General Aspects

Essential Biomonitoring Methods. DFG, Deutsche Forschungsgemeinschaft

ISBN: 3-527-31478-4

Preliminary remarks

Introduction

It is the duty of occupational and environmental medicine to evaluate the health risk posed by hazardous chemicals in order to guard against impairment of health. In the last 20 years biological monitoring has proven extremely valuable for this purpose.
The following definition of biological monitoring, which has been adopted in a slightly modified form by the "Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area" of the Deutsche Forschungsgemeinschaft, was formulated by the international seminar "Assessment of Toxic Agents at the Work Place", organized in 1980 by *CEC, OSHA,* and *NIOSH.* This definition reflects how biological monitoring is viewed by the countries of the European Community, where it has been practiced for a number of years:

"Biological monitoring is the directed systematic continuous or repetitive health-related activity for collection of biological samples for the measurement and assessment of hazardous chemical compounds, their metabolites of their specific biochemical effect-parameters. The objective is to evaluate the exposure and health risk of exposed persons by comparing the obtained data with appropriate reference values, leading to corrective actions if necessary."

The difficulties of biological monitoring are primarily analytical because occupational and environmental medicine must measure
- a minute quantity of substance (down to the picogram level)
- in a small volume (a few milliliters)
- of complex specimen material (blood, urine, etc.)

This demands a high-performance analytical method. Often only the most up-to-date instrumental techniques are capable of achieving the required level of specificity and sensitivity. The lower the amount of analyte present and the higher the instrumental complexity, the more important is a continuous check on analytical reliability. For this reason both internal and external quality control are essential for investigations within the realm of occupational and environmental toxicology. The so-called preanalytical phase (specimen collection, transport, storage, sample aliquotation etc.) is also important within the framework of trace analyses and deserves more attention than it has received up to now.
An evaluation of the results is necessary from an analytical as well as a medical viewpoint and should be based on the collaborative efforts of a body of experts.

Analysis in biological materials

Biological specimen

The biological material used for the analysis must be representative of the exposure to the particular hazardous substance. The concentration of the substance in the critical organ would provide the optimum measure for an individual exposure, but this is usually not

Essential Biomonitoring Methods. DFG, Deutsche Forschungsgemeinschaft

ISBN: 3-527-31478-4

possible. Furthermore, within the framework of biological monitoring, the collection of the biological specimen should not place any noticeable strain on the donor.
The preferred biological materials for occupational health are therefore
- blood
- urine
- exhaled air.

The determination of a hazardous substance in the blood is one of the diagnostically most reliable ways to quantify an exposure. Since the organ concentrations of the hazardous substance are maintained in equilibrium with that of the circulating blood, its concentration in the critical organ can be estimated from its concentration in the blood. Moreover, the risk of exogenous contamination or manipulation of the specimen is considerably less for blood than for other biological test materials.
Urine has the advantage of being easier to collect and more available than blood. However, urinary analyses also have drawbacks stemming from, for example, the different functions of the kidneys, the variation in volumes drunk and excreted, and the fact that the metabolic products of some industrial substances are also excreted physiologically. Under working conditions, spontaneous urine has to be used because unfortunately the collection of urine over a 24 h period is usually impracticable. This leads to difficulties in the interpretation of analytical results from spontaneous urine specimens. Parallel analyses of a reference parameter such as creatinine, osmolality, or density are in practice still unable to resolve this problem satisfactorily. This is especially true for very dilute or highly concentrated urine specimens. Parallel analyses of the above-mentioned parameters could, however, make possible the preselection of urine specimens suitable for occupational health studies.
From an analytical and medical viewpoint the analysis of exhaled air offers several advantages. However, because of difficulties in collection, transport, and storage the analysis of exhaled air is still of limited practical importance.
In structures like hair, fingernails, and teeth an exposure to a hazardous chemical can be determined only after a longer period of time. Hence these materials are far better suited for environmental than occupational health studies.

