurements, TCE glucuronide in urine is first hydrolyzed and oxidized to TCA by heating in the presence of chromium trioxide. The combination of TCA and TCE (i.e., total trichloro-compounds) can then be measured together after making the solution alkaline. There are many variations in the procedures of conducting urinalysis by colorimetry (19, 23–26). The following is one example (19, 25).

(b) Reagents required

Trichloroacetic acid as standard, chromium trioxide, concentrated nitric acid, potassium hydroxide, pyridine and ice. Anhydrous TCA is used for standard preparation. Care must be taken to ensure that the TCA is kept in its anhydrous state, as it is extremely hygroscopic.

Use of chromium trioxide and pyridine needs **caution** because chromium [VI] is carcinogenic to humans (25), and pyridine is both pungent and toxic.

(c) Equipment required

A spectrophotometer (or colorimeter) and, if available, a recorder. A local exhaust system is needed to vent the pyridine vapour. A boiling water bath and an air condenser are also needed.

(d) Procedure and calibration

The whole procedure should be carried out in a fume cupboard or similar ventilated cabinet, as pyridine is both pungent and toxic. To measure total trichloro-compounds (TCA+TCE), 0.5 mL of urine is mixed with 0.5 mL of an oxidizing reagent (8 g chromium trioxide dissolved in 5 mL water and 15 mL concentrated nitric acid) in a tube with an air condenser attached. The tube is heated for 15 min in a boiling water bath to complete the hydrolysis and oxidation. After cooling to 0°C in ice, 2.5 mL of 7.8 mol/L potassium hydroxide is added followed by mixing, and then 5 mL of pyridine is added. After mixing by shaking, the mixture is heated at 65°C for 1 hour, and then ice-chilled. Three mL of pyridine layer is transferred to another tube and clarified by the addition of 0.5 mL water. The colour intensity is measured at 530 nm.

The measurement of TCA alone can be achieved by omitting the hydrolysis and oxidation step (i.e., no addition of oxidizing agent nor heating).

Calibration standards are prepared by adding known amounts of anhydrous TCA to blank urine which is then taken through the analytical procedure along with the samples.

(e) Criteria for analytical reliability

i) Trueness
Trueness, based on recovery studies, was 93% (19, 25).

ii) Precision
The day-to-day precision of the analysis at 2–20 mg/L (0.012–0.12 mmol/L) was 2% relative standard deviation (19, 25).

iii) Detectability
The detection limit of TCA in urine was 0.5 mg/L (0.003 mmol/L) (19, 25).

(f) Quality assurance

i) Special precautions
Participation in external proficiency testing programmes is recommended (see Chapter 2 of this volume). Internal quality control using split samples, pooled urine and blanks is necessary to ensure quality results.

(g) Sources of possible errors

i) Pre-analytical
The time of urine sampling is important (see 4.7.7.2).

ii) Analytical
As noted above, TCA is highly hygroscopic and the TCA standard must be desiccated thoroughly, e.g. in presence of conc. sulfuric acid overnight, before weighing. In the colour-forming reaction at 65°C, prolonged heating may result in fading of the colour. The coloured pyridine layer must be made clear before measurement; more water can be added, if necessary.

(h) Reference to the most comprehensive description of the method

Tanaka S, Ikeda M. A method for determination of trichloroethanol and trichloroacetic acid in urine. British Journal of Industrial Medicine 1968;25:214–9.

Imamura T, Ikeda M. A time-saving procedure for determination of total trichloro-compounds in human urine samples. Internationales Archiv für Arbeitsmedizin 1973;31: 333–8.

(i) Evaluation of the method

Although the method is sensitive, it gives a colour reaction with unidentified metabolite(s) of other chlorinated hydrocarbon solvents (27).

4.7.7.3.2 Gas ghromatography method

(a) Principle of the method

TCE glucuronide in urine is hydrolysed by pretreatment with *β*-glucuronidase (20, 28–29) or with sulphuric acid (18) to liberate volatile TCE. TCA is made volatile by methyl esterification with dimethyl sulphate (20–21), diazomethane (28), or methanol and sulphuric acid (18). Several methods have been described (18, 20–21, 28–31). The following is one example (18).

(b) Reagents

TCA and TCE as standards, methanol and concentrated sulphuric acid.

(c) Equipment required

A GC equipped with an electron capture detector (ECD), a head-space air sampler, a temperature controlled equilibration bath are required. A recorder or data processor is also needed.

(d) Procedure and calibration

In a vial for head-space GC (kept in a chilled water bath), 0.1 mL of the urine sample is mixed with 0.6 mL of the esterification reagent [an ice-chilled mixture of methanol (1 volume), concentrated sulphuric acid (5 volume) and water (6 volume), kept refrigerated in the dark when not in use]. The vial is immediately sealed with a Teflon-coated septum. The mixture is heated at 85°C for 40 min in an equilibration bath. The head-space air is introduced to a ECD-GC by means of an automatic sampler and analysed on a 30 m-long DB-WAX capillary column at 130°C. Nitrogen as a carrier gas is allowed to flow at 2 mL/min. The split ratio is set at 40:1.

(e) Criteria for analytical reliability

i) Trueness
Trueness, based on recovery studies, was 96–105% for TCA and 99–103% for TCE, when samples containing 3 to 32 mg TCA and 1 to 100 mg TCE/L were assayed (18).

ii) Precision
The overall precision of the analysis was less than 4% relative standard deviation for both TCA and TCE, when samples containing 3–32 mg/L (0.02–0.2 mmol/L) of TCA and 1–100 mg/L (0.0067–0.67 mmol/L) of TCE were assayed (18).

iii) Detectability
The estimated detection limit for TCA in urine was 0.02 mg/L (1 x 10^{-4} mmol/L) and

for TCE 0.05 mg/L (3×10^{-4} mmol/L).

(f) Quality assurance

i) Special precautions
Participation in external proficiency testing programmes is recommended (see Chapter 2 of this volume). Internal quality control using split samples, pooled urine and blanks is necessary to ensure quality results.

ii) Interferences
None reported.

(g) Sources of possible error

The use of an electron capture detector is essential, as a flame ionisation detector is not sensitive enough.

(h) Reference to the most comprehensive description of the method

Ohara A, Michitsuji H, Jyoyama Y, Yamaguchi K, Fujiki Y. Determination of trichloroacetic acid and trichloroethanol by head-space gas chromatography (HS-GC). Japanese Journal of Industrial Medicine 1991;33:94–103 (in Japanese with English abstract).

(i) Evaluation of the method

The ECD-GC method is specific and sensitive enough to detect occupational exposure to trichloroethylene. The results obtained by colorimetric and GC methods agree well with each other (18).

4.7.7.4 Other analytical methods

A number of other GC methods have been published (20–21, 28–31).

4.7.7.5 Guide to interpretation

There are several studies to show dose-dependent excretion of TCA and total trichloro-compounds in urine of subjects exposed to trichloroethylene, either experimentally (9, 32) or occupationally (10, 33).

(a) Measured values in groups without occupational exposure

Levels of TCA and total trichloro-compounds in urine of subjects without occupational exposure are essentially nil (18–19), provided that there is no specific exposure from local environmental pollution or deliberate abuse of solvents containing trichloroethylene.

(b) Published biological action levels

The American Conference of Governmental Industrial Hygienists (16) has adopted a value of 100 mg/g creatinine for TCA in urine collected at the end of a shift at the end

of a work week, and 300 mg/g creatinine for total trichloro-compounds (TCA+TCE) in urine collected at the end of a shift at the end of a workweek as a 'Biological Exposure Indices (BEI)'. These values are set in reference to the time-weighted average concentration of occupational exposure to trichloroethylene at 50 ppm (269 mg/m^3).

Deutsche Forschungsgemeinschaft (17) has adopted a value of 100 mg/L for TCA in urine collected at the end of exposure or the end of a workshift after several shifts (or for long-term exposures) as a 'Biological Tolerance Value (BAT-value)'.

(c) Non-analytical interference

i) Exposure to other chemicals (the same metabolite), co-exposure, ethanol intake, medication

Tetrachloroethylene and 1,1,1-trichloroethane (methylchloroform), the two common solvents for degreasing or dry-cleaning in industry, will also be metabolized *in vivo* to TCA and TCE (10, 12, 27). Trichloroethylene metabolism may be influenced by alcohol intake.

(d) Ethnic differences (enzyme deficiency, environment, diet)

Ethnic differences in trichloroethylene metabolism have been suggested (33–35).

4.7.8 Other indices

TCE in blood can be used as a biological indicator of exposure to trichloroethylene (16–17). The half-time for the disappearance of TCE from blood after cessation of exposure is short [about 12 hours (36)]. Therefore, the choice of optimum time for blood sampling is important for quantitative evaluation of exposure intensity. The GC methods described for urinalysis are also applicable to the analysis of TCE in blood, and the GC methods combined with head-space technique (29) should be more convenient and practical.

The American Conference of Governmental Industrial Hygienists (16) has adopted a value of 4 mg/L (0.027 mmol/L) for free TCE (i.e., TCE without hydrolysis) in blood collected at the end of shift at the end of a work week as a 'Biological Exposure Index (BEI)'; about a half of TCE in blood is conjugated with glucuronic acid (20).

Deutsche Forschungsgemeinschaft (17) has adopted a value of 5 mg/L (0.034 mmol/L) for TCE in blood collected at the end of exposure or the end of a workshift after several shifts (or for long-term exposures) as a 'Biological Tolerance Value (BAT-value)'.

4.7.9 Research needs

The intensity of trichloroethylene exposure in the occupational setting has been dramatically reduced in recent years due to improvements in industrial hygiene. The validity of the tests needs to be re-examined under the present lower exposure conditions encountered in modern industries.

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4.8 Xylene

4.8.1 Introduction

Xylene is a common organic solvent used in a wide range of industries, but usually present in combination with toluene (1–3), and single, unmixed use is rather limited. Among the three isomers, m-xylene is the dominant constituent in commercial xylene as an industrial solvent (1–2). Xylenes are present also in automobile gasoline, especially unleaded fuel (4). The chemical is also used as raw material for synthesis of plasticizers, resins, etc. Comprehensive reviews on toxicology and biological monitoring are available (5–8).

4.8.2 Physical-chemical properties

Xylene is a liquid at room temperature.
Molecular formula $C_6H_4(CH_3)_2$
Molecular weight   106.17

o-Xylene: CAS No. 95-47-6
Specific gravity   0.880 at 20°C
Vapour pressure   0.91 kPa at 25°C
Solubility   The logarithm of the octanol/water partition coefficient is 2.71.

m-Xylene: CAS No. 108-38-3
Specific gravity   0.864 at 20°C
Vapour pressure   1.11 kPa at 25°C
Solubility   The logarithm of the octanol/water partition coefficient is 3.20.

p-Xylene: CAS No. 106-42-3
Specific gravity   0.861 at 20°C
Vapour pressure   1.19 kPa at 25°C
Solubility   The logarithm of the octanol/water partition coefficient is 3.15.

Conversion factors
Xylene in air: 1 ppm = 4.34 mg/m^3; 1 mg/m^3 = 0.230 ppm at 25°C
Xylene in blood: 1 mg/L = 0.0094 mmol/L; 1 mmol/L = 106 mg/L
Methylhippuric acid : 1 mg/L = 0.0052 mmol/L; 1 mmol/L = 193 mg/L

4.8.3 Possible occupational and non-occupational exposures

Xylene is among the most commonly used solvents and is also a material for organic synthesis in industry. It is a constituent of unleaded automobile gasoline. Xylene is present in general atmosphere [e.g. 40 μg/m^3 (9)], but the extent of non-occupational expo-

sure via the general environment should be negligible compared with occupational exposure.

4.8.4 Summary of toxicokinetics

4.8.4.1 Absorption

(a) Inhalation

The major route of entry for xylene is via the lungs.

(b) Dermal

Xylene can also be absorbed through the skin.

4.8.4.2 Metabolic pathways and biochemical interaction

The metabolism of xylene in humans is very similar to that of toluene. Namely, the major portion of the absorbed xylene is oxidized primarily at one of the two side-chain methyl moieties. The oxidized intermediate, methylbenzoic acid (or toluic acid) undergoes glycine conjugation and is excreted in urine as methylhippuric acid. A minor portion of the absorbed xylene is excreted as xylenol (in the forms of sulphates or glucuronides) (fig. 4.8.1) (8).

$CH_3C_6H_4CH_3$
Xylenes (dimethylbenzene)
|
(Side chain oxidation)   (Aromatic oxidation as a minor pathway)
↓
$CH_3C_6H_4CH_2OH$
Methylbenzyl alcohol
↓
$CH_3C_6H_4COOH$
Methylbenzoic acid
|
(Glycine conjugation)
↓
$CH_3C_6H_4CONHCH_2COOH$
Methylhippuric acid
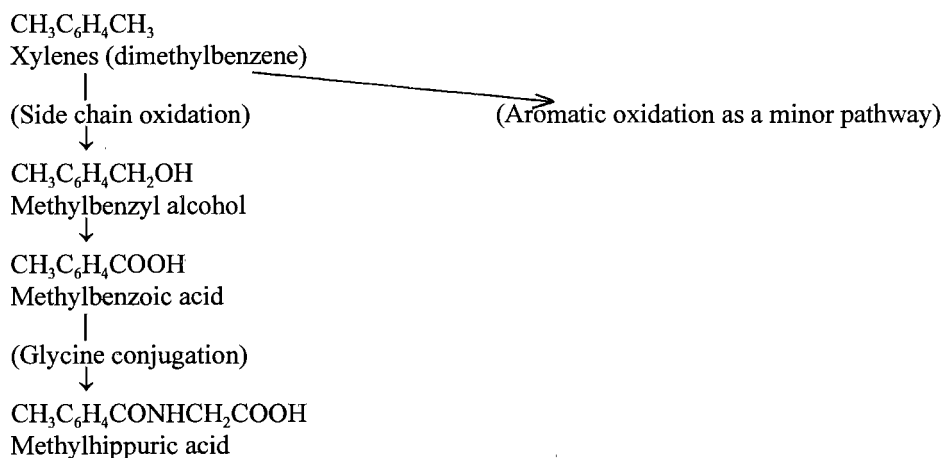
Figure 4.8.1. Schematic metabolism of xylenes (simplified from ref. 8)

4.8.5 Summary of toxic effects

The toxicity profile of the three xylene isomers is similar to that of toluene (10); namely, the most important toxic effect under occupational conditions is suppression of the function of the central nervous system, as evidenced by increases in various subjec-

tive symptoms (11).

4.8.6 Biological monitoring indices

Available biological monitoring indices are summarized in table 4.8.1.

Table 4.8.1. Available biological monitoring indices

Indicator	Suitability for use	Ref
Sum of methylhippuric acids in urine	Non-invasive	(12)
Individual methylhippuric acid isomers in urine	Non-invasive	(12)
Xylene in blood	Specific, but invasive	(13)

4.8.7 Methylhippuric acids in urine

Both the sum of methylhippuric acids, as well as individual methylhippuric acid isomers in urine are applicable for biological monitoring of occupational exposure to xylenes. Since exposure can be to either a mixture of the three xylene isomers or to individual isomers of xylene, depending on the xylene preparation in use, analysis of the solvent preparation or the workroom air is recommended to identify the leading isomer.

4.8.7.1 Toxicokinetics

This is summarized in section 4.8.4 above.

4.8.7.2 Biological sampling

(a) Sampling time and specimen

Because of the short half-time of methylhippuric acid excretion, the timing of sample collection is of critical importance for exposure assessment. Urine samples should be collected at the end of a workshift, and Monday afternoon should be excluded as in the case of toluene monitoring.

(b) Contamination possibilities

As the analytes are metabolites of xylene, there is little possibility of contamination.

(c) Sampling device and containers

Urine specimens should be collected into clean containers.

(d) Preservatives, shipment and stability

No preservatives are necessary. Samples should be stored under refrigeration, or more preferably by freezing, if analysis cannot be performed on the day of collection.

4.8.7.3 Recommended analytical methods

(a) Principle of the methods

The methods valid for determination of hippuric acid in urine are generally applicable to the analysis for methylhippuric acids. The most popular method of analysis is high performance liquid chromatography (HPLC) which allows separation and determination of the three methylhippuric acid isomers (13–15). Addition of ß-dextrin [e.g. at 1.5% (15)] to the mobile phase of the HPLC analysis will improve the separation of the isomers on the column. GC analysis after derivatization [e.g. methyl esterification (16)] is also applicable but less widely used. The following is one example (12) of HPLC procedures.

(b) Reagents required

Methylhippuric acids as standards, KH_2PO_4, acetonitrile, sodium 1-decanesulphonate, ß-dextrin (if necessary).

(c) Equipment required

A high pressure liquid chromatograph (HPLC) equipped with a UV detector, a recorder and, if available, an automatic liquid sampler are required. A clinical centrifuge is also needed.

(d) Procedure and calibration

An aliquot of the urine sample (1 mL) is mixed with 1 mL methanol and the mixture is centrifuged (e.g. at 1600 x g for 5 min with a clinical centrifuge) for removal of the precipitates. The supernatant solution (10 µL/injection) is introduced to a HPLC by means of an automatic liquid sampler. A stainless steel column (4.6 mm in diameter and 150 mm in length) packed with octadecyl silanized silica gel (TSK gel, ODS-80 TM, 5µm) is used for chromatography, and the column is kept at 25°C. The mobile phase is a mixture of 20 mmol/L KH_2PO_4 (pH 3.3; containing 3 mmol/L sodium 1-decanesulphonate) and acetonitrile (85:15), and is allowed to flow at 0.7 mL/min producing a pressure of 100 kg/cm^2. The fractions are monitored at a wavelength of 225 nm.

Standards are prepared by adding known amounts of methylhippuric acids to blank urine, which are then taken through the analytical procedure.