Toxicological investigation in occupational health

In occupational medicine, as for the field of medicine in general, the goal of every investigation is a diagnosis related to the individual patient. In the case of an exposure to hazardous (chemical) industrial materials this diagnosis is usually based on a chemical analysis of the substance, its metabolites, or other parameters of its intermediary metabolism in the biological material and on the analytical-medical evaluation of the obtained value.
Thus, in occupational medicine a diagnosis is reached essentially in three steps:
- the preanalytical phase
- the analytical determination and assessment
- the medical evaluation.

Preanalytical phase

There is a growing recognition of the importance of the preanalytical phase for the accuracy of the results. Any factor that can alter the analytical result between the time of specimen

collection up to its analysis in the laboratory is termed an *interference factor.* Such a change may occur for example as a result of evaporation or chemical transformation of the analyte. Interference factors are independent of the reliability of the method used and are, therefore, not covered by the statistical quality control. They can be minimized only by the appropriate standardization of:

- specimen collection (syringes, needles, containers, etc.)
- transport and storage (duration, temperature, etc.)
- aliquotation of the biological specimen (homogenization, etc.).

Which preventive measures are to be taken during the preanalytical phase depends on the kind of biological test material as well as the substances to be determined. To satisfy these requirements and the practical demands of monitoring, the preanalytical phase should be designed to be applicable to as many analytes as possible.

The Working Group "Analytical Chemistry" has worked out some recommendations along these lines. These suggestions for occupational health and toxicological monitoring investigations on urine and blood specimens can be summarized as follows:

Time of specimen collection. Whole blood or spontaneous urine specimens should be collected at the end of the work shift, if possible after 3 workdays. For special working materials different conditions for specimen collection have to be accepted. Information pertaining to this can be found in the annual report of the Commission of the Deutsche Forschungsgemeinschaft for the Investigation of Health Hazards of Chemical Compounds in the Work Area and in the most recent Arbeitsmedizinisch-toxikologische Begründung von BAT-Werten (Occupational Hygiene and Toxicological Justification of BAT Values).

Collection of urine specimens. Plastic containers (about 200 mL, wide-mouthed) that have been rinsed with acid are used for urine collection. The urine is voided directly into the container. Care must be taken that the hands have been washed and the work clothes exchanged for street clothes before the specimen is collected. Contamination by dust, gas, or vapor from the work area must be strictly avoided.

Specimen volume: If possible the volume of urine should be at least 50 mL.

Collection of whole blood specimens. Venous blood to which anticoagulant has been added is used for the analytical investigations. *Coagulation must be scrupulously avoided by thorough rotation of the specimen containers!*

Determination of inorganic substances (metals):

Disposable needles, syringes, and containers (e.g. Monovettes and Vacutainers), can be used for collection. Monovettes and Vacutainers already contain anticoagulants (e.g., K-EDTA) in the required amounts. They can also serve as containers for transport and storage.

Specimen volume: For most analyses 5 mL whole blood is sufficient, but the volume of blood should be at least this amount.

Determination of highly volatile organic substances:

Disposable syringes (5 mL) and needles are used for collection. Usually disinfection of the puncture site with alcohol must be waived.

Specimen volume: A 4 mL sample of venous blood from the arm is divided equally between two 20 mL "head-space sample flasks" containing an anticoagulant, e.g., 50 mg ammonium oxalate, and the flasks are sealed airtight with PTFE-coated butyl rubber stoppers. The flasks serve also as storage and transport containers.

In a single case it may be necessary to use a procedure that deviates from the one described here. It is then presented in detail in the method descriptions.

Storage and shipping of the specimens. Arrangements should be made to ship the blood and urine specimens as soon as possible after collection. If it is not possible to ship them right after collection, the specimens can be stored at 4 °C for up to 5 days.

Sample aliquotation. A major source of error in the analysis of blood and urine is the inhomogeneity of the biological material due to coagulation or sedimentation. To avoid gross analytical errors appropriate precautions must be observed in the treatment of the blood and urine specimens to ensure that the analyzed portion (sample) is representative of the specimen.