(e) Criteria for analytical reliability

i) Trueness
Trueness, based on recovery studies at 0.5–2.0 g/L, was 97–100% (12).

ii) Precision
The day-to-day precision of the analysis at 0.5–2.0 g/L was 2% relative standard deviation (12).

iii) Detectability
The estimated detection limit of MHA in urine was 4 mg/L.

(f) Quality assurance ˎ

i) Special precautions
Participation in external proficiency testing programmes is recommended (see Chapter 2 of this volume). Internal quality control using split samples, pooled urine and blanks is necessary to ensure quality results.

(g) Sources of possible error

i) Pre-analytical
Selection of the optimum time for urine sampling in relation to exposure is important (see 4.8.7.2a). When stored samples are analysed, care should be taken to mix the sample thoroughly before analysis to prevent any loss of the analytes by co-precipitation.

(h) Reference to the most comprehensive description of the method

Ogata M, Taguchi T. Simultaneous determination of urinary creatinine, and metabolites of toluene, xylene, styrene, ethylbenzene and phenol by automated high performance liquid chromatography. International Archives of Occupational and Environmental Health 1988;61:131–40.

(i) Evaluation of the method

The method is sensitive enough to detect occupational exposure to xylene.

4.8.7.4 Other analytical methods

Other HPLC methods are available (13–15).

4.8.7.5 Guide to interpretation

There are several volunteer exposure studies (17–18) and occupational health studies (19–25) which all indicate a good correlation between time-weighted average intensity of exposure to xylenes and concentrations of methylhippuric acids in urine samples collected at the end of a shift. This is true for the three isomers, both separately and in combination.

(a) Measured values in groups without occupational exposure

Biological levels without occupational exposure are very low and essentially nil.

(b) Published biological action levels

The American Conference of Governmental Industrial Hygienists (26) has adopted a value of 1.5 g/g creatinine (880 mmol/mol creatinine) for methylhippuric acids in urine collected at the end of a workshift as a 'Biological Exposure Index (BEI)'. This value is set in reference to the time-weighted average concentration of occupational exposure to xylenes at 434 mg/m^3 (100 ppm).

Deutsche Forschungsgemeinschaft (27) has adopted a value of 2 g/L (10.4 mmol/L) for

methylhippuric acids in urine collected at the end of exposure or the end of a workshift as a 'Biological Tolerance Value (BAT-Value)'.

(c) Non-analytical interferences

i) Exposure to other chemicals (the same metabolite), co-exposure, ethanol intake, medication

Because the metabolism of xylenes is very similar to that of toluene (see 4.8.4, Summary of toxicokinetics), metabolism of xylene may interact with that of toluene especially when the exposures are high (28), although such may not be the case with low exposures (29). It is also probable that smoking and drinking suppress metabolism of xylenes to methylhippuric acids as is the case with toluene (30–33).

4.8.8 Other indices

It has been observed that the xylene concentration in venous blood reflects the intensity of exposure to xylene (17, 34, 35). Xylene in urine is also found to correlate with xylene exposure (36). In practice, timing for both blood and urine sampling is critical, and care should be taken to avoid contamination of, or loss from, samples, e.g. absorption of xylene in atmospheric air into blood or urine, leakage of xylene in biological materials into air. Because xylene in blood or urine is specific with non-detectable background levels, its presence when properly analysed can be regarded as the evidence of exposure to xylene.

Deutsche Forschungsgemeinschaft (27) has adopted a value of 1.5 mg/L for xylenes in blood collected at the end of exposure or the end of a workshift as a 'Biological Tolerance Value (BAT-value)'.

4.8.9 Research needs

The measurement of xylenes in blood and urine is highly specific to exposure to xylenes. Further occupational health studies are needed to validate these measurements, particularly at low levels of exposure.

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M. Ikeda
see section 4.2

Chapter 5. Selected pesticides

5.1 Organophosphorus pesticides

5.1.1 Introduction

Organophosphorus pesticides have been produced since the 1940s and as the more persistent organochlorine pesticides have been phased out, there has been widespread usage of this family of compounds. The basic chemical structure can be represented by

$$R_1O\diagdown\;_{\diagup}O(S)$$
$$P$$
$$R_1O\diagup\;\diagdown(OX\ or\ SX)$$
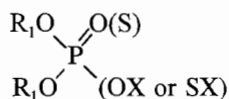
in which X is the leaving group. These compounds have a range of physical-chemical properties and toxicities and their routes of metabolism can vary to a considerable extent. The chemistry and toxicology of over 50 of the major organophosphorus pesticides in use are reviewed by Gallo and Lawryk (1) in 'Handbook of Pesticide Toxicology'. The mode of action of these pesticides is based on their anticholinesterase properties although their mammalian toxicity can also be mediated by other mechanisms. A large number of the organophosphorus pesticides in use are metabolized to one or more water soluble dialkyl phosphates and are excreted in the urine after exposure. Shafik and Enos (2) first reported the measurement of alkyl phosphates in urine of workers exposed to organophosphorus pesticides. Although several research groups have measured alkyl phosphates in urine as an index of organophosphorus pesticide absorption, it is only recently that advances in analytical methods have allowed this to be considered a possible technique for routine monitoring.

There are essentially four subclasses of organophosphorus (OP) pesticides of interest:

Phosphate
$$R_1O\diagdown\;_{\diagup}O$$
$$P$$
$$R_1O\diagup\;\diagdown OX$$
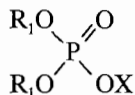
Phosphorothiolate
$$R_1O\diagdown\;_{\diagup}O$$
$$P$$
$$R_1O\diagup\;\diagdown SX$$
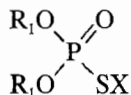
Phosphorothionate

$$R_1O \diagdown \mathllap{} P \diagup^{S} \diagdown OX \diagup R_1O$$
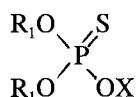
Phosphorodithioate

$$R_1O \diagdown P \diagup^{S} \diagdown SX \diagup R_1O$$
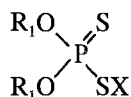
R_1;   the majority of the organophosphorus pesticides in use are dimethyl or diethyl compounds.

5.1.2 Physical-chemical properties

The physical form and physicochemical properties of the numerous OP pesticides vary with the size and structure of the leaving group X. The majority have some solubility in water but are considerably more soluble in organic solvents.

Details of physical-chemical properties of individual organophosphorus pesticides can be found in the 'Handbook of Pesticide Toxicology' Chapter 16 (1) and in the 'The Pesticide Manual' (3).

5.1.3 Possible occupational and non-occupational exposures

Organophosphorus pesticides are sold in a range of formulations for use for pest control on crops, in crop and seed storage and in animal husbandry. In agricultural operations it is quite possible for a farm worker to use several formulations containing differing OP pesticides over a period of a few days. On the other hand, an individual, perhaps engaged in pesticide production or packaging, may only be exposed to one compound at a time. It is clear from detection of trace amounts of alkyl phosphates in some "pre-exposure" urine samples or in samples from "control" individuals, that occasional uses of small amounts of pesticides often are unreported. However, the majority of samples from unexposed individuals or those taken to establish a pre-exposure baseline have no metabolites detectable.

5.1.4 Summary of toxicokinetics

5.1.4.1 Absorption

(a) Inhalation

Spray application of pesticides provides excellent opportunities for inhalation.

(b) Dermal

The majority of OP pesticides can be absorbed to a significant extent through the skin and this is reflected in the LD_{50} values reported following dermal application. Absorption through the skin tends to be prolonged compared with other routes and this

results in an extended time course for the excretion of the metabolites in the urine.

(c) Gastrointestinal

Gastrointestinal absorption in humans has been regularly documented following non-accident ingestion for many of these pesticides.

5.1.4.2 Metabolic pathways and biochemical interaction

Several biotransformation reactions are involved in the degradation of OP pesticides. Studies in several species have shown rapid breakdown of the majority of OP pesticides tested with the appearance of water soluble metabolites in the urine. Typically 90 to 95% of radioactivity from a labelled pesticide can be recovered in 24 hours after oral or parenteral administration (1). The majority of OP pesticides in use are metabolized to six simple alkyl phosphorus compounds, i.e., dimethylphosphate (DMP), diethylphosphate (DEP), O,O-dimethylphosphorothioate (DMPT), O,O-diethylphosphorothioate (DEPT), O,O-dimethylphosphorodithioate (DMPDT) and O,O-diethylphosphorodithioate (DEPDT), (see figure 5.1.1). Measurement of these metabolites is the basis of the biological monitoring method described below.

As the metabolism of these OP compounds involves a variety of hydrolysis, oxidation and conjugation reactions, there is clearly the opportunity for a range of biochemical interactions. The involvement of cytochrome P450 enzymes in the oxidation stages suggests possible interactions with concurrent drug administration. The large number of OP pesticides in use and the possibility of interactions between many of them and common medications suggests that interactions must have occurred but the evidence is difficult to find.

5.1.4.3 Distribution

Experimental studies in several species have shown widespread tissue and organ distribution of radioactivity from appropriately labelled OP compounds before most of the activity appears in the urine as water soluble metabolites. However, there is evidence from these studies and human experience that a small proportion of the more lipid soluble pesticides can be stored in fat depots to be released later (see (1) for individual compounds).

5.1.4.4 Elimination

Experimental oral administration of several different pesticides to human volunteers has shown that when urinary metabolites are measured, the rate of elimination is similar to that seen in animal studies. It appears that 90% of the compound is eliminated in between 6 and 24 hours after administration. There is evidence though, that after self-poisoning, patients may still be excreting detectable levels of metabolites up to 14 days later.

5.1.5 Summary of toxic effects

The acute toxicity of the organophosphorus pesticides is related to the inhibition of the enzyme acetyl cholinesterase. Clinical symptoms include headache, giddiness, blurred vision, nausea, weakness, cramps, chest discomfort and nervousness. Clinical changes include sweating, salivation, vomiting, loss of sphincter control, miosis, muscular twitching, convulsions, coma and death.

Chronic toxicity is induced by some OP pesticides as the well defined organophosphate-induced delayed neuropathy associated with the inhibition of the enzyme, neuropathy target esterase (NTE). More recently an 'intermediate syndrome' has been described in which neurotoxic symptoms occur within days of acute poisoning, i.e., before the typical onset of delayed neuropathy associated with NTE inhibition. There is some evidence that chronic neuropsychiatric changes can be detected following episodes of acute poisoning, but the causes and extent have not been established. Details of the mechanisms of OP toxicity and the associated clinical effects are well described by Gallo and Lawryk (1).

5.1.6 Biological monitoring indices

The measurement of erythrocyte acetyl cholinesterase and/or plasma cholinesterase has been used for monitoring of workers exposed to organophosphorus pesticides for many years (1). Erythrocyte acetyl cholinesterase activity (AcChE) has been taken as a surrogate target for the nervous system activity. Although measurement of AcChE (and also the less specific plasma cholinesterase enzyme) does reflect the absorption of OP compounds, the relationship between the depression in enzyme activity and early symptoms is not always close enough for monitoring of moderate levels of exposure. The measurements become progressively more useful in the diagnosis of frank toxicity. However, modern automated analytical methods allow high precision in the measurement of these two enzymes so that a relatively small fall in enzyme activity from one measurement to another can be ascribed to exposure to the OP pesticide between the measurements. Mason and Lewis (4) have compared the effects of intra-individual variation and analytical imprecision on cholinesterase measurements. A fall of 15% in erythrocyte AcetylChE and 7.5% in plasma ChE between two successive samples suggests significant inhibition, if the laboratory's analytical performance is such that the coefficients of variation for the two assays are 3.5 and 2.5%, respectively. A detailed review of the measurement of erythrocyte and plasma cholinesterases is presented by Gallo and Lawryk (1).

The observation that many OP pesticides are metabolized in humans to one or more of six dialkyl phosphates (figure 5.1.1) and these are preferentially excreted in the urine usually within 24–48 hours of exposure led to the suggestion by Shafik and Enos (2) that exposed workers could be monitored by measurement of these metabolites. Any analytical method has to be able to extract and analyse dialkyl phosphates, phosphorothioates and phosphorodithioates; compounds with differing reactivity, solubility and stability. Although several authors have published a range of analytical approaches to the measurement of these "alkyl phosphates" in urine, the technical difficulties in-

volved have been such that this form of biological monitoring has not yet been adopted into routine occupational health practice. Experience in the use and interpretation of cholinesterase measurements has obscured the potential usefulness of alkyl phosphate measurement. However, as exposure levels are reduced and attention turns to the chronic effects of lower levels of exposure, cholinesterase measurements are seen as less useful and the use of alkyl phosphate measurements to assess exposure is being reassessed. Improvements in analytical technology have made it possible to increase the precision of the analyses and throughput of samples making these measurements available for routine screening (see below). The advantages and disadvantages of alkyl phosphate and cholinesterase measurement are summarized in table 5.1.1.
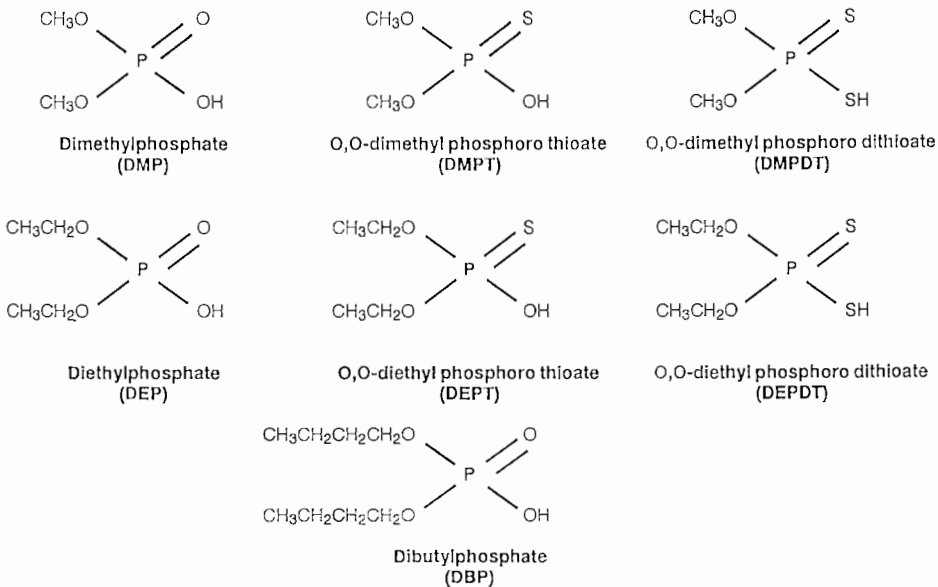
Figure 5.1.1. Structure of dialkyl phosphate metabolites and internal standard

Suitable methods for the measurement of both urinary alkyl phosphates and blood cholinesterases are described below; sections 5.1.7 and 5.1.8, respectively.

Table 5.1.1. Comparison of the use of urinary alkyl phosphates and blood cholines-
terases for monitoring organophosphorus pesticide exposure

	Alkyl phosphates	Cholinesterases
Specimen	Urine (non-invasive, but dilution/ concentration effects)	Blood (invasive, infective risk)
Intra-individual variation	unknown	high
Analytical methods		
Stability	good	good
Precision	medium	high
Convenience	manual analysis, several stages	automated analysis (also field method)
Sampling time	end of shift or? next morning	pre- and post-exposure samples required
Interpretation	able to detect low levels of exposure BUT interpretation in terms of health risk is not known	large % falls relate to clinical symptoms but measurements are insensitive to lower exposure levels
Advantages	sensitive to recent and low exposures, non-invasive	considerable experience in use
Disadvantages	little practical experience, analytically more demanding	insensitive to medium to low exposures, do not reflect clinical symptoms

5.1.7 Urinary alkyl phosphates

5.1.7.1 Toxicokinetics (or physiology)

The kinetics of excretion of alkyl phosphates will depend on the physical-chemical properties and the manner and extent of the absorption and metabolism of the parent OP pesticide. A considerable number of animal studies using radioactively labelled OP pesticides have been reported and a limited number of human volunteer studies have also been performed (see individual pesticides reviewed in (1)). In general it appears that 75 to 100% of oral or parenteral doses of OP pesticides given to rats or humans are metabolized to water soluble metabolites which appear in the urine in the first 24 (or 48) hours after administration. The rate of appearance of water-soluble metabolites in urine is considerably slower following dermal application and so measurement of urinary alkyl phosphates will reflect integrated skin absorption over the previous few days.

5.1.7.2 Biological sampling

(a) Sampling time and specimen

The balance between skin absorption and inhalation will affect the time course of alkyl phosphate excretion in relation to exposure as will differences in working practices (occasional versus regular exposures). Sampling strategy will depend on the aims of the monitoring exercise. Monitoring may be performed, for example, to identify those ex-

posed, to establish the sources of exposure, to quantitate uptake, or to monitor remedial activity. Field studies in the literature have shown that 24 to 48 hour full urine collections are needed for near quantitative recovery. However, a study monitoring the clean-up of domestic premises (5) has shown the usefulness of isolated morning urine samples. In view of the large number of pesticides used, opportunities for dermal and/or inhalation exposure and different temporal patterns of exposure, it is difficult to recommend a standard sampling time for all circumstances. As exposures become more chronic and the dermal route is involved, excretion patterns will be more damped and sampling time less important; the measured concentration of alkyl phosphates being a more general index of longer term exposure than just absorption during the previous shift. It is suggested though that, in the absence of information concerning the pesticide involved and the detailed pattern of exposure, the best approach is to take a urine sample the next morning after exposure at the beginning of the first workshift, (see below).

(b) Contamination possibilities

The normal precautions should be taken when collecting urine samples during workplace activities to minimize contamination by the parent compounds or other workplace materials.

(c) Sampling device and container

A plain urine collection bottle of glass, or preferably plastic, is suitable.