Blood

Blood specimens to be analyzed for nonvolatile organic and inorganic constituents are homogenized by careful mixing. The so-called roller mixer has proven especially well suited for this purpose. The specimen containers are placed on their side in the roller mixer and are turned continuously around their long axis. At the same time they are tipped slightly back and forth so that the test material is swished around in the container. After the blood specimens have been mixed on this apparatus for 1 h, a sample is taken for analysis.
To measure volatile substances in the blood the head-space sample flasks containing the specimens are incubated at a constant temperature of, for example, 60 °C. By 60 min the concentration of the substance in the blood and in the head space of the flask have reached an equilibrium.

Urine

Before aliquotation the urine specimens are agitated on a shaking machine for 30 min. Directly before the samples are pipetted, each urine specimen must be shaken thoroughly again by hand.

Analytical determination and assessment

Laboratories studying problems concerning occupational health and toxicology rely mainly on those techniques of analytical chemistry that distinguish themselves by especially high specificity and selectivity.
The quality of the investigative method can be characterized by reliability criteria. Practical application of a method must be accompanied by statistical quality control. This is the only way to ensure that the analytical results are consistently reliable and is also a prerequisite for the comparison of results from different laboratories.

Analytical reliability criteria

The analytical reliability of a method can be satisfactorily described by the criteria
- sensitivity
- imprecision
- inaccuracy
- detection limit
- specificity.

Sensitivity

In chemical analysis the sensitivity H is the differential quotient of the calibration function. A graph of the calibration function is obtained by plotting the observed value of the measure x (ordinate) as a function of the analyte concentration c (abscissa). The sensitivity H is then the slope of this calibration function:

$$H := \frac{dx}{dc}$$

Imprecision

Imprecision is a measure for the reproducibility of the results from a given experimental design. The 1980 IFCC definition for imprecision is:
"Imprecision: Standard deviation or coefficient of variation of the results in a set of replicate measurements. The mean value and number of replicates must be stated, and the design used must be described in such a way that other workers can repeat it. This is particularly important whenever a specific term is used to denote a particular type of imprecision, such as within-day, between-day, or between-laboratory."
To establish the **within-series imprecision** one person performs several successive analytical determinations on a ready-made sample pool using the same reagents, instruments, and technical aids. Within-series imprecision is a measure for the reproducibility of an individual determination under identical conditions. The length of the series should be set so that the possibility of time-dependent effects can be excluded, but the number of analyses *(n)* should be at least 10. Ideally this check on imprecision should be carried out at three different concentrations, chosen to encompass as much of the linearity range of the method as possible. Preferably the specimens should be from donors who have been exposed to the hazardous substance in question.
To establish the **between-day imprecision** determinations on the same material are carried out on different days. The preparation of the sample pool should be described in detail, and information should be provided as to whether the same reagents, standards, and instruments were used and whether all determinations were performed by the same person.
The **between-laboratory imprecision** is given for some of the methods. In these cases samples from the same pool were analyzed by workers in different laboratories.
Imprecision is defined by the relative standard deviation s and the prognostic range u. The **relative standard deviation** s is the standard deviation relative to the mean and is given in percent.
The **prognostic range** u defines an interval that contains the analytical result for an identical sample with a probability $P = 95\%$. Due to practical considerations the prognostic range u is given in relative units (percent) with respect to the analytical results. The prognostic range u is determined by the Student's t_p factor for $P = 95\%$ and the standard deviation s of the complete method, which can be calculated in two ways:

1. By replicate analyses at a given analyte concentration (standard deviation s_w)

$$s_w = \sqrt{\frac{\sum_{i=1}^{n} (c_i - \bar{c})^2}{n-1}} \cdot \frac{1}{\bar{c}}$$

where

n = number of analyses
c_i = analytical result of the i^{th} analysis
$\bar{c}$ = mean of n analytical results

2. By double analyses at different concentrations within a defined concentration range (standard deviation s_d)

$$s_d = \sqrt{\frac{\sum_{i=1}^{n} (c_{i1} - c_{i2})^2}{2z}} \cdot \frac{1}{\bar{c}}$$

where

z = number of double analyses
c_{i1}, c_{i2} = results of the i^{th} double analysis
$\bar{c}$ = mean of the $2z$ analytical results

For the first case (replicate analyses) the prognostic range is given by:

$u = t_p \cdot s_w$

For the second case (double analyses) the prognostic range is given by:

$u = t_p \cdot s_d$

where t_p = Student's t factor for $P = 95\%$.
The effect of the number of analyses, n or z, on the Student's t factor for $P = 95\%$ is shown in the following table.