(d) Preservatives, shipment and stability

No preservatives are necessary. Ito et al. (6) have established that alkyl phosphates are stable in deep frozen urine samples for a minimum of 20 weeks, while Kummer and van Sittert (7) report that dimethylphosphate is stable in urine stored at 4°C for at least six months. Recent studies have shown that these compounds are stable in urine during postal transmission to the laboratory (Nutley B, Health and Safety Executive, UK, unpublished).

5.1.7.3 Recommended analytical method

(a) Principle of the method

The method involves concentrating the six major alkyl phosphate metabolites from an aliquot of urine by removal of water by azeotropic distillation with acetonitrile. The dry residue is treated with pentafluorobenzylbromide and the derivatized alkyl phosphates analysed by high resolution gas-chromatography with flame-photometric detection. This method is a recent modification by Nutley and Cocker (8) of the method described by Reid and Watts (9).

(b) Reagents required

Standards: dimethylphosphate (DMP), diethylphosphate (DEP), O,O-dimethylphosphorothioate (DMPT), O,O-diethylphosphorothioate (DEPT), O,O-dimethylphosphorodithioate (DMPDT) and O,O-diethylphosphorodithioate (DEPDT).

Internal standard: dibutyl phosphate (DBP)

Stock solutions of the standards and internal standard are prepared in HPLC grade acetonitrile and a working solution of mixed dimethyl and diethyl phosphates (0.01mmol/L) prepared in this solvent for each batch of analyses. A separate working solution of the internal standard DBP is also prepared in HPLC grade acetonitrile.

The following reagents are required for the derivatization procedure: pentafluorobenzylbromide (CARE! – a potent lacrimator), anhydrous potassium carbonate and 2–3 mm antibumping granules. Oxygen-free nitrogen is required to evaporate the acetonitrile/water mixture.

(c) Equipment required

A heated dry-block and an oxygen-free nitrogen-gas manifold are needed for the extraction/derivatization stages.

Gas chromatographic analysis requires an autosampler, a split-splitless injector, a temperature programmable gas chromatograph, a flamephotometric detector and a data handling system. The column is BP10 25 m x 0.33 mm i.d. (0.5μm thick film) capillary column.

(d) Procedure and calibration

Calibration curves (0–0.003mmol/L) are prepared using urine from an individual with no known exposure to OP pesticides, see below. Samples (1mL) of test and quality control urine samples are dispensed into clean tubes and the internal standard DBP working solution (250μL) added to all tubes and mixed. Acetonitrile (7mL) is added to all tubes, the tubes mixed and centrifuged for 10 mins at 2000 rpm to remove precipitated salts. After centrifugation the supernatant is transferred to clean labelled culture tubes (16X100mm) and 15–20 antibumping granules added to each tube. The tubes are heated to 90°C in the dryblock and the contents evaporated to dryness under a stream of nitrogen. To ensure a completely dry residue, 4mL of acetonitrile is added and the sample taken to dryness again. It is important that the samples are thoroughly dried. When dry, anhydrous potassium carbonate (c. 50mg) is added to each tube together with acetonitrile (0.5mL) and pentafluorobenzylbromide (25μL).

NOTE – Pentafluorobenzylbromide is a potent and corrosive lacrimator! Handle in a fume cupboard.

Cap the tubes securely and heat in a dry-block at 50°C overnight (12 to 16 hours). Allow tubes to cool and then transfer an aliquot of the derivatized sample to GC vials for analysis.

Injections (1 μL) are made with an injection temperature of 280°C. Initial oven temperature of 140°C is held for 1 min, then increased at 8°C min^{-1} to 280°C and held for 2 min.

Separate stock solutions of each dialkyl phosphate (and dibutyl phosphate, the internal standard) in acetonitrile are prepared at a concentration of 1 mol/L and from these a working solution is prepared containing all 7 dialkyl phosphates at a concentration of 0.01mmol/L. A standard curve is prepared from a urine specimen from a volunteer unexposed to OP pesticides, adding 0, 50, 100, 150, 200, 250 and 300 µL of the working alkyl phosphate solution to 1.0mL aliquots of the urine sample. These are analysed in the same way as unknown urine specimens.

(e) Criteria of analytical reliability

This method is designed as a compromise to give optimum recovery of the six differing alkyl phosphates and internal standard in one analysis and the precision and recovery varies from one alkyl phosphate to another. Performance details of this method have been reported by Nutley and Cocker (8), see table 5.1.2.

Table 5.1.2. Estimated recoveries, limits of detection (LOD) and intra- and inter-assay variation. From Nutley and Cocker (8) with permission

	DMP	DEP	DMTP	DETP	DMDTP	DEDTP
Recovery[a] %	73	82	79	88	83	89
LOD[b] (µmoles/l)	0.05	0.01	0.02	0.01	0.01	0.01
intra-assay cv %	11.6	6.5	8.7	4.4	4.5	3.8
inter-assay cv %	14.2	13.3	22.6	6.3	6.4	7.4

a Recovery estimated by comparison with tributylphosphate response
b Limit of detection

(f) Quality assurance

Urine from workers not exposed to OP pesticides is spiked with the six metabolites at a nominal 0.001mmol/L. Aliquots of this urine sample are stored deep frozen and enough thawed for each analytical run. These quality control samples should be included after each calibration curve and after each set of five duplicate samples.

i) Special precautions
It is important to use the purest grade reagents commercially available.

ii) Interferences
Good chromatographic separation is achieved for all 6 metabolites and the internal standard with no interferences from other urinary compounds, see figure 5.1.2.

(g) Source of possible errors

i) Pre-analytical
Contamination of the urine sample by the parent pesticide or other organophosphorus compounds during sample collection should be avoided (7).

(h) Reference to the most comprehensive description of the method

Nutley B, Cocker J. Biological monitoring of workers occupationally exposed to organophosphorus pesticides. Pesticide Science 1993;38:315–22.
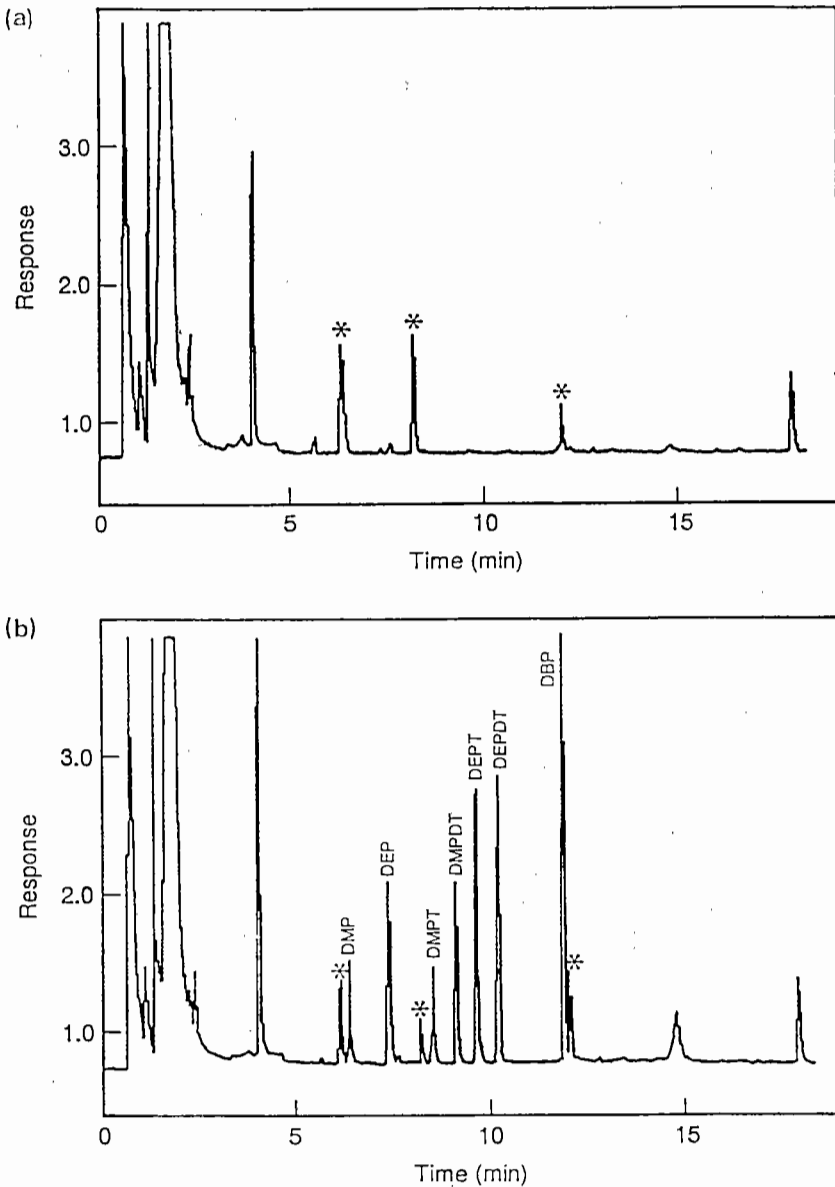

Figure 5.1.2. Typical chromatogram of urine extracts, (a) from a subject with no known exposure to organophosphate pesticides, (b) urine sample spiked with DMP, DEP, DMPT, DEPT, DMPDT, DEPDT and DBP at 2.5μmole litre⁻¹. Peaks marked with * are urinary compounds of unknown identity. From Nutley and Cocker (8) with permission

(i) Evaluation of the method

There have been considerable advances in analytical methods since the original description of the measurement of alkyl phosphates in human urine in 1969. Even so, the quantitative extraction, derivatization and analysis of all six metabolites at the same time remains a problem. The method described here (8) is a modification of the one developed by Reid and Watts (9). Nutley and Cocker have taken advantage of the greater resolving power of capillary gas chromatography and so have been able to dispense with the silica gel chromatography stage. By compromising on the derivatization conditions (now 50°C overnight) they have been able to combine the high and low temperature stages suggested by Reid and Watts thereby halving the number of gas chromatographic injections required. The use of an internal standard (dibutyl phosphate) has improved quantitation. These modifications in derivatization, chromatography and measurement make this method less time-consuming and more suitable for routine monitoring. Nutley and Cocker (8) have shown that it can take its place among other methods in a toxicology service laboratory; however, further experience is required in other laboratories to demonstrate its general applicability.

5.1.7.4 Other analytical methods

Other extraction and derivatization methods have been employed. However, these other methods all have some disadvantages, including the toxicity and carcinogenicity of reagents.

5.1.7.5 Guide to interpretation

In contrast to the numerous studies available correlating changes in blood cholinesterases with clinical symptoms, it is difficult to find equivalent studies comparing alkyl phosphate excretion with clinical effects. Davies et al. (10) reported the excretion of DEP and DETP in 7 cases of parathion poisoning and compared these with cholinesterase depression while Richter et al. (5) followed urinary DEP levels in 5 members of a household affected by diazinon. This group has also measured alkyl phosphate excretion in workers and kibbutzim exposed to pesticide spray (11). However, many of the subjects of their investigations were selected on the basis of non-specific symptoms and not as a cross-sectional sample designed to relate symptoms to biochemical indices of absorption. Two field studies of spraymen exposed to monocrotophos have been used to study the relationship between dimethylphosphate excretion, cholinesterase depression and clinical effects (7, 12). In both studies, the authors reported a correlation between dimethylphosphate excretion and depression of plasma cholinesterase activity, but in neither was there a fall in erythrocyte acetylcholinesterase nor were adverse health effects reported.

The lack of large-scale systematic studies relating alkyl phosphate excretion to the signs and symptoms of acute intoxication is probably due to: 1) the earlier lack of availability of a usable routine analytical method; and 2) clinicians' familiarity and satisfaction with cholinesterase monitoring during acute poisoning episodes. However, the usefulness of alkyl phosphate monitoring will be for monitoring populations of exposed workers and

residents not absorbing sufficient pesticide to cause acute poisoning, but enough to suggest that the pesticide might be responsible for low grade non-specific symptoms. Several of the earlier publications exploring the use of alkyl phosphate excretion reported significantly raised concentrations in those exposed to OP pesticides below levels sufficient to affect cholinesterase activity. No studies have yet been reported comparing alkyl phosphate excretion with possible long-term neurological or neuropsychiatric changes.

In the absence of data to correlate clinical events with biological monitoring measurements, a knowledge of the typical values found in workers from a variety of working situations allows the occupational health practitioner to evaluate results reported to him/her from the group currently being investigated (13). Nutley and Cocker (8) have reported alkyl phosphate concentrations in agricultural pesticide users and formulators (figure 5.1.3). These individuals were not selected on the basis of any symptoms and only one had depressed cholinesterase activity. These results will not necessarily be directly extrapolatable to other situations or climates but do give the occupational health practitioner some guide.

(a) Measured values in groups without occupational exposure

Several of the reported studies have indicated that trace levels of alkyl phosphates are detectable in a proportion of samples from unexposed control subjects or in pre-exposure urines (e.g. 14, 15, 8). Whether these alkyl phosphate residues come from food, water or environmental contamination is not clear. Agricultural workers may be using small amounts of OP pesticide regularly in undisclosed circumstances.

(b) Published biological action levels

There has not been sufficient information available to establish levels of urinary metabolites associated with the onset of overt clinical changes, lesser adverse effects or other indices of exposure. This is reflected in the lack of maximal advisable or allowable levels being set by any regulatory organization. Tentative action levels might be set according to the upper 90th percentile values observed across a range of occupational settings. There has been little discussion whether values should be recommended for individual alkyl phosphates and/or for the total alkyl phosphate excretion. Ideally any decision should be related to detailed clinical studies.

(c) Non-analytical interferences

Knowledge of the range of metabolic reactions involved in the degradation of OP pesticides to alkyl phosphates suggests that drugs and alcohol must have some modifying effects on the rate of alkyl phosphate appearance in the urine. However, this does not appear to have been investigated. It may be that the modifying effects of the route of absorption and the formulation of the pesticide will have a much greater effect upon the kinetics of excretion.
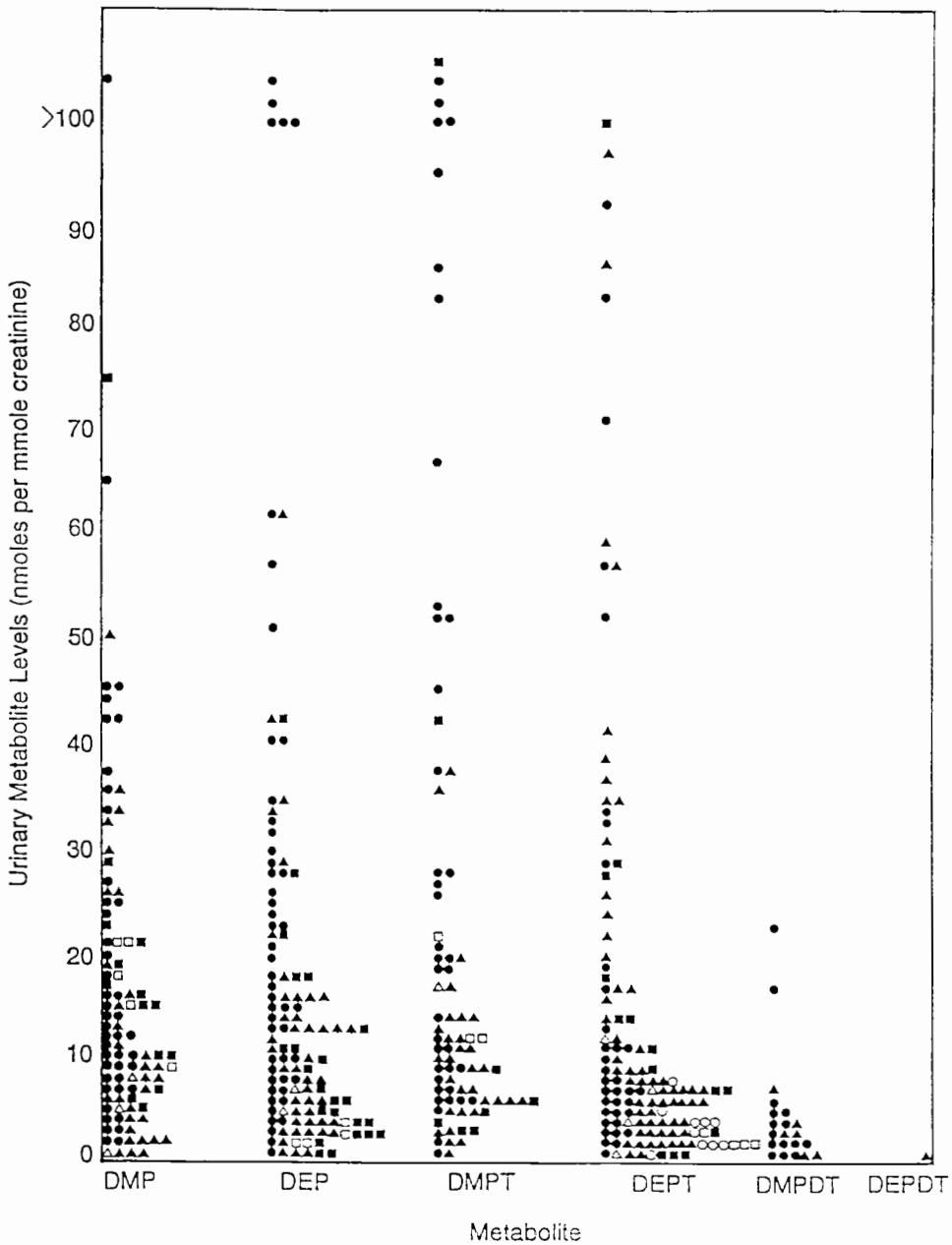
Figure 5.1.3. Distribution of urinary metabolites in urine samples from workers exposed to organophosphorus pesticides. Samples from workers are classified as sheep dippers -preexposure Δ, and postexposure ▲, sheep dippers - other exposures o, agricultural workers preexposure □, and postexposure ■ and formulation workers postexposure o. From Nutley and Cocker (8) with permission

(d) Sampling representative of recent or long-term exposure or biological effect

The animal and human studies of OP pesticide metabolism and kinetics indicates that alkyl phosphate excretion reflects absorption over the previous day or days, (see Section 5.1.4.4). There is a suggestion that some OP pesticides are retained to some extent in adipose tissue and that this may account for trace levels of metabolites in those currently unexposed. There is evidence, though, of accumulation following self-poisoning with a significant overdose.

(e) Ethnic differences (enzyme deficiency, environment, diet)

No populations have been identified as being especially suitable for alkylphosphate monitoring. However, there has been some concern that, although those with abnormal cholinesterase variants are not at increased risk from OP pesticide exposure, their low level of plasma enzyme activity makes biological effect monitoring difficult. Measurement of alkyl phosphates would add to the information available in protecting these individuals.

There is no evidence of ethnic differences in alkyl phosphate excretion although it is known that there are ethnic differences in hydrolytic reactions responsible for OP pesticide metabolism.

5.1.7.6 Research needs

There are two primary research needs. The first is to determine the optimum sampling strategy for acute and chronic exposure monitoring. This requires both experimental volunteer and worker studies to demonstrate the effects of inhalation versus skin absorption over one or multiple shifts. The second and most urgent need is for well designed clinical-epidemiological studies of sufficient power to establish the relationship between metabolite concentrations in urine and the onset of early toxic effects and cholinesterase inhibition.

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5.1.8 Erythrocyte and plasma cholinesterases

Erythrocyte cholinesterase (AcChE) and plasma cholinesterase are both inhibited by organophosphorus (OP) pesticides and this inhibition has been used as an index of OP exposure and toxicity in a variety of occupational and environmental settings. The usefulness of these assays performed both in the laboratory and the field has been confirmed in many studies and by their adoption in virtually all countries. A number of methods have been described, but nowadays most assays are based on the method of Ellman (1), i.e., hydrolysis of acetylthiocholine and measurement of liberated thiol. In this section both a field method using a microprocessor-based dedicated handheld instrument (Test-Mate OP kit) (2) and an automated laboratory-based procedure are described (3). The field method has recently been evaluated for the WHO (4).

5.1.8.1 Toxicokinetics

The signs and symptoms observed after poisoning by OP pesticides are the result of an accumulation of acetylcholine at neuromuscular junctions and other sites of action. Acetylcholine is the chemical mediator responsible for physiological transmission of nerve impulses from (a) preganglionic to postganglionic neurons of both the parasympathetic and sympathetic nervous systems, (b) postganglionic parasympathetic fibres to effector organs and postganglionic sympathetic fibres to sweat glands, (c) motor nerves to skeletal muscles and (d) some nerve endings within the central nervous system (5).

Under normal physiological conditions, acetylcholine is released from synaptic vesicles at the axonal nerve endings where it diffuses across the neuroeffector or neuromuscular junction to bring about transmission of a nerve impulse. Once this transmission has occurred the acetylcholine is rapidly hydrolysed by the enzyme AcChE to acetic acid and choline.

Esterases are divided into three classes. 'A' esterases are normally responsible for the hydrolysis of OP pesticides and carboxylic acid esters, 'B' esterases which are susceptible to progressive covalent inhibition by a variety of OP esters, and 'C' esterases which do not react with OP or carbamate compounds. The 'B' esterases include such important enzymes as chymotrypsin, acetylcholinesterase, various enzymes involved in the blood clotting cascade and immune responses and neuropathy target esterase.

In the course of inhibition of a 'B' esterase by an OP compound a reversible complex is first formed which can then be converted by a progressive covalent reaction to a stable phosphorylated enzyme. These products may then be hydrolysed to regenerate active enzyme plus hydrolysed OP: the latter products have no inhibitory power. This process may take hours or days so that organophosphorylated AcChE activity may only be restored over a long period.

Whereas AcChE is vital for the hydrolysis of acetylcholine and transmission of nerve impulses, other cholinesterases, such as plasma cholinesterase (butyrylcholinesterase or

pseudocholinesterase) have no known physiological role and their inhibition does not result in toxicity (6).

5.1.8.2 Biological sampling

(a) Sampling time and specimen

Because of the wide inter-individual variation in both plasma and AcChE activity, it is recommended that individuals should have at least one estimation of both plasma and erythrocyte cholinesterase activities before coming into contact with OP compounds. These figures provide a baseline against which any subsequent exposure estimations can be compared for their significance.

During an extended OP spraying programme at least twice weekly estimations are recommended, normally in the morning prior to the commencement of the day's spraying programme. Where reactivation of inhibited enzyme is a problem, as with dimethoxy-OP compounds, sampling and testing may have to be carried out immediately after completion of the day's spraying schedule. More frequent estimations will be required when exposure is to a particularly toxic pesticide, or for particularly hazardous spraying operations, e.g. spraying inside huts, or when an individual already shows some evidence of excessive exposure. A dedicated field instrument is particularly useful in these circumstances. Where exposure is more likely to be low and well controlled, less frequent estimations are acceptable.

For estimation of cholinesterase activity using a field biomonitoring technique, a blood sample is normally obtained by pricking the finger pad with a disposable blood lancet. The first drop of blood is rejected and blotted away with a gauze pad and the second drop used to obtain the sample for analysis. Before sampling, the finger must be thoroughly cleaned using an alcohol swab and allowed to air dry for 30–60 sec. The ear lobe may also be used as an uncontaminated sampling site. For the field methods described herein, a 10 µL sample of blood is taken directly into a pipette. When larger blood samples are required for transfer to a laboratory, blood is taken from the median basilic vein by syringe.

When taking a blood sample, to prevent transmission of infectious disease, the operator must wear gloves. Blood lancets, hypodermic needles and blood collection containers must NEVER be re-used. All contaminated swabs, lancets, needles, etc. should be safely disposed of by an appropriate method for medical waste.

(b) Contamination possibilities

When blood is collected by means of a finger prick two forms of contamination can occur: 1) Excessive squeezing of the finger to obtain an adequate blood sample may alter the ratio of erythrocytes to plasma in the sample; 2) Contamination of the blood sample with pesticide may occur, if cleaning of the sampling site is inadequate, or when sampling is carried out too near a pesticide packing or spraying operation.

(c) Sampling device and container

Blood is taken either directly in a syringe and transferred to an EDTA (ethylenediamine tetraacetic acid) blood vial, or by means of a blood collection system. These incorporate both needle and an EDTA treated blood storage container and are filled either by vacuum, or by a syringe principle.

For field monitoring kits a blood sample, usually 10 µL, is taken up directly by a positive displacement, or air displacement pipette, or into a heparinized capillary tube. It is then transferred into a tube or cuvette containing either sample buffer or distilled water, depending on the method of cholinesterase monitoring employed.

(d) Anticoagulant

Where samples of blood are taken for transfer to a laboratory for analysis, EDTA is the anticoagulant of choice.

(e) Preservative, shipment and stability

No special preservatives are required. Samples can be transferred by post. No special arrangements need be made to cool samples, except under extreme conditions. Normally no more than 24h should elapse between obtaining the blood sample and its receipt by the analytical laboratory.

When stored as washed packed erythrocytes at -20°C the AcChE activity is stable for at least 2 years (3).

5.1.8.3 Recommended analytical methods

(a) Principle of the method

Both the laboratory (3) and field methods (2) described below are based on the method of Ellman et al. (1). In this method the substrate, acetylthiocholine, is hydrolysed by AcChE. The thiocholine released reacts with DTNB [5,5-dithiobis(2-nitrobenzoic acid)] to produce the yellow anion of 5-thio-2-nitro-benzoic acid. The rate of colour production is measured at a wavelength of 412nm, in a spectrophotometer, and is proportional to the acetylcholinesterase activity. Quinidine sulphate is added to suppress any plasma cholinesterase activity originating from trapped plasma. For the measurement of plasma cholinesterase quinidine is omitted.

(b) Reagents required

i) Laboratory method (3)
Sodium/potassium phosphate buffer 0.lmol/L, pH 8.0 and pH7.0. Acetylthiocholine iodide 7.3 mmol/l in distilled water is used as the substrate for both erythrocyte and plasma cholinesterase determinations.

DTNB [5,5-dithiobis(2-nitrobenzoic acid)] 10 mmol/L. For the stock solution, dissolve 99.1 mg of DTNB in 25mL of 0.lmol/L sodium/potassium phosphate buffer pH 7.0,

then add 37.5mg of $NaHCO_3$. For the working solution 2mL of stock solution is diluted with 3mL of distilled water and 57.5mL of 0.1mol/L sodium/potassium phosphate buffer pH 8.0. For erythrocyte cholinesterase estimations 0.1mL of a 1.94g/L quinidine sulphate solution is added to 50mL of DTNB working solution. Saponin 10mg in 100mL of 0.1mol/L phosphate buffer, pH 8.0 Physiological saline (9.0g/L)

ii) Field method (2)

In the cholinesterase field kit all reagents are supplied prepacked into aliquots required for a single estimation. Butyrylthiocholine iodide is used as substrate for plasma cholinesterase and acetylthiocholine iodide for erythrocyte cholinesterase.

(c) Equipment required

i) For the laboratory method (3)

A bench centrifuge is required to separate plasma and erythrocytes, a discrete kinetic enzyme automated analyser capable of adding starter reagents is required for the analysis, and a pH meter for adjusting the buffer pH. A vortex mixer may also be required.

ii) Field method (2)

Test-Mate OP kit. (EQM Research Inc. 68 Junefield Avenue, Cincinnati, Ohio 45218, USA) is supplied as a complete system in a sturdy carrying case. It contains all the components and reagents required to carry out 96 plasma and erythrocyte cholinesterase estimations plus a comprehensive instruction manual.

(d) Procedure and calibration

i) Laboratory method (3)

The whole blood is centrifuged, and plasma collected. The packed erythrocytes are then resuspended in approximately an equal volume of physiological saline and the haematocrit of the suspension obtained. The erythrocytes are then haemolysed by diluting 20μL of the cell suspension, 50-fold, with buffered saponin solution and vortex mixed. For erythrocyte estimations, 30μL of haemolysate plus 520μL of the DTNB working solution containing quinidine sulphate are placed in a reaction tube on the analyser. For plasma cholinesterase estimations, 10μL of plasma plus 540μL of the DTNB working solution without added quinidine sulphate are placed in reaction tubes on the analyser. After a pre-incubation period of 4 min 50 sec the reaction is started by the addition of 40 μL of acetylcholine substrate to 550μL of reaction mixture, this is done automatically in the analyser.

The change in absorbance at 410nm is followed for 18 sec at 37°C and the rate of reaction and degree of linearity are calculated by the instrument.

The calculation of enzyme activity is as follows:

$$
\begin{aligned}
\text{Erythrocytes} &= [\,A/\text{min}\cdot E)\cdot(V_t/V_1)\cdot(100D/H) \\
&= (\,A/\text{min})\cdot(7.338\cdot10^6/H) \text{ U/L of erythrocytes} \\
\text{Plasma} &= [\,A/\text{min}\cdot E\,)\cdot(V_t/V_p) \\
&= (\,A/\text{min})\cdot4403 \text{ U/L of plasma}
\end{aligned}
$$

U^* = enzyme activity, $\mu mol/min$
Vt = volume of reaction mixture ($590\mu L$)
V_1 = volume of lysate ($30\mu L$)
V_p = volume of plasma ($10\mu L$)
D = dilution of lysate ($\times 50$)
H = haematocrit of erythrocyte suspension (%)
E = molar absorptivity at 410nm = $1.34 \cdot 10^4 L \cdot mol^{-1} \cdot cm^{-1}$

* Enzyme activity in this chapter is expressed as 'U/L' of erythrocytes or plasma, or 'U/g' haemoglobin. The international unit of enzyme activity 'U' is defined as the number of micromoles of substrate transformed per min.

ii) Field method (2)

All the components and instructions for carrying out this procedure are contained within the Test-Mate OP kit. The Test-Mate blood analyser is a computer controlled LED-source colorimeter, powered by a standard 9-volt alkaline battery, which uses key activated instructions and audible "beeps" to guide the operator through the test. Dry reagent for carrying out the procedure is contained on 96-well assay plates and the contents of a single well are dissolved in water for each test. The reaction mixture for AcChE activity contains: 1 mmol/L acetylthiocholine, 20mmol/L potassium phosphate, 0.1% Triton X-100, 0.3mmol/L DTNB and 0.02mmol/L quinidine and is carried out at pH 7.4.

The colorimeter is first zeroed against a buffer solution (4 drops of buffer reagent diluted to 2 mL with water in a cuvette). Blood, obtained from the finger pad by stabbing with a sterile lancet and filling a 10μL capillary pipette, is then dispensed into the buffer-filled cuvette and mixed. Pressing the test button on the colorimeter then initiates a haemo-globin analysis at 450nm. All reagent within the test well, dissolved in 4 drops of water, is then transferred to the cuvette and mixed in response to an audible 'beep' from the colorimeter and a display of 'add reagent'. The colorimeter then carries out an 80-second pre-incubation, followed by a 50-second analysis at 450nm. The final calculations are carried out by the machine and expressed as units of cholinesterase activity /g haemo-globin. The haemoglobin concentration and the cholinesterase activity /mL of blood may also be obtained. All results are corrected to 25°C. A similar sequence is used to obtain the plasma cholinesterase activity, although without a haemoglobin measurement and using butyrylthiocholine as substrate. Quinidine is omitted from the reagent mixture for this assay. The Test-Mate OP kit may also be used to monitor exposure to carbamates.

(e) Criteria of analytical reliability

i) Trueness

Estimates of enzyme rate determined with acetylthiocholine are considered to be an accurate estimate of the rate for acetylcholine hydrolysis (1), and the substrate concentration used does not inhibit enzyme hydrolysis (3). The laboratory method has been compared with the Michel method (7) and a correlation coefficient of 0.85 (y=134x+3062) was obtained for erythrocyte cholinesterase and 0.95 (y=26x+286) for plasma cholinesterase (3). When the field method was compared with the Tintometric method (a semi-quantitative field method) a correlation coefficient of 0.84 (y=0.834x+9.4), n=24

was obtained (2). Erythrocyte cholinesterase activity is considered to be a reflection of the AcChE activity at nerve endings (6).

ii) Precision
For the laboratory method (3), the long-term precision of the erythrocyte cholinesterase assay is reflected in a coefficient of variation of 3.8% for n=20 over a 76-day period.

For the field method (2) the between run coefficient of variation ranged from 2.1–6.0% with a mean of 4.1% for erythrocyte cholinesterase and 3.0–8.7% with a mean of 5.6% for plasma cholinesterase. The instrument to instrument response for 7 instruments gave a coefficient of variation of 0.95%.

iii) Detectability
For cholinesterase measurement the issue is the minimum degree of change between two measurements that can be ascribed to OP inhibition and this depends on analytical imprecision. For the laboratory method, minimal detectable changes have been assessed by Mason and Lewis (8). They said that a fall of 15% in erythrocyte cholinesterase and 7.5% in plasma cholinesterase between two successive samples suggests significant inhibition if the laboratory's analytical performance is such that the coefficients of variation for the two assays are 3.5% and 2.5%, respectively.

For the field method a fall in erythrocyte cholinesterase activity of 22% or more from the normal range, or initial baseline value, is indicative of overexposure to OP compounds (9).

(f) Quality assurance

i) Special precautions
For the laboratory method storage of the saponin lysate at 4°C resulted in a daily fall of 10%, whereas storage of the saponin lysate at -18°C resulted in no loss of activity over a 2-month period. Washed whole cells can be stored for at least 2 years at -20°C and are suitable for quality control. The plasma enzyme system also has good stability and pooled plasma samples, as well as commercially available quality control sera can be used for internal control procedures (3). Samples of quality control material, washed cells or plasma depending on the assay, should be analysed with each batch of samples.

For the field method a blood sample taken from the technician carrying out the assays, who should avoid any exposure to pesticide, can be measured at the start of each run to establish a reference baseline activity. This may be helpful where an individual's own baseline level is not available.

When taking samples for the laboratory method special care must be taken to avoid haemolysis of the blood sample prior to centrifugation.

For the field method, great care must be taken to ensure that all the solid reagent is completely dissolved and transferred from the assay plate to the cuvette for the analysis.

iii) Interferences

The release of thiol material from cells could interfere with this method. Some non-enzymic hydrolysis of the AcChE may also occur. In spite of the use of quinidine to suppress non-specific esterases a small component of the activity may reflect esterase activity originating from trapped plasma. For the field method 93% of the activity of trapped non-specific esterases is inhibited by quinidine and the cross-reactivity between the plasma and erythrocyte assays was therefore considered to be negligible.

(g) Sources of possible error

i) Pre-analytical

In analyses by the laboratory method, any haemolysis of the blood sample will be a source of error for the plasma measurements. Spontaneous reactivation may also occur. Reactivation of dimethoxy inhibited erythrocyte cholinesterase has a half-time of about 50 min *in vitro* at 37°C (10), while reactivation of dimethoxy inhibited plasma cholinesterase can occur up to a maximum of 25% of the initial inhibition. Reactivation of diethoxy inhibited plasma cholinesterase was negligible (11).

When using the field kit, contamination of the blood with pesticide, while sampling, will increase inhibition. Excessive squeezing of the finger will result in an artificially high plasma/erythrocyte ratio.

ii) Analytical

Major errors are unlikely to occur during the laboratory analysis. When using the field kit, failure to dissolve and completely transfer all the reagent mixture to the cuvette will result in a low value. The colorimeter may take up to 30 min to equilibrate to a sudden change in temperature. When used in the field, it is therefore important to keep the colorimeter away from direct sunlight.