$n-1$ or z	5	10	15	20	25	30	35	50	60
t_p	2.57	2.23	2.13	2.09	2.06	2.04	2.03	2.01	2.00

Since imprecision can be effected not only by the chemical preparation of the biological material and the physical technique used but also by individual variations in the composition of the sample matrix, the second method for calculating imprecision is preferable. The biological samples from exposed donors should cover as much as possible of the range of concentrations relevant to occupational medicine.

Inaccuracy

The inaccuracy of a method is defined by the deviation of the analytical results from the true value. It is a measure for the agreement between the amount of substance actually present and the amount determined by the analysis. The IFCC gives the following definition for inaccuracy:
"Inaccuracy: Numerical difference between the mean of a set of replicate measurements and the true value. This difference (positive or negative) may be expressed in the units in which the quantity is measured, or as a percentage of the true value."
The following ways of determining the inaccuracy of a method are arranged in descending order of validity as applied to this criterion:

- Comparison of results from the method in question with results from a definitive method.
- On the basis of reference materials having a certified value for the concentration of analyte in the matrix to be analyzed.
- Comparison of results from the method in question with results from a second method that differs as much as possible from the first in all procedural steps.

- By recovery experiments. In this case the analyte must be added to the biological material in at least three concentrations, covering the concentration range of interest. The analysis must be based on aqueous calibration standards, and the experimental conditions should be described in detail.

Detection limit

A generally applicable definition of the limit of detection must be based on mathematical statistics, involving a "signal-to-noise ratio" (in a generalized sense).
At very low concentrations it is uncertain whether an observed value is due to the presence of the substance to be determined or to uncontrolled chance perturbations. Perturbations can be caused by umpurities in reagents, losses from sputtering or absorption, weighing or titration errors, temperature fluctuations in light sources, secondary reactions, thermal electronic noise in amplifiers, observational error, and contamination. **All** such uncontrolled fluctuations are subsumed under the concept of **"analytical noise".** This "analytical noise" can be grasped only in statistical terms. For example, it is essentially the scatter of the **"blank measures"** from "blank analyses" and can be numerically described by the standard deviation $s_{bl,abs}$ of the blank measures.
Because the blank scatter of an analytical procedure is a very complex phenomenon, it cannot be calculated by theory. However, in practice it can be found **experimentally** by making a sufficiently larger number of blank analyses (at least 20) and then treating the measured x_{bl} statistically, i.e., the mean x_{bl} and the absolute standard deviation $s_{bl,abs}$ are calculated.
Whether an observed value of the measure x can be accepted as genuine or whether it must be rejected because it is suspected to be only an accidentally high blank measure is decided according to the following inequality:

$$x \geq \bar{x}_{bl} + 3\, s_{bl,abs}$$

The detection limit can be read from the calibration curve as the concentration, corresponding to the lowest observed measure, that still satisfies this inequality. This is, therefore the **lowest concentration that is acceptable with the given analytical procedure.**
This definition of detection limit may appear questionable when the observed reagent blank values are of the same order of magnitude as the concentrations to be measured in the biological material. The Working Group "Analytical Chemistry" of the Commission of the Deutsche Forschungsgemeinschaft for the Investigation of Health Hazards of Chemical Compounds in the Work Area is considering this problem and discussing a redefinition of detection limit as the lowest still detectable concentration. This would be accompanied by the introduction of the concept *"determination limit"* to designate the lowest concentration that can still be determined with the degree of reliability that characterizes the method.

Specificity

Specificity refers to the power of a method to differentiate one chemical compound from other potentially detectable compounds. The specificity of an analytical method depends heavily on the type of chemical procedure used for sample preparation and on the physicochemical principles of the method.