(h) Reference to the most comprehensive description of the method.

i) Laboratory method

Lewis PJ, Lowing RK, Gompertz D. Automated discrete kinetic method for erythrocyte acetylcholinesterase and plasma cholinesterase. Clinical Chemistry 1981;27:926–9.

ii) Field method

Magnotti Jr. RA, Dowling K, Eberly JP, McConnell R. Field measurement of plasma and erythrocyte cholinesterases. Clinica Chimica Acta 1988;315:315–32.

(i) Evaluation of the methods

The automated laboratory assay based on the method of Ellman et al. (1), is now a well established method for the measurement of erythrocyte and plasma cholinesterase. Previous attempts to adapt this method for field use have been unsatisfactory for two reasons: 1) The requirement for a centrifuge to obtain packed erythrocytes; and 2) The instability of the battery-powered tungsten light source. The Test-mate OP kit makes use of a solid state battery powered colorimeter with a silicon carbide light emitting diode in place of a tungsten lamp. This light source avoids the problems of limited lamp life,

electrical noise, instability and energy inefficiency inherent in conventional lamps. This colorimeter is also used to obtain the haemoglobin value for each blood sample which allows the consideration of variations in erythrocyte number or inaccurate blood sampling. The final cholinesterase value is expressed as units of activity/g of haemoglobin.

5.1.8.4 Other analytical methods

For many years a semi-quantitive method for the measurement of whole blood cholinesterase, the Tintometric method (12), has been used, reasonably successfully, in the field. In this method, the change in pH with time that results from the liberation of acetic acid following the hydrolysis of acetylcholine, is correlated with cholinesterase activity. A small blood sample is incubated with a pH indicator solution (bromo-thymol blue) and substrate (acetylcholine perchlorate) in CO_2 free distilled water. The duration of the incubation is dependent on the temperature and is obtained from time-temperature tables provided with the kit. The final colour is matched, by eye, with a Lovibond comparator disc in which colour change is shown in steps of 12.5%. The interpretation of results obtained with the Tintometric method has been discussed by Vandekar (13).

The main difficulties with this method are the generation of CO_2 free distilled water at the required pH and the accurate comparison of the final colour with the comparator disc.

5.1.8.5 Guide to interpretation

Inhibition of erythrocyte and plasma cholinesterase reflects absorption of significant amounts of OP pesticide. However, there is not always a direct and close relationship between the degree of inhibition and the occurrence of nervous system symptoms. Changes in the activity of the circulating enzymes do not necessarily reflect the inhibition of nervous system enzyme, no doubt due to differences in tissue distribution and kinetics of activation and elimination. At high exposure levels, however, measured changes in enzyme activity are an indicator of possible toxicity and have proved useful in monitoring workers at risk in a variety of occupational situations.

(a) Measured values in groups without occupational exposure

There is considerable inter-individual variation in cholinesterase activity from one person to another and therefore it is desirable to measure the normal level in each person prior to occupational exposure. The actual levels of cholinesterase activity in unexposed persons are thus of less importance than the knowledge of the baseline activity in any one individual and the normal day-to-day variation in activity that can be expected in the absence of exposure. More precise automated methods have now allowed studies of day-to-day variation, distinguishing this from analytical variation (8).

Plasma and erythrocyte cholinesterase activities were measured, by the automated laboratory assay, in 9 healthy laboratory workers 6–11 times over a 13-month period. The range of the individual means for plasma cholinesterase over this period was from 1980–3700 U/L and for erythrocyte cholinesterase 14,970–18,150 U/L whole blood. The

mean coefficient of variation for these workers, over the 13-month period, was 4.63% for plasma cholinesterase and 2.26% for erythrocyte cholinesterase (8).

The normal mean activity for erythrocyte cholinesterase, using the TestMate OP kit, in a group of 23 unexposed Mexican pesticide workers was 42.9 U/g haemoglobin with a 90% range from 37.7–48.1 U/g haemoglobin (9). A lower activity, mean±SE of 34.3± 0.85 U/g haemoglobin was obtained preexposure from a group of 38 spraymen in Pakistan (4). In both Mexico and Pakistan the haemoglobin concentration has been high, with means of 162 and 168 g/L, respectively.

(b) Published biological action levels

While inhibition of plasma cholinesterase can be used as an indicator of exposure, it is less useful as an indicator of poisoning. A number of OP compounds can preferentially inhibit plasma cholinesterase (6). Measurement of erythrocyte cholinesterase is a more useful monitor of the extent of exposure, since the activity of acetylcholinesterase in the erythrocyte is assumed to mirror the effects in the target organs. For any exposed individual, a fall in their erythrocyte cholinesterase activity to 70% of the pre-exposure value is an indication of overexposure. In these circumstances the worker concerned should be removed from any further exposure until his/her cholinesterase activity returns to at least 80% of the pre-exposure activity (1, 2, 6).

A 70% action level for cholinesterase monitoring is also laid down by the American Conference of Governmental Industrial Hygienists (ACGIH) biological exposure indices for 1993–1994 (14) and the Deutsche Forschungsgemeinschaft (DFG), Maximum Concentrations at the Workplace and Biological Tolerance Values for Working Materials (15).

Symptoms of poisoning may occur when the erythrocyte cholinesterase activity is less than 35% of normal. The erythrocyte enzyme recovers approximately 1% daily after OP exposure, plasma cholinesterase recovers much more rapidly. Measurement of cholinesterase cannot be used to monitor the effectiveness of treatment.

(c) Non-analytical interference

i) Exposure to other chemicals (the same metabolite), co-exposure, ethanol intake, medications

Exposure to carbamate compounds can also cause significant depression of erythrocyte cholinesterase. Chemicals, such as mixed function oxidase inhibitors, which interfere with OP metabolism may increase the concentration of circulating OP thus increasing cholinesterase inhibition. Succinylcholine and related drugs widely used in surgery and electroconvulsive therapy will compete with butyrylcholine as a substrate for plasma cholinesterase. Although alcohol intake is not directly linked to cholinesterase inhibition, chronic liver disease is associated with decreased basal plasma cholinesterase levels (1).

ii) Diet and environment

Plasma cholinesterase activity is reduced in protein and thiamine deficiency. There are no known environmental factors influencing cholinesterase activity.

(d) Sampling representative of recent or long-term exposure, or biological effect

Measurement of erythrocyte cholinesterase was carried out in five grain terminal workers exposed to fenitrothion. Erythrocyte cholinesterase activity was reduced from a range of 28–40 U/g Hb to 16–29 U/g Hb with a mean value of 23 U/g Hb. After modification of work practices erythrocyte cholinesterase activity recovered to a mean of 33.6 U/g Hb with a range of 32–36 U/g Hb (16).

A group of children given metrifonate (trichlorphon) orally for the treatment of schistosomiasis all showed cholinesterase depression as measured by the Ellman method (1) for cholinesterase. Erythrocyte cholinesterase activity was inhibited by 40–60% while plasma cholinesterase was reduced by up to 80% (17).

Cholinesterase activity was measured in a group of 13 factory workers exposed to dichlorvos at air concentrations of 0.7 mg/m^3 air for 8 months using a modification of the Ellman method. Erythrocyte cholinesterase was inhibited by 35%, while plasma cholinesterase was inhibited by 60% (18).

Measurement of erythrocyte cholinesterase, using the field method, was carried out in 3 males poisoned by methamidophos. One day after admission to hospital erythrocyte cholinesterase activity was reduced by over 80% in 2 of the patients and by 66% in the third. Complete recovery of activity was not evident until 80–100 days after exposure (2).

(e) Ethnic differences (enzyme deficiency, environment, diet)

No ethnic variation has been recorded with respect to erythrocyte cholinesterase activity. Plasma cholinesterase activity of North American blacks tends to be lower than whites of the same sex (1).

5.1.8.6 Research needs

Measurement of cholinesterase by the method of Ellman (1) is the accepted technique which has been extensively used, for many years, for monitoring OP exposure. Published information on cholinesterase activity measured in the field by the Ellman method is needed for occupationally exposed individuals. At present, much of the published information refers to measurements made using the Tintometric method.

The Test-Mate OP Kit has so far performed well in the field use, but haemoglobin estimations carried out by this method seem unusually high and should be compared with values obtained by a more accepted method. More published measurements carried out in workers occupationally exposed to OP compounds are also required for the Test-Mate OP kit.

5.1.8.7 References

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Chapter 6. Selected compounds

6.1 Carbon monoxide

6.1.1 Introduction

Carbon monoxide is ubiquitous in our environment, being a product of incomplete combustion. Common non-occupational sources include space heaters, furnaces, and internal combustion engines. It is also produced endogenously by the catabolism of hemoglobin and other heme proteins. It is a product of tobacco smoke and a metabolite of the common solvent dichloromethane (methylene chloride). Exposure to carbon monoxide results in tissue hypoxia. This hypoxia produces effects on the brain, cardiovascular system, exercising skeletal muscles, neurobehavioural systems, and the developing fetus. Effects on the cardiovascular system occur at HbCO levels of less than 5%.

The toxicity of carbon monoxide has been extensively reviewed by the National Academy of Sciences (1), the National Institute for Occupational Safety and Health (2), the World Health Organization (3), and most recently, by the US Environmental Protection Agency (EPA) (4).

Published biological action limits are based on the relationship between health effects and biological indicator.

6.1.2 Physical-chemical properties

Carbon monoxide (CO, CAS 630-08-0) is a flammable, colourless, odourless gas.
Molecular weight   28.01
Solubility   Sparingly soluble in water (2.3 mL/100mL at 20°C) (4)

Conversion factors:
CO   (at 25°C, 1 atm) $1.0 \text{ mg/m}^3 = 0.87$ ppm;
                        $1.0 \text{ ppm} = 1.1 \text{ mg/m}^3$

6.1.3 Possible occupational and non-occupational exposures

Occupational exposure to carbon monoxide is common where combustion occurs. Examples are blast furnace operations, and emissions from automotive exhaust. Automotive garage workers, traffic policemen, tunnel workers, and firemen are at particular

risk. Carbon monoxide is also a metabolite of dichloromethane (methylene chloride), a solvent and paint stripper in common industrial and home use (5).

Carbon monoxide is formed endogenously from the catabolism of hemoglobin and other heme proteins. Being a combustion product of carbonaceous material, it is ubiquitous in the environment. Common sources of non-occupational exposure to carbon monoxide include automobile engines, space heaters, and furnaces. Tobacco smoking is another source of exposure (5).

6.1.4 Summary of toxicokinetics

6.1.4.1 Absorption

(a) Inhalation

Carbon monoxide is absorbed via the lungs. It readily diffuses across capillary and placental membranes (4).

(b) Dermal

Dermal absorption of carbon monoxide has not been reported.

(c) Gastrointestinal

Gastrointestinal absorption of carbon monoxide has not been reported.

6.1.4.2 Metabolic pathways and biochemical interaction

Approximately 80%–90% of the absorbed amount of carbon monoxide binds with hemoglobin forming carboxyhemoglobin (HbCO). HbCO causes the dissociation of oxyhemoglobin, thus reducing the oxygen-carrying capacity of the blood (4, 6). The affinity of hemoglobin for carbon monoxide is 200–250 times that for oxygen. HbCO shifts the oxyhemoglobin dissociation curve to the left, thus interfering with the delivery of oxygen to tissues. Carbon monoxide also binds reversibly to other heme proteins, such as myoglobin, cytochrome oxidase, cytochrome P-450, and hydroperoxidases. Another site of toxicity may be the binding of carbon monoxide to cytochrome a_3 oxidase, which results in inhibition of mitochondrial respiration and impairment of oxygen diffusion into mitochondria (5).

6.1.4.3 Distribution

Carbon monoxide rapidly binds to heme in blood forming HbCO. About 15% of the absorbed dose is stored in extravascular cells, primarily the heart and muscle tissue bound to myoglobin. As discussed above, binding also occurs with various cytochromes (5).

6.1.4.4 Elimination

Carbon monoxide is eliminated unchanged via the lungs. The rate of elimination depends on the rate of its release from heme proteins, alveolar ventilation, duration of exposure, inspired oxygen concentration, and carboxyhemoglobin (HbCO) saturation (5).

6.1.5 Summary of toxic effects

The combination of decreased oxygen-carrying capacity of the blood, impaired release of oxygen to tissues, and interference with intracellular oxidation processes results in tissue hypoxia that is proportional to the HbCO saturation and oxygen demand. The brain, cardiovascular system, exercising skeletal muscle, and the developing fetus are the tissues most sensitive to this hypoxia. Toxic effects thus are of concern to neurobehavioural function, cardiovascular exercise capacity, and developmental effects.

Carbon monoxide does not appear to have large and consistent behavioural effects on healthy, young volunteers with HbCO levels below 10%. However, exposure to carbon monoxide resulting in a prolonged HbCO level of 5–10% may affect the performance of tasks requiring a high degree of vigilance, such as flying an aircraft or attending a control panel. Moreover, carbon monoxide exposure can reduce the capacity to perform strenuous physical activity at HbCO levels above 2.5%. Persons with coronary artery disease are particularly sensitive to carbon monoxide. Decreased exercise time to onset of angina or ischemia was observed at HbCO levels as low as 3% and increased ventricular arrhythmias at HbCO levels of 6%. Carbon monoxide exposure during pregnancy may result in low birth weight and possibly diminish the mental ability of children (5).

6.1.6 Biological monitoring indices

Table 6.1.1 lists biological monitoring indices that have been suggested for monitoring occupational exposure to carbon monoxide.

Table 6.1.1. Biological monitoring indices for carbon monoxide

Index	Suitability for use	Reference
HbCO in blood	Most common index with well documented relationship between HbCO and both health effects and exposure. Suitable only on fresh blood.	2, 7–11
Carbon monoxide in blood	A related measurement which is used to calculate % HbCO; requires a separate hemoglobin measurement. Suitable for clotted blood.	8–10
Carbon monoxide in exhaled air	A suitable screening method which is related to HbCO	8–10

Carboxyhemoglobin (HbCO) in blood, determined directly by spectrophotometric methods is, by far, the most common index for carbon monoxide exposure. Limitations on the sensitivity and performance characteristics of some spectrophotometric methods, however, do not permit accurate determination of HbCO levels below 3%. Alternate methods with improved sensitivity include the gas chromatographic determination of carbon monoxide following its release from hemoglobin. These two indices are actually a measure of the same constituent, HbCO, and differ only in the methods of measurement.

Carbon monoxide in exhaled air can also be used as an index of exposure. Measurement of carbon monoxide in end-exhaled air by common occupational hygiene air measurement devices is common. The determination of carbon monoxide in expired air is recommended only when a blood specimen cannot be obtained.

6.1.7 Carboxyhemoglobin in blood (HbCO)

6.1.7.1 Toxicokinetics

The concentration of HbCO rises from the start of exposure to carbon monoxide. The increase, rapid at onset, starts to level off after 3 hours of exposure. After the end of exposure, HbCO declines with a half-life of 5 hours (5). The Coburn-Forster-Kane exponential equation (CFK equation) describes the rising of HbCO concentration in blood during carbon monoxide exposure, taking into account all known physiological variables affecting the carbon monoxide uptake, such as endogenous carbon monoxide production, diffusion in the lung, alveolar ventilation, blood volume, barometric pressure, and partial pressures of carbon monoxide and oxygen in the lung (12). At the end of an 8-hour exposure, HbCO approaches steady-state.

There have been few valid studies on the elimination of HbCO under normal working conditions. HbCO appears to be eliminated with an initial rapid phase followed by a longer exponential elimination phase; the elimination kinetics seem to be influenced by the magnitude of exposure and the degree of physical activity (6). Others have suggested that the elimination is monophasic with a half time of 4–5 hours (11).

6.1.7.2 Biological sampling

(a) Sampling time and specimen

To evaluate daily time-weighted average (TWA) exposure, blood samples should be taken after at least 3 hours of exposure, preferably at the end of the shift. Whole blood samples should be collected by venipuncture as soon as possible following exposure because of the exponential elimination of HbCO.

(b) Contamination possibilities

Contamination of blood is not likely.

(c) Sampling device and container

Blood collection using a vacuum tube system is preferred to avoid losses of CO that could occur in an open syringe collection system.

(d) Anticoagulant

Whole blood specimens obtained by venipuncture should be collected with ammonium oxalate anticoagulant.

(e) Preservative, shipment and stability

Whole oxylated blood should be stored in the dark at 4°C in order to avoid bacterial action which can result in both the production of carbon monoxide and the denaturation of hemoglobin. Blood samples to be analysed by direct reading spectrophotometric methods depend on the integrity of the erythrocytes and must be thoroughly mixed with anticoagulant immediately after collection. These specimens should be shipped and stored at 4°C and analysed within 48 hours of collection. Blood specimens for analysis by gas chromatographic methods do not depend on the integrity of the erythrocyte and can be kept at least 5 days at room temperature. Samples can be frozen for longer storage.

6.1.7.3 Recommended analytical methods

There are a wide variety of spectrophotometric methods available and commonly used for measurement of HbCO. However, most of the methods do not have sufficient trueness and precision to accurately determine concentrations of HbCO of less than 3%. However, these methods have considerable value for screening populations with exposures above 5% HbCO. They all require fresh, unclotted whole blood. References for some of these spectrophotometric methods plus a brief description of one method are given in Section 6.1.7.4.

In order to analyse blood specimens with HbCO levels between 0.5–5%, gas chromatographic methods are recommended, even though these methods are more elaborate and generally require more equipment and time to perform analyses (13–17). Blood specimens, including clotted blood, can be analysed by gas chromatographic methods. A separate determination of hemoglobin is also required. The selection of methods must be based on the expected range of HbCO. The gas chromatographic method described below was selected because it is well documented (18).