In 1980 the IFCC gave the following definition for specificity:
"Specificity: The ability of an analytical method to determine solely the component(s) it purports to measure. It has no numerical value. It is assessed on the evidence available on the components that contribute to the result and on the extent to which they do."

Statistical quality control

Statistical quality control in the Federal Republic of Germany follows the guidelines established by the Bundesärztekammer (German Medical Association) in 1971. Similar recommendations have also been formulated by other countries.
Detailed procedural directives can be found in, e.g., Regulation TRgA 410 of the ArbStoffV; Richtlinien der Bundesärztekammer (1971); and *Angerer* and *Schaller* (1976).
Quality control provides for:

internal quality control: the continual monitoring of
- imprecision
- inaccuracy

external quality control: analysis of the same specimens by different laboratories to check
- inaccuracy

Internal quality control. A control sample, whose analyte concentration is known to the analyst, is processed with each analytical series. These control values are recorded in a control chart with their mean and the two- and threefold standard deviations of the mean (Fig. 1). A control sample with an analyte concentration known only to the director of the laboratory is analyzed after every fourth series.

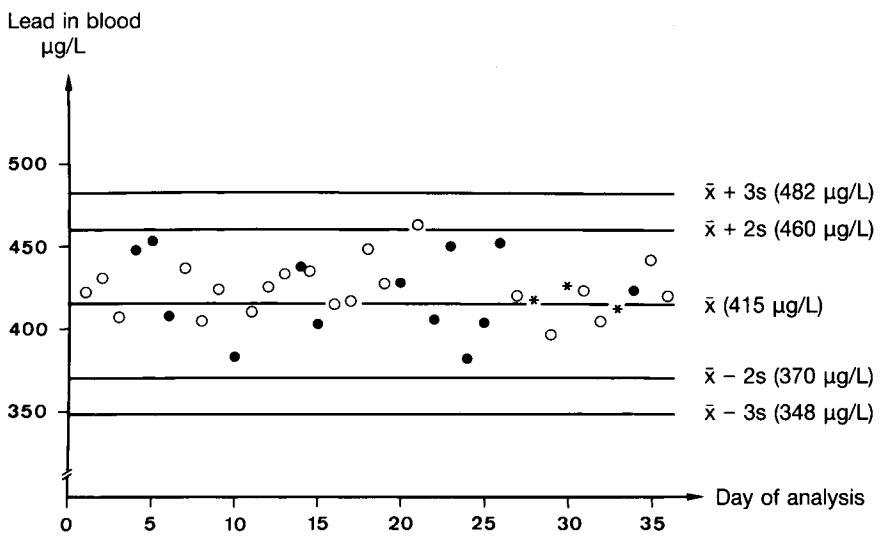

Figure 1. Control chart (Determination of lead in blood, see p. 161, internal quality control).

External quality control. The inaccuracy of the results is checked by round-robin experiments on the national and international level in which the same specimens are analyzed by different laboratories. The analyte concentration of these circulating specimens is known only to the organizer of these experiments.
In the Federal Republic of Germany statistical quality control falls under the jurisdiction of the Arbeitsstoffverordnung (Code on Hazardous Working Materials), TRgA 410 (Regulation 410). Control materials are commercially available for these purposes in concentration ranges relevant to environmental and occupational medicine (Behring, Marburg).
When no control material is commercially available for the determination of a particular parameter, control samples must be prepared in the laboratory from pooled biological material. A year's supply of such samples can be made up at one time and stored in the deep-freeze until needed.

Evaluation and interpretation of analytical results in biological material

In the evaluation and interpretation of analytical results in occupational medicine and toxicology certain particulars specific to these fields must be taken into consideration. An example of this is the variation from individual to individual of sensitivity and metabolizing ability. In addition to such individual-specific parameters, in occupational medicine an evaluation is based on biological limit values. These are set on the basis of the relationships between external exposure to internal exposure or between internal exposure to effects. In the Federal Republic of Germany this has been codified as the BAT value (Biological Tolerance Value for Working Materials).
The BAT values are based on the Arbeitsmedizinisch-toxikologische Begründung von BAT-Werten (Occupational Hygiene and Toxicological Justifications of BAT Values) published by the Commission of the Deutsche Forschungsgemeinschaft for the Investigation of Health Hazards of Chemical Compounds in the Work Area. A list of updated BAT values is put out yearly.

Supplementary literature:

General:
- *A. Berlin, R. E. Yodaiken,* and *D. C. Logan:* Summary report of the international seminar on the assessment of toxic agents at the workplace. Roles of ambient and biological monitoring, Luxembourg, 8 – 12 December, 1980. Int. Arch. Occup. Environ. Health *50, 197 – 207* (1982)
- *R. Roi, W. G. Town, W. G. Hunter,* and *L. Alessio:* Occupational health guidelines for chemical risk. Commission of the European Communities. EUR 8513, Brüssel/Luxemburg, 1983
- *R. R. Lauwerys* (ed.): Industrial chemical exposure: Guidelines for biological monitoring. Biomedical Publications, Davis, California, 1983

Preanalytical phase:

- *J. Angerer, K. H. Schaller,* and *H. Seiler:* The pre-analytical phase of toxicological monitoring examinations in occupational medicine. TrAC (Trend in Analytical Chemistry) *2,* 257–261 (1983)

Reliability criteria:

- *J. Büttner, R. Borth, J. H. Boutwell, P. M. G. Broughton,* and *R. C. Bowyer* (International Federation of Clinical Chemistry, Committee on Standards, Expert Panel on Nomenclature and Principles of Quality Control in Clinical Chemistry): Approved recommendation (1978) on quality control in clinical chemistry. Part 1. General principles and terminology. J. Clin. Chem. Clin. Biochem. *18,* 69–77 (1980)
- *International Union of Pure and Applied Chemistry,* Analytical Chemistry Division, Commission on Spectrochemical and Other Optical Procedures for Analysis: Nomenclature, symbols, units and their usage in spectrochemical analysis. II. Terms and symbols related to analytical functions and their figures of merit. Information Bulletin, Appendices on Tentative Nomenclature, Symbols, Units and Standards. Number 26, Oxford 1972

Statistical quality control:

- TRgA 410: Statistische Qualitätssicherung. Ausgabe April 1979. In: *W. Weinmann,* and *H.-P. Thomas:* Arbeitsstoffverordnung Teil 2: Technische Regeln (TRgA) und ergänzende Bestimmungen zur Verordnung über gefährliche Arbeitsstoffe. Carl Heymanns Verlag KG, Köln, 8th supplement 1982

- *J. Angerer* and *K. H. Schaller:* Erfahrungen mit der statistischen Qualitätskontrolle im arbeitsmedizinisch-toxikologischen Laboratorium. Arbeitsmed. Sozialmed. Präventivmed. *11,* 311–312 (1976) and *12,* 33–35 (1977)
- Richtlinien der Bundesärztekammer zur Durchführung der statistischen Qualitätskontrolle und von Ringversuchen im Bereich der Heilkunde. Deutsches Ärzteblatt, Heft 31, 2228–2231 (1971)
- *D. Stamm:* A new concept for quality control of clinical laboratory investigations in the light of clinical requirements and based on reference method values. J. Clin. Chem. Clin. Biochem. *20,* 817–824 (1982)

Evaluation and interpretation:

- *Deutsche Forschungsgemeinschaft:* Maximale Arbeitsplatzkonzentrationen und Biologische Arbeitsstofftoleranzwerte. Mitteilung XIX der Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe. Verlag Chemie, Weinheim 1983
- *D. Henschler* and *G. Lehnert (ed.):* Biologische Arbeitsstoff-Toleranz-Werte (BAT-Werte). Arbeitsmedizinisch-toxikologische Begründungen. Verlag Chemie, Weinheim, 1st edition 1983
- *American Conference of Governmental Industrial Hygienists:* Threshold limit values for chemical substances in the work environment, 1983–84. Cincinnati, U.S.A. 1983