(a) Principles of the method

Carbon monoxide is released from hemoglobin by potassium ferricyanide (III), separated from other sample constituents on a molecular sieve gas chromatography column, reduced to methane by hydrogen with a nickel catalyst and determined with a flame ionization detector. Hemoglobin is determined separately using the cyanomethemoglobin spectrophotometric method, using Drabkins reagent.

(b) Reagents required

Potassium ferricyanide(III)
Pre-column: Chromosorb G/AW coated with methanolic KOH
Analytical column: molecular sieve 5 Å, 30–80 mesh.
Catalytic column: Chromosorb G/AW coated with nickel (II) nitrate
Nitrogen, hydrogen and purified air (80% nitrogen, 20% oxygen)
0.01% Carbon monoxide standard in nitrogen, carbon monoxide (> 98.6%)
Cyanomethemoglobin reagent (Drabkins reagent) containing potassium ferricyanide (III), potassium cyanide, and sodium bicarbonate [commercially available]
Cyanomethemoglobin standards [commercially available].

(c) Equipment required

Gas chromatograph with flame ionization detector adapted for use of a 0.64 cm (ID) 20 cm pre-column, 0.64 cm (ID) 2 m molecular sieve separation column and a 0.64 cm (ID) 20 cm catalytic reduction column.
Spectrophotometer or colorimeter equipped with a 540 nm filter, for determination of hemoglobin.
Tonometer for equilibrating standards, "head-space vials", gas-tight syringe, and other miscellaneous laboratory equipment.

(d) Procedures and calibration

i) Calibration
Calibration standards for carbon monoxide are made up in whole blood taken from a non-smoking, unexposed person. A stock solution of 100% carbon monoxide in blood is prepared by passing carbon monoxide through the blood. Since carbon monoxide also dissolves in blood, excess unbound carbon monoxide is removed by flushing the container with nitrogen producing 'nearly 100%' HbCO. The exact concentration of this high standard can be determined using a spectrophotometric method described later, since spectrophotometric methods are insensitive to dissolved carbon monoxide. Stock solutions can then be made over the range of 0.2–40% HbCO by mixing the 'nearly 100%' HbCO with blood from non-smokers, using a tonometer to mix the solutions. Diluted standards are analysed, along with specimens using whole untreated blood, as blanks. Blank corrected standards are plotted against HbCO concentration.

ii) Procedure
A venous blood sample containing oxalate anticoagulant, is split between two head-space vials. After the vials are flushed with nitrogen, one vial receives a solution of potassium ferricyanide; the other serves as a blank. After equilibration, an aliquot of head space is removed with a gas tight syringe and injected into the gas chromatograph. After separation of the other components, carbon monoxide is catalytically reduced to methane and determined by flame ionization detection. Gas chromatographic conditions are: Injector and pre-column (20 cm): 100°C; analytical column (2 metre): 100°C; catalytic column (20 cm) and detector: 300°C; Nitrogen carrier: 25 mL/min.

A separate determination of hemoglobin is required to correct for the hemoglobin content present in the blood used for calibration and in the blood from the exposed worker. The determination is performed by mixing 0.02 mL blood with 5 mL of the cyanomethemoglobin reagent, allowing the mixture to stand for 20 minutes. The absorbance is read at 540 nm against a calibration curve prepared by dilutions of the cyanomethemoglobin standard with the cyanomethemoglobin reagent (19). Calibration for hemoglobin determinations are usually provided by commercially available hemoglobin determination kits based on the Drabkins reagent. There are commercially available hemoglobin quality control materials. The corrected HbCO percent is then calculated by multiplying the observed HbCO% by the ratio of the hemoglobin content of the specimen to hemoglobin content of the blood used for standard preparation.

(e) Criteria of analytical reliability

i) Trueness
Trueness, based on recovery studies at 5% HbCO, was 101%.

ii) Precision
The within day precision, based on analysis of 10 samples at 5.07% HbCO, was 2.5% relative standard deviation. The day-to-day precision, based on 20 days of analyses at a 5% HbCO was 8.8% relative standard deviation.

iii) Detectability
The estimated detection limit, based on three times the standard deviation of the blank was 0.17% HbCO.

(f) Quality assurance

i) Special precautions
Blood should be treated as a biohazard, using universal precautions to avoid the transmission of HIV or HBV viruses[1]. The blood used as a stock solution must be from a non-smoker who is not exposed occupationally or environmentally to carbon monoxide or dichloromethane (methylene chloride). If this blood specimen has appreciable HbCO, then results may be higher due to higher background blanks.

ii) Interferences
The procedure is specific and is not subject to interference.

(g) Sources of possible errors

i) Pre-analytical
Bacterial action which may result in carbon monoxide formation and denaturation of hemoglobin must be avoided. EDTA was reported to increase the level of HbCO and

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[1] Centers for Disease Control, "Guidelines for Prevention of Transmission of Human Immunodeficiency Virus and Hepatitis B Virus to Health-Care and Public Safety Workers," *Morbidity and Mortality Weekly Reports* (1989) 36:(S-6), iii–vi, 1–37.

should be avoided as an anticoagulant (16). Atmospheric contamination of the sample with environmental carbon monoxide is minimized by flushing vials with nitrogen prior to releasing carbon monoxide with potassium ferricyanide.

ii) Analytical

The molecular sieve analytical column must be kept dry and free of excess carbon dioxide. The use of a pre-column is effective in maintaining a dry, efficient analytical column. Should the column become overloaded, it can be baked out overnight.

(h) Reference to the most comprehensive description of the method

DFG. Carboxyhemoglobin, In: Analysis of Hazardous Substances in Biological Materials: Methods for biological monitoring. Volume 1. Angerer J, Schaller HK. (Eds.) Weinheim, VCH Publishers, 1985 [English].

(i) Evaluation of the method

The method described above (18) included a laboratory validation study of precision, trueness, and estimates of the limit of detection. Precision, assessed by analysis of a blood specimen containing 5% HbCO was 2.5% relative standard deviation. Day-to-day precision, over a 3-month period was 8.8%. Trueness, as assessed by recovery studies containing 5% HbCO, was 101%. The detection limit was 0.17% HbCO based 3 times the standard deviation of the blank. No interlaboratory studies were reported.

6.1.7.4 Other analytical methods

Specific spectrophotometric methods for measurement of HbCO utilize two or more wavelengths in the visible region (4). These include automated visible spectrophotometry (20) and manual spectrophotometry at two or more wavelengths (3, 21–25). A number of automated instruments are available to determine fractions of hemoglobin present in blood as HbCO, oxyhemoglobin, reduced hemoglobin, and methemoglobin. The CO-oximeter, described by Dubowski and Luke (20) has been widely used to assess occupational exposures. It is a good method if blood samples are fresh and if HbCO levels exceed 3%. Measurements using oximeters were compared with measurements using gas chromatography (13). The difference between measured values was only ± 0.2% HbCO. However, the trueness of ± 1% HbCO, claimed by manufacturers of oximeters, is unacceptable for determination of background levels (< 2% HbCO) and is questionable for measurements of levels approaching 3.5% HbCO (5).

A representative manual spectrophotometric method suitable for assessment of HbCO at concentrations exceeding 2% is described by Commins and Lawther (23), a method that was evaluated many years ago in Europe and found to be acceptable (26). It must be used on fresh, unclotted blood. A summary of the method follows.

0.01 mL of whole blood taken from a finger prick is dissolved in 10 mL of a 0.04% ammonia solution. The solution is divided into two halves. Carbon monoxide is displaced from one half by bubbling oxygen through the sample. The sample containing HbCO is

placed in a spectrophotometer and read against the oxyhemoglobin sample as a blank. Readings are taken at 414, 420 and 426 nm to estimate HbCO. Readings are taken at 575 and 559 nm to estimate total hemoglobin. The percent HbCO is then calculated from the two results according to a formula described by the authors. Calibration is done by determining the difference in absorption of blood treated with 100% oxygen and blood treated with 100% carbon monoxide. The performance characteristics, as described by the author included determination of precision at HbCO concentrations of 1.4% HbCO (3.5% RSD), 6% HbCO (2.2% RSD), and 9.7% HbCO (1.2% RSD). Precision at 0.66% HbCO was reported as 10.6%. As discussed before, the method was evaluated in 1977 by the European Commission and found to be acceptable.

6.1.7.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

HbCO in individuals not occupationally exposed to carbon monoxide varies, depending on endogenous production of carbon monoxide, smoking habits, and environmental exposures. Such variation can affect the interpretation of HbCO measurements below 5%. Workers commuting via congested roadways may arrive at work with HbCO levels of 5% or more. Table 6.1.2 shows typical levels of HbCO in populations without occupational exposure to carbon monoxide (4, 27, 28).

Table 6.1.2. Background carboxyhemoglobin levels

Group/Source of exposure	Average HbCO levels
Endogenous production	0.4–0.7% increasing up to 2.6% during pregnancy and up to 4–6% in patients with hemolytic anemia
Urban population	1–2%
Commuters on urban highways	5 % or more (CO levels on highways average 28.6 mg/m^3 (25 ppm) rising up to 114.5 mg/m^3 (100 ppm) during temperature inversions)
Tobacco smokers	Cigarettes: One pack per day 5–6%, two to three packs per day 7–9%
	Cigars: up to 20% HbCO

(b) Published biological action levels

Table 6.1.3 shows published biological action levels by organizations and recommendations by individual authors.

Table 6.1.3. Published biological action levels

Index	Biological action level, basis and collection time	Reference
HbCO in blood	3.5% collected at end of shift, corresponding to TWA exposure at 28.6 mg/m^3 (25 ppm)	ACGIH BEI (10)
HbCO in blood	5% collected at end of shift or end of exposure, based on prevention of adverse health effects (MAK = 30 ppm, 34.3 mg/m^3)	DFG BAT (7)
HbCO in blood	Not to exceed 5%, not to exceed 2.5% in susceptible persons, based on prevention of adverse health effects	WHO (6)
HbCO in blood	3.5% tentative maximum permissible concentration in non-smokers, based on health effects	Lauwerys et al. (8)

(c) Non-analytical interferences

i) Exposure to other chemicals (the same metabolite), co-exposure, ethanol intake, medications

Dichloromethane (methylene chloride) is metabolized to carbon monoxide. An exposure to dichloromethane at 57.3 mg/m^3 (50 ppm) for 8 hours will produce HbCO levels of 1.5–2.5%. Tobacco smoking, including side stream exposure to tobacco smoke, will add to the background level of HbCO.

Any population with decreased oxygen-carrying capacity or decreased oxygen availability may be at increased risk of carbon monoxide toxicity. Workers with respiratory disease that impairs oxygen delivery and pregnant workers and their fetuses may also be at an increased risk. Populations not adapted to living at high elevations (over 1,500 meters above sea level) are more sensitive to carbon monoxide because of reduced atmospheric oxygen. Native populations may not be more sensitive because of adaptation to lowered atmospheric oxygen (1–3, 29). Heavy labour, high temperature, or high altitude contribute to the health risk of workers exposed to carbon monoxide.

ii) Diet and environment

Smoking habits and exposure to dichloromethane (methylene chloride) should be noted as a source of error. However, since HbCO is a measure of exposure by all routes and from all sources, monitoring of HbCO may be of value. Samples taken from current tobacco smokers or workers exposed to dichloromethane should not be used for evaluation of occupational exposure to carbon monoxide because of the other sources of carbon monoxide assessed by HbCO measurements (5).

(d) Sampling representative of recent or long-term exposure, or biological effect

HbCO measurements represent exposure during the last few hours prior to sample collection. Measurements in samples taken during the first 3 hours of exposure or later than 15–30 minutes after the end of exposure cannot be used for the evaluation of 8-hour TWA exposures.

(e) Ethnic differences (enzyme deficiency, environment, diet)

No ethnic differences that affect HbCO production were found in the literature.

6.1.7.6 Research needs

Further clarification of the effects of low level chronic exposures to carbon monoxide on neurobehavioural and cardiovascular systems are needed. In addition, simplified methods are needed that have sufficient sensitivity to detect HbCO accurately at levels less than 2%.

6.1.7.7 References

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6.1.8 Carbon monoxide in expired air

6.1.8.1 Toxicokinetics

Carbon monoxide in exhaled air is in equilibrium with HbCO and thus parallels the toxicokinetics of HbCO (1–7). The concentration of carbon monoxide in exhaled air rises from the start of exposure to carbon monoxide. The increase, rapid at onset, starts to level off after 3 hours of exposure. After the end of exposure, carbon monoxide de-clines with a half-life of 5 hours (8). The elimination kinetics seem to be influenced by the magnitude of exposure and the degree of physical activity (9).

6.1.8.2 Biological sampling

(a) Sampling time and specimen

End-exhaled air, collected as described below, is collected after at least 3 hours of expo-sure and as near the end of exposure as possible.

(b) Contamination possibilities

Carbon monoxide from the ambient air is not likely to be a contaminant using the proce-dures described below.

(c) Sampling device and container

There are several reports on the collection of end-expired air. They all are based on the same principle. Workers take a deep breath, and hold it for 20 seconds. The first few hundred mL of expired air (physiological dead space) is discarded and the last portion of the expired air expelled into a gas sampling bag equipped with a valve closure (6, 7, 10).

(d) Shipment and stability

Samples in gas bags can be immediately expelled into a portable infrared analyser and analysed on site. Alternately, bags of gas can be shipped and stored at room temperature with no losses reported up to 49 days (10).

6.1.8.3 Recommended analytical methods

There are two components of an acceptable expired air carbon monoxide method. The first is the breath-holding technique for collecting the sample. The second is the method used to perform the analysis of the expired air sample for carbon monoxide. Methods for collection of the expired air sample are described above (6, 7, 10). The procedure described by Ringold (10) is presented in detail. Methods for the analysis of carbon monoxide expelled from the collection bag have primarily used infrared analysers, the same type of equipment used to analyse ambient carbon monoxide samples (11–12).

(a) Principle of the method

The worker takes a deep breath, holds it for 20 seconds, expels the first few hundred mL, and expires the last portion, the alveolar air or end-expired air into a previously evacuated polyvinyl bag. The contents of the bag can be analysed on site or shipped to a laboratory where the contents of the bag are passed through an infrared analyser, cali-brated with gas mixtures of carbon monoxide. Results are read from either a calibration curve or directly on a digital display.

(b) Reagents required

The only reagents required are calibration gases containing carbon monoxide used to calibrate the infrared analyser. A drying agent can be used to trap moisture prior to passing the sample into the gas collection bag.

(c) Equipment required

Polyvinyl or Milar ® gas collection bags equipped with valves. Sizes are dependent on the sensitivity and flow cell dimensions of the infrared analyser but usually are in the range of 1.5–8 L.

Portable infrared gas analyser equipped with flow through cells and suitable for analysis of carbon monoxide in ambient air.

(d) Procedures and calibration

i) Calibration
The infrared analyser is calibrated according to manufacturer's instructions using gas mixtures over the range of 0–114.5 mg/m^3.

ii) Procedure
Workers are asked to take a deep breath, hold it for 20 seconds, and expire the first few hundred mL to the air and the remaining into a previously evacuated gas sampling bag equipped with a valve. The gas bag can be analysed in the field or sent to a laboratory where the expired air is passed through the infrared analyser at a flow rate of 300–500 mL/min. Some authors use a tube of drying agent to remove moisture. Results are read on the digital readout or from a plot of the analog signal versus the carbon monoxide concentration, depending on the instrument used.

(e) Criteria of analytical reliability

i) Trueness
Trueness, as assessed by analysis of 15 bags filled with a standard gas (57.8 µL/L), was 95.2% (13).

ii) Precision
The overall precision of the entire measurement system, including the sampling was estimated at 4% RSD. The precision at 28.6 mg/m^3 was 3.1% RSD; the precision at 1.6 mg/m^3 was 6.8% RSD (13). The within-run precision of the analytical method, as assessed with duplicate standards was < 1% (11, 13).

iii) Detectability
Limits of detection are dependent on the infrared analyser but should be less than 1.1 mg/m^3 (10).

(f) Quality assurance

i) Special precautions
It is critical that the expired air sample is collected using the breath-holding technique described above and that the end-expired sample is collected for analysis. Moisture may interfere and can be removed using a drying agent in the tube leading to the collection bag.

ii) Interferences
Moisture is the only reported interference.

(g) Sources of possible errors

i) Pre-analytical
The details of the collection procedure must be followed, including selection of gas sampling bags. The instrument must be calibrated according to manufacturer's directions.

ii) Analytical
Calibration drift must be determined for each type of infrared analyser. The frequency of recalibration must be based on instrument drift.

(h) Reference to the most comprehensive description of the method

Ringold A, Goldsmith JR, Helwig HL, Finn R, Schuette F. Estimating Recent Carbon Monoxide Exposure, a Rapid Method. Archives of Environmental Health 1962;5:308–18.

(i) Evaluation of the method

There have been several evaluations of the method published. Unfortunately, each paper used slightly different collection apparatus and different commercial brands of infrared analysers. The overall precision and trueness are stated above. There have also been several papers which compared expired air measurements and HbCO measurements. These papers have generally shown a linear correlation with excellent correlation coefficients (6–8, 10, 11–15).

6.1.8.4 Other analytical methods

There are many variations and apparatus used for collection of the sample (6, 7, 10). Methods for the analysis of carbon monoxide expelled from the collection bag have primarily used infrared analysers, the same type of equipment used to analyse ambient carbon monoxide samples (11–12).

6.1.8.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

Carbon monoxide in individuals not occupationally exposed to carbon monoxide varies, depending on endogenous production of carbon monoxide, smoking habits, and environmental exposures. Such variation can affect the interpretation of carbon monoxide measurements below 22.3 mg/m^3. Workers commuting via congested roadways may arrive at work with end-expired CO levels of 34.4 mg/m^3 or more. Table 6.1.4 shows typical levels of end-expired CO in populations without occupational exposure to carbon monoxide (4, 16–17).

Table 6.1.4. Background carbon monoxide levels

Group/Source of exposure	Average CO levels
Endogenous production	Less than 22 mg/m^3 increasing up to 17.0 mg/m^3 during pregnancy and up to 34.4 mg/m^3 in patients with hemolytic anemia
Urban population	6.9–13.7 mg/m^3
Commuters on urban highways	34.4 mg/m^3 or more (CO levels on highways average 28.6 mg/m^3 rising up to 115 mg/m^3 during temperature inversions)
Tobacco smokers	Cigarettes: One pack per day 34–40 mg/m^3, Two to three packs per day 52–57 mg/m^3 Cigars: up to 143 mg/m^3

(b) Published biological action levels

Table 6.1.5 shows published biological action levels by organizations and recommendations by individual authors.

Table 6.1.5. Published biological action levels

Index	Biological action level, basis and collection time	Reference
CO in end-exhaled air	22.8 mg/m^3 collected at end of shift and related to 3.5% HbCO	ACGIH BEI (18)
CO in expired air	13 mg/m^3 tentative maximum permissible concentration in non-smokers based on bioequivalent to HbCO	Lauwerys et al. (19)

(c) Non-analytical interference

i) Exposure to other chemicals (the same metabolite), co-exposure, ethanol intake, medications

Dichloromethane (methylene chloride) is metabolized to carbon monoxide. An exposure to dichloromethane at 57 mg/m^3 for 8 hours will produce end-exhaled CO levels of 10–17 mg/m^3. Tobacco smoking, including side stream exposure to tobacco smoke, will add to the background level of CO.

ii) Diet and environment

Smoking habits and exposure to dichloromethane (methylene chloride) should be noted as a source of error. However, since end-exhaled CO is a measure of exposure by all routes and from all sources, monitoring of end-exhaled CO may be of value. Samples taken from current tobacco smokers or workers exposed to dichloromethane should not be used for evaluation of occupational exposure to carbon monoxide because of the other sources of carbon monoxide assessed by end-exhaled CO measurements (5).

Populations not adapted to living at high elevations (over 1,500 meters above sea level) are more sensitive to carbon monoxide because of reduced atmospheric oxygen. Native populations may not be more sensitive because of adaptation to lowered atmospheric oxygen (1–3, 20). Heavy labour, high temperature, or high altitude contribute to the health risk of workers exposed to carbon monoxide.

(d) Sampling representative of recent or long-term exposure or biological effect

End-exhaled CO measurements represent exposure during the last few hours prior to sample collection. Measurements in samples taken during the first 3 hours of exposure or later than 15–30 minutes after the end of exposure cannot be used for the evaluation of 8-hour TWA exposures.

(e) Ethnic differences (enzyme deficiency, environment, diet)

No ethnic differences that affect CO exhalation were found in the literature. Individuals with diminished pulmonary function may not be able to provide the required sample. Any population with decreased oxygen-carrying capacity or decreased oxygen availability may be at increased risk of carbon monoxide toxicity. Workers with respiratory disease that impairs oxygen delivery and pregnant workers and their fetuses may also be at an increased risk.

6.1.8.6 Research needs

Further clarification of the effects of low level chronic exposures to carbon monoxide on neurobehavioural and cardiovascular systems are needed.

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3. *Environmental Health Criteria for Carbon Monoxide*. Geneva, World Health Organization, 1979.
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6.2 Fluorides (including metallic fluorides, hydrogen fluoride and fluorine)

6.2.1 Introduction

Fluorides, including metallic fluorides, hydrofluoric acid and fluorine, are a diverse group of compounds. They are widespread in nature, being a key component of the production of aluminum. Fluorides are also used as fluxes in welding. Soluble fluorides are naturally present in drinking water and in foods, particularly tea. The principal route of occupational exposure is inhalation. Fluorides produce a number of toxic effects, depending on the physical form. For instance, hydrofluoric acid is very corrosive and produces burns on contact. Soluble fluorides ingested or inhaled can lead to increased deposition in the bone, and in long-term exposures at high levels, to bony fluorosis, a disorder of the bone characterized by increased bone density. There are reviews on fluoride (1–5).

There are adequate human data and published analytical methods to support biological monitoring for urinary fluoride. Recommended published biological action limits are based on health effects.

6.2.2 Physical-chemical properties

Fluorides are a diverse group of substances, the physical properties of which are dependent on the individual compound. Metallic fluorides are solids of variable solubility in water. Salts of monovalent metals are fairly soluble; salts of divalent metals are sparingly soluble. Hydrogen fluoride, or hydrofluoric acid, is a reactive gas which readily dissolves in water, reacts with glass, and is corrosive. Fluorine is a highly reactive gas, attacking all elements with the exception of oxygen and nitrogen and forming both covalent and electrovalent bonds with metals and other elements (6).

Conversion units: 1 mmol fluoride/L = 19 mg/L as fluoride
                1 mg/L = 0.053 mmol/L
                1 mg/g creatinine = 5.95 mmol/mol creatinine
                1 mmol/mol creatinine = 0.168 mg/g creatinine

6.2.3 Possible occupational and non-occupational exposures

Fluorides have widespread use in industry with the primary sources being fluorspar (calcium fluoride), cryolite (sodium aluminum fluoride) and phosphate rock. In addition to occupational exposure during the mining of these minerals, widespread occupational exposure occurs during their use in the production of steel and aluminum. Occupational exposures also occur during the use of fluoride containing fluxes used in alloy production and welding. Fluorides are also present in brick and refractory material which pre-

sent a potential for occupational exposure. The principal route of exposure in the work-place is inhalation (4, 7).

Non-occupational exposure to fluorides is widespread. As a result of its widespread distribution in the earth's mantle, fluoride is present in most foods. It is particularly prominent in tea (5, 8). In the United States, the dietary intake of fluoride by an adult is estimated to be 0.2–0.3 mg/day (0.011–0.016 mmol/day) in food with an increment of about 1.0 mg/day (0.053 mmol) from drinking water (5). Water and vegetation harvested in regions rich in phosphate rocks and cryolite and in regions near phosphate and aluminum plants can be very high in fluoride (9–11). In some communities, fluoride concentrations in water greater than 4 mg/L (0.2 mmol/L) (up to 33 mg/L [1.7 mmol/L]) have been measured (5). Small concentrations of airborne fluoride ($\mu g/m^3$ levels) were measured in some cities; however, these exposures were too low to significantly influence the urine concentrations of fluoride (8).

6.2.4 Summary of toxicokinetics

6.2.4.1 Absorption

(a) Inhalation

The main route of absorption of both particulate and gaseous fluorides in many industrial settings is inhalation. Gaseous compounds are readily absorbed. Up to 99.9% of inhaled hydrogen fluoride can be absorbed in the upper respiratory tract (12). The absorption of inhaled particulate fluorides increases with their water solubility.

(b) Dermal

Dermal exposure to hydrofluoric acid results in skin burns. Systemic toxicity resulting from dermal absorption of hydrofluoric acid has been reported (13, 14).

(c) Gastrointestinal

Absorption of ingested particulate fluorides increases with their solubility (12, 15, 16). It has been found that 90% to 97% of ingested water-soluble salts are readily absorbed (16). The sparingly soluble salts are absorbed more slowly and to a lesser extent. For example, only 62% of ingested calcium fluoride is absorbed (15).

6.2.4.2 Metabolic pathways and biochemical interactions

Inorganic fluoride, and elemental fluoride are not metabolized in the body, however, organic fluorides are metabolized to some extent and release fluoride ion into the body. Fluorides which are absorbed are deposited mostly in the bone. It has been reported that fluorides at high concentrations can inhibit vital enzymes (12).

6.2.4.3 Distribution

Soluble fluoride is taken up by the blood and rapidly distributed to the entire body. About 75% of fluorides in the blood are found in the plasma. In addition to distribution to organs, such as liver, heart and lungs, the principal site of deposition is the bone where 99% of all deposited fluorides accumulate. Binding to bone is reversible and is dependent on plasma concentrations (4, 17).

6.2.4.4 Elimination

The kidney is the major route of elimination with about 50% of the daily intake appearing in the urine (16). Approximately 5% to 30% of the amount ingested is excreted in the faeces (16, 18). Sweat may be a significant route of elimination in hot environments (19). Fluoride is also eliminated in mothers' milk (15). Fluoride elimination in urine is dependent on urine flow and pH and shows an elimination half-life of 4 to 7 hours (20–24). Fluoride retained in soft tissues is excreted very rapidly while fluoride retained in the skeleton is eliminated very slowly. Skeletal elimination is biphasic. Hodge (25) suggested that the rapid process, lasting weeks, involves an ionic exchange escape mechanism, and the slow process, lasting years, involves osteoclastic resorption of bone. Neither one of the skeletal elimination phases can be measured by urinary excretion because of the relatively small concentration increase compared to the large variability of background levels.

6.2.5 Summary of toxic effects

Acute exposures to hydrogen fluoride gas results in skin burns, in necrosis of the lungs and eyes, and death. Long-term exposures to soluble, bioavailable fluorides, result in bony fluorosis, a condition which produces rigid bone structure. There are reports of skeletal fluorosis in aluminum potroom workers exposed for more than 5 years (1–2, 26).

6.2.6 Biological monitoring indices

Table 6.2.1 shows a listing of available biological monitoring media for monitoring fluoride exposure.

Table 6.2.1. Biological monitoring indices

Parameter	Suitability for use	Reference
Fluoride in urine	Best indicator of exposure to soluble fluorides. Excretion dependent on urine flow and urine pH	17, 27–30
Fluoride in plasma	Reflects exposure to soluble fluorides, but not in common use.	4, 27, 28
Fluoride in hair	Potentially acceptable, but limited due to exogenous contamination of the hair	27, 31, 32
Fluoride in nails	Limited to one publication	32

The determination of fluoride in urine is the most widely accepted technique for evaluation of exposure to soluble and inorganic fluorides. The excretion of fluoride in the urine is dependent on urine flow rate, and is influenced by the pH of the urine (23–24). Other biological monitoring indices that have been used for exposure assessment are measurements of fluoride in plasma, hair and nails. Plasma concentrations are one-tenth that found in urine, so exogenous contamination becomes more of a problem when measuring fluorides in plasma. Measurements of fluorides in hair and nails are also hampered by highly variable exogenous contamination.

6.2.7 Fluoride in urine

6.2.7.1 Toxicokinetics

Almost one-half of the absorbed amount of fluoride is excreted very rapidly in urine (16). The remaining absorbed amount is deposited in the mineral matrix of bone from where it is eliminated very slowly (16, 25). Fluoride concentration in urine peaks at the end of exposure or shortly thereafter and declines with a half-time of 4 to 7 hours (20–22). Neither one of the skeletal elimination phases can be measured by urinary excretion because of the relatively small concentration increase compared to the large variability of background levels.

6.2.7.2 Biological sampling

(a) Sampling time and specimen

Urine samples should be collected before the shift and at the end of the shift. The pre-exposure sample should be collected after 2 days without exposure.

(b) Contamination possibilities

Since fluoride is ubiquitous, precaution must be taken to avoid contamination from the workplace and clothing. It is preferable to remove workplace clothing that may be contaminated with particulate fluorides to avoid contamination of the urine sample. Use of tap water, which contains fluoride, for the rinsing of collection bottles must be avoided.

(c) Sampling device and container

Samples should be collected in clean plastic containers, such as high density polyethylene bottles. Containers made of fluorocarbons must be evaluated using blanks for the leachability of residual fluorides from the container. Glass containers should not be used because of the affinity of fluoride for glass.

(d) Preservatives, shipment and stability

EDTA should be added to the collection bottle prior to collection to chelate cations that may interfere with the specific ion-electrode. Measurement of fluoride in urine by ion specific electrodes is affected by microbial growth and adsorption of fluoride onto the sampling container. Samples should be refrigerated or frozen until analysed (17).

6.2.7.3 Recommended analytical method

Fluoride is best determined by an electrometric method, using a specific fluoride ion electrode (33–36). The method with the best documentation and validation is that by Angerer and Schaller (36). Measurements must be taken in specimens in which a chelating agent and total ionic strength adjuster are added and the pH is adjusted so that it is between 5 and 7. The determination of creatinine would appear to be required, based on published reference values (29, 30). Since the excretion of fluoride in the urine is influenced by both urine flow rate and pH, the correction for creatinine may actually introduce an unwanted error, as creatinine excretion is not affected to the same extent by these variables (23–24). In practice, creatinine is best used to assess the dilution of the urine. If urine samples have creatinine levels below 0.3 g/L (2.65 mmol/L), an additional urine sample should be collected.

(a) Principles of the method

The urine sample is diluted with a Total Ionic Strength Adjustment Buffer (TISAB) which serves to establish a defined pH and a constant ionic strength. The fluoride level is determined directly with a fluoride ion-specific electrode using aqueous calibration standards.

(b) Reagents required

TISAB (total ion-strength-adjustment buffer) – This buffer is made from glacial acetic acid (57 mL), sodium citrate dihydrate (300 mg), and sodium chloride (58 g). The pH is adjusted to 5.5 with 10 M NaOH. The solution is diluted to the 1 L mark with deionized distilled water. It is available commercially. Potassium fluoride working standards in deionized distilled water are required over the range of 0.2–10 mg/L (0.012–0.53 mmol/L), and can be prepared from commercially available stock solution of potassium fluoride (1 g/L). Deionized distilled water must be used for all reagent preparation, electrode rinsing and labware final rinses, since most tap water contains fluorides.

(c) Equipment required

Ion-specific fluoride electrode, reference electrode and a digital pH/millivolt meter with accuracy of ± 0.1 mV are required. A magnetic stirrer with polyethylene coated stirring bar, as well as polyethylene bottles, tubes, transfer pipets, volumetric flasks (avoid glass) are also required.

(d) Procedures and calibration

i) Calibration
Calibration is carried out by plotting the mV response versus the logarithmic concentration of freshly prepared aqueous fluoride standards over the range of 0.2–10 mg/L (0.012–0.53 mmol/L).

ii) Procedure
A portion of urine is mixed with an equal portion of TISAB and allowed to equilibrate for 2 minutes. The mV reading is then taken using an appropriate deionized distilled

water blank. The electrodes must be rinsed between samples, using deionized distilled water.

(e) Criteria of analytical reliability

i) Trueness
Trueness, determined by recovery of spiked samples, ranged between 92 and 99% for samples in the normal, occupational, and toxic ranges.

ii) Precision
The within-day precision was 1.6% relative standard deviation for 20 replications of a urine sample containing 2.15 mg/L (0.114 mmol/L). The between-day precision over 14 days was 2.8% relative standard deviation for a urine sample containing 1.16 mg/L (0.0615 mmol/L).

iii) Detectability
The practical detection limit was estimated at 0.1 mg/L of urine (0.0053 mmol/L).

(f) Quality assurance

i) Special precautions
In addition to normal, within laboratory quality assurance using frozen aliquot of pooled urine, a variety of commercially available fluoride in urine controls are available. These controls should be used in every batch with appropriate control charting performed to monitor performance (36). There is one international proficiency testing programme available for fluoride in urine operated by the Centre for Toxicology in Québec.[1]

ii) Interferences
The use of EDTA to chelate cations from the container reduces the possible influence of cations on the method (35).

Exposure to organic fluorine-containing chemicals which are metabolized to fluoride must be avoided for 2 days or longer before sampling, otherwise the occupational exposure could be overestimated. Such chemicals are some chlorinated fluorocarbons (e.g. Freons®), fire extinguishing agents, insecticides and fluorosteroids, and some drugs, such as inhalation anesthetic agents or ointments.

(g) Sources of possible errors

i) Pre-analytical
Urine samples that have been acidified with glacial acetic acid may produce false low results. The pH of such samples, after addition of buffer, should be checked to verify that the pH is in the appropriate range.

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[1] Programme operated by Jean-Philippe Weber, Ph.D., Center de Toxicologie du Québec, 2705, Laurier Blvd, Sainte-Foy, Québec, CANADA G1V 4G2.

ii) Analytical

The pH must be between 5 and 7 to produce reliable results. The TISAB should maintain the pH between these limits. Contamination from the laboratory must be avoided by the use of deionized distilled water in all reagents. Proteins and other blood components affect the performance of the membrane of the fluoride ion electrode. Rinsing of the electrode between samples with deionized distilled water is recommended.

(h) Reference for the most comprehensive description of the method

DFG. Fluoride. In: Analysis of Hazardous Substances in Biological Materials: Methods for biological monitoring, Volume 2. Angerer J, Schaller HK. (Eds.) New York. VCH Publishers, 1988 [English].

(i) Evaluation of the selected method

The method described above (36) included a laboratory validation study of precision, trueness, and estimates of detectability. Within-day precision was determined by analysis of 20 specimens at 2.15 mg/L (0.114 mmol/L); the relative standard deviation was 1.6%. Day-to-day precision was determined using commercially available control materials with a mean concentration of 1.16 mg/L (0.061 mmol/L) over a 14-day period; the relative standard deviation was 2.8%.

Trueness, as assessed by recovery studies of urine samples in the normal, occupational and toxic levels spiked at three different levels ranged from 92–99% over a range of 0.37 to 52 mg/L (0.0020–2.8 mmol/L). The limit of detectability, under conditions used, was 0.1 mg/L (0.0053 mmol/L). Below this concentration, the calibration curve was not linear. No inter-laboratory comparisons or method comparisons were reported.

6.2.7.4 Other analytical methods

Fluoride in urine can be determined by flow injection analysis using ion-specific electrodes (37). Fluoride in urine has also been reported by gas chromatography (38). However, these methods do not offer any advantages to ion-specific electrode methods and are more costly to perform.

6.2.7.5 Guide to interpretation

(a) Measured values in groups without occupational exposure

Fluoride concentrations in urine of occupationally unexposed populations depend on dietary and environmental conditions. There is generally a balance between daily fluoride intake and fluoride concentrations in urine. Values measured in the US population are usually below 1 mg/L (0.053 mmol/L) (8). Hogstedt reported the maximal level of 2 mg/L (0.11 mmol/L) (4). A close correlation between fluoride concentrations in the water supply and in urine has been noted by a number of investigators (8, 12, 39, 40).

(b) Published biological action levels

Biological monitoring action levels for fluoride in urine have been recommended by a variety of organizations and individuals. Table 6.2.2 summarizes those recommendations.

Table 6.2.2. Biological monitoring action levels

Pre-shift urine	Post-shift urine	Net change	Basis	Reference
3 mg/g creatinine (17.9 mmol/mol creatinine)	10 mg/g creatinine (59.5 mmol/mol creatinine)		Health-based	ACGIH BEI (29)
4 mg/g creatinine[a] (24 mmol/mol creatinine)	7 mg/g creatinine (42 mmol/mol creatinine)		Health-based	DFG-BAT (30)
4 mg/L (1.024) [0.2 mmol/L]	7 mg/L (1.024) [0.4 mmol/L]		Health-based	NIOSH (7, 41)
		3–4 mg/g creatinine (18–24 mmol/mol creatinine)	Health-based	Lauwerys (27)

[a] At the beginning of the next shift

Since fluorides are ubiquitous in the environment and the background levels of fluoride in world populations is expected to be more variable than background levels within a single country, a biological action level based on the net change from urine specimens collected post shift minus the concentration in a pre-shift urine specimen may be more appropriate. In order to provide a measure of protection against fluorosis to populations which may have a high background from environmental and dietary sources of fluoride, a maximum level of fluoride in the urine from specimens collected at the end of the shift is recommended.

(c) Non-analytical interferences

i) Exposure to other chemicals (the same metabolite) co-exposure, ethanol intake, medication

Fluoride can be released by the metabolism of numerous industrial chemicals and drugs containing organic fluorine. An increased fluoride concentration in urine can also be caused by exposure to some fluorinated hydrocarbons and their derivatives which are metabolized to fluoride.

ii) Diet and environment

Fluoride levels in urine can be temporarily elevated after consumption of tea or salt water fish (5, 8). Concentrations of fluoride in urine are dependent on the fluoride content of drinking water, and populations consuming water with a high fluoride content will have higher concentrations of fluoride in urine.

The fluoride elimination rate in urine is decreased by increased intake of magnesium and calcium and is increased by increased intake of iron (39). The elimination rate is also affected by the pH of urine which is affected by diet (42, 43).

Fluoride concentrations in urine can be temporarily elevated after using preparations for the prevention of dental caries (mouthwashes, toothpastes, pills, and lozenges).

Some household products contain fluoride, such as rust removing gels. Since fluoride is ubiquitous in the environment, fluoride concentrations in the urine will reflect total exposure, including occupational and non-occupational sources.

Populations living in areas with high concentrations of fluoride in drinking water and/or high environmental fluoride concentrations may be at higher risk to occupational exposures not considered excessive in individuals living in areas of low fluoride background levels.

(d) Sampling representative of recent or long-term exposure or biochemical effect

Fluoride measurements in urine can provide two kinds of information depending on when the sample is collected (17).

i) Measurements in pre-shift samples indicate the amount of fluoride accumulated in the body over a long time regardless of the source of exposure and route of absorption. Such measurements are an indicator of skeletal burden, and an elevated level can be an indicator of the increased health risk of recurring exposure.

ii) Measurements in samples collected at the end of the shift indicate the magnitude of the recent exposure. When compared to pre-exposure levels, results from these measurements should correspond to the concentration increase produced by an exposure during the workshift.

(e) Ethnic differences (enzyme deficiency, environment, diet)

No ethnic differences were found that would effect fluoride levels in urine.

6.2.7.6 Research needs

There is a good data base relating airborne exposures to fluorides with fluoride concentrations found in urine samples collected before and after a workshift. There is also information relating the levels of fluoride in urine and the incidence of bony fluorosis; however, there is little information relating past exposures, past urinary concentrations of fluoride and the incidence of bony fluorosis.

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Biological monitoring methods	Sampling time	Biological limit values as proposed by		
		ACGIH BEI	DFG BAT	TMPC
Acetone				
acetone in urine	ES	100 mg/L●		30 mg/g creat
acetone in blood	ES			5 mg/100 ml
Acetylcholinesterase inhibitors				
acetylcholinesterase activity in red blood cells		30% inhibition (discretionary)	30 % inhibition (ES, EW)	30 % inhibition (ES)
Aluminium				
aluminium in urine	ES		200 μg/L	150 μg/g creat
Aniline				
aniline in urine	ES, EW		1 mg/L (free)	
p-aminophenol in urine	ES	50 mg/g creat (total)		30 mg/g creat
aniline Hb adducts	ES, EW		10 μg/100 ml	10 μg/100 ml
methemoglobin in blood	DS/ES	1.5 %		2 %
Antimony				
antimony in urine	ES			35 μg/g creat
Arsenic				
iAs metabolites in urine	ES, EW	50 μg/g creat●		50 μg/g creat[1] 30 μg/g creat[2]
Benzene				
phenol in urine	ES	50 mg/g creat (total)		45 mg/g creat[3] <20 mg/g creat[4]
muconic acid in urine	ES			1.4 mg/g creat[4]
benzene in blood	DS			<5 μg/100 ml[4]
benzene in mixed-exhaled air	PS	0.08 ppm		
benzene in end-exhaled air	PS	0.12 ppm		
	DS			0.022 ppm[4]
Biphenyl				
2,4-hydroxybiphenyl in urine	ES			1.5 mg/g creat
p-t-Butylphenol				
p-t-butylphenol in urine	ES		2 mg/L	2 mg/g creat
Cadmium				
cadmium in urine	NC	5 μg/g creat●	15 μg/L	5 μg/g creat
cadmium in blood	NC	0.5 μg/100 ml●	1.5 μg/100 ml	0.5 μg/100 ml
Carbaryl				
1-naphtol in urine	ES			10 mg/g creat

Biological monitoring methods	Sampling time	Biological limit values as proposed by		
		ACGIH BEI	DFG BAT	TMPC
Carbon disulfide				
2-thiothiazolidine-4-carboxylic acid in urine	ES	5 mg/g	8 mg/L	5 mg/g creat
Carbon monoxide				
carboxyHb in blood*	ES	3.5 %●	5 %	3.5 %
CO in end-exhaled air*	ES	20 ppm●		12 ppm
CO in blood*				7 ml/100 ml
Carbon tetrachloride				
carbon tetrachloride in end-exhaled air	ES$^+$		1.6 ml/m^3	
carbon tetrachloride in blood	ES, EW		7 µg/100 ml	
Chlorobenzene				
4-chlorocatechol in urine	ES	150 mg/g creat (total)	300 mg/g creat	
	PS		70 mg/g creat	
p-chlorophenol in urine	ES	25 mg/g creat (total)		
Chromium (VI) (soluble compounds)				
chromium in urine	IDS	10 µg/g creat		
	ES, EW	30 µg/g creat (total)		30 µg/g creat
Cobalt				
cobalt in urine	ES			30 µg/g creat
Cyclohexane				
cyclohexanol in urine	ES			3.2 mg/g creat
cyclohexane in blood	DE			45 µg/100 ml
cyclohexane in exhaled air	DE			220 ppm
Cyclohexanone				
cyclohexanol in urine	ES			20 mg/g creat
p-Dichlorobenzene				
p-dichlorobenzene in urine	ES			250 µg/g creat
Dichloromethane				
dichloromethane in blood	ES		0.1 mg/100 ml	0.05 mg/100 ml
dichloromethane in exhaled air	ES			15 ppm
carboxyHb in blood*	ES		5 %	2 %
Dieldrin				
dieldrin in blood	ES			15 µg/100 ml

Biological monitoring methods	Sampling time	Biological limit values as proposed by		
		ACGIH BEI	DFG BAT	TMPC
Dimethylethylamine (DMEA)				
DMEA + DMEA-N-oxide in urine	ES			90 mg/g creat[5]
N,N-Dimethylformamide				
N-methylformamide in urine	ES	40 mg/g creat		30 mg/g creat
N-methylformamide in blood	ES			0.1 mg/100 ml
dimethylformamide in blood	ES			0.15 mg/100 ml
dimethylformamide in exhaled air	DE			2.5 ppm
Dinitroorthocresol				
dinitroorthocresol in blood	ES			1 mg/100 ml
Endrin				
endrin in blood	ES			5 µg/100 ml
anti-12-hydroxy endrin in urine	ES			0.13 mg/g creat
2-Ethoxyethanol (acetate)				
2-ethoxyacetic acid in urine	ES, EW	100 mg/g creat●	50 mg/L	150 mg/g creat[5]
Ethyl benzene				
mandelic acid in urine	ES, EW	1.5 g/g creat		1 g/g creat
ethylbenzene in blood	DE			0.15 mg/100 ml
Ethylene oxide				
ethylene oxide in blood	DS			0.8 µg/100 ml
ethylene oxide in exhaled air	DS			0.5 mg/m^3
Fluoride				
fluoride in urine	PS	3 mg/g creat	4 mg/g creat	
	ES	10 mg/g creat	7 mg/g creat	
	ES-PS			3-4 mg/g creat
Furfural				
furoic acid in urine	ES	200 mg/g creat (total)		80 mg/g creat
Halothane				
trifluoroacetic acid in blood	ES, EW		0.25 mg/100 ml	0.25 mg/100 ml[5]
trifluoroacetic acid in urine	ES, EW			10 mg/g creat[5]
halothane in urine	ES			90 µg/g creat[6]
				10 µg/g creat[5]
halothane in exhaled air	ES			0.5 ppm[5]
Hexachlorobenzene				
hexachlorobenzene in blood			15 µg/100 ml plasma/serum (NF)	30 µg/100 ml

Biological monitoring methods	Sampling time	Biological limit values as proposed by		
		ACGIH BEI	DFG BAT	TMPC
γ-Hexachlorocyclohexane (lindane)				
γ-hexachlorocyclohexane in blood	ES		2 µg/100 ml	2 µg/100 ml
γ-hexachlorocyclohexane in plasma/serum	ES		2.5 µg/100 ml	
n-Hexane				
2,5-hexanedione in urine	ES	5 mg/g creat		4 mg/g creat (EW)
	ES, FD			2 mg/g creat
2-hexanol in urine	ES			0.2 mg/g creat
n-hexane in blood	DS			15 µg/100 ml
n-hexane in exhaled air	DS			50 ppm
2,5-hexanedione+4,5-dihydroxy-2-hexanone in urine	ES		9 mg/L	
Isopropyl alcohol				
acetone in urine	ES		50 mg/L	
acetone in blood	ES		5 mg/100 ml	
Isopropylbenzene (cumene)				
2-phenylpropanol in urine	last 2 hr of shift			10 mg/hr
Lead				
lead in blood	NC	50 µg/100 ml	70 µg/100 ml o 30 µg/100 ml o	40 µg/100 ml
lead in urine	NC	150 µg/g creat		50 µg/g creat
zinc protoporphyrin in blood	after 1 month exposure	100 µg/100 ml 250 µg/100 ml RBC		40 µg/100 ml 3 µg/g Hb
δ-aminolevulinic acid in urine			15 mg/L o 6 mg/L o	5 mg/g creat
Mercury inorganic				
mercury in urine	PS	35 µg/g creat●	200 µg/L (NF)	50 µg/g creat
mercury in blood	ES, EW	1.5 µg/100 ml●	5 µg/100 ml (NF)	2 µg/100 ml
Methylmercury				
mercury in blood			10 µg/100 ml (NF)	10 µg/100 ml
Methanol				
methanol in urine	ES	15 mg/L	30 mg/L (EW)	25 mg/g creat
formic acid in urine	PS, EW	80 mg/g creat		
Methyl-n-butylketone				
2,5-hexanedione in urine	ES			4 mg/g creat
2,5-hexanedione + 4,5-dihydroxy-2-hexanone in urine	ES		9 mg/L	

Biological monitoring methods	Sampling time	Biological limit values as proposed by		
		ACGIH BEI	DFG BAT	TMPC
Methyl ethyl ketone (2-butanone) methyl ethyl ketone in urine	ES	2 mg/L	5 mg/L	2.5 mg/g creat (EW)
Methyl isobutyl ketone (MIBK) MIBK in urine	ES	2 mg/L●	3.5 mg/L	0.5 mg/g creat
2 or 3 Methyl-pentane 2 (or 3) methyl-pentane in exhaled air				1,500 µg/L
2 (or 3) methyl-pentane in blood				35 µg/100 ml
Nickel (soluble compounds) nickel in urine				30 µg/g creat
nickel in plasma				1 µg/100 ml
Nitrobenzene p-nitrophenol in urine	ES, EW	5 mg/g creat (total)		5 mg/g creat
methemoglobin in blood	ES	1.5 %		5 %
aniline Hb adducts in blood	EW		10 µg/100 ml	
Nitrous oxide nitrous oxide in urine				60 µg/g creat
Parathion p-nitrophenol in urine	ES	0.5 mg/g creat (total)	0.5 mg/L (EW)	0.5 mg/g creat
cholinesterase activity in RBC		70 % of individual baseline value	70 % of individual baseline value	
Pentachlorophenol PCP in urine		2 mg/g creat (PS, EW) (total)		1 mg/g creat
PCP in plasma	ES	0.5 mg/100 ml (free)		0.05 mg/100 ml
Phenol phenol in urine	ES	250 mg/g creat (total)	300 mg/L	250 mg/g creat
Styrene mandelic acid in urine	ES PS	800 mg/g creat 300 mg/g creat	2 g/L	800 mg/g creat
phenylglyoxylic acid in urine	ES PS	240 mg/g creat 100 mg/g creat		250 mg/g creat
styrene in blood	ES PS	55 µg/100 ml 2 µg/100 ml		100 µg/100 ml 2 µg/100 ml

Biological monitoring methods	Sampling time	Biological limit values as proposed by		
		ACGIH BEI	DFG BAT	TMPC
mandelic + phenylglyoxylic acid in urine	ES		2.5 g/L	
Tetrachloroethylene				
trichloroacetic acid in urine	EW	7 mg/L		5 mg/g creat
tetrachloroethylene in blood	PS	100 µg/100 ml (EW)	0.1 mg/100 ml	100 µg/100 ml creat
tetrachloroethylene in urine	PS			100 µg/g creat
tetrachloroethylene in end-exhaled air	PS	10 ppm (EW)	9.5 ml/m^3	8 ppm
	DS			60 ppm
Toluene				
hippuric acid in urine	ES	2.5 g/g creat		2.5 g/g creat
o-cresol in urine	ES	1 mg/g creat●		1 mg/g creat
toluene in blood	ES	100 µg/100 ml	170 µg/100 ml	
	DS			100 µg/100 ml
toluene in end-exhaled air	DS			20 ppm
Trichloroethane (methylchloroform)				
trichloroacetic acid in urine	ES, EW	10 mg/L		10 mg/g creat
trichloroethanol in urine	ES, EW	30 mg/L (total)		30 mg /g creat
trichloroethane in blood	ES, EW		55 µg/100 ml	100 µg/100 ml
trichloroethane in urine				800 µg/g creat
trichloroethanol in blood	ES, EW	100 µg/100 ml (total)		100 µg/100 ml
trichloroethane in end-exhaled air	PS, EW	40 ppm	20 ml/m^3	30 ppm
Trichloroethylene				
trichloroacetic acid in urine	EW	100 mg/g creat	100 mg/L (ES)	75 mg/g creat
trichloroethanol in urine				150 mg/g creat
trichloroacetic acid + trichloroethanol in urine	ES, EW	300 mg/g creat		
trichloroethanol in blood	ES, EW	400 µg/100 ml (free)	500 µg/100 ml	0.25 mg/100 ml plasma
trichloroethylene in end-exhaled air	DS			10 ppm
	PNS			0.5 ppm
trichloroacetic acid in plasma	ES, EW			5 mg/100 ml
trichloroethylene in blood	DS			60 µg/100 ml
Triethylamine (TEA)				
TEA + TEA-N-oxide in urine				60 mg/g creat[7]
Vanadium				
vanadium in urine				50 µg/g creat
Xylene				
methylhippuric acid in urine	ES	1.5 g/g creat	2 g/L	1.5 g/g creat

Biological monitoring methods	Sampling time	Biological limit values as proposed by		
		ACGIH BEI	DFG BAT	TMPC
xylene in blood	ES DS		150 µg/100 ml	300 µg/100 ml

1.   if TWA exposure: 50 µg/m^3
2.   if TWA exposure: 10 µg/m^3
3.   if TWA exposure: 10 ppm
4.   if TWA exposure: 1 ppm
5.   if TWA exposure: 5 ppm
6.   if TWA exposure: 50 ppm
7.   if TWA exposure: 2.5 ppm

*non-smokers

ES   end of shift
EW   end of workweek
PS   prior to shift
DS   during shift
IDS   increase during shift
NC   not critical
NF   not fixed
ES-PS   end shift minus preshift
FD   first day
ES$^+$ 1 hour after the end of the shift
•   on the "Notice of Intent to Establish"
BEI   Biological Exposure Indice (ACGIH 1992–1993)
BAT   Biological Tolerance Value (DFG 1991)
TMPC   Tentative maximum permissible concentration (from reference Bernard A, Lauwerys R.
            Les méthodes biologiques d'évaluation de l'exposition aux solvants. *Cahiers de Médecine du
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