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Analyses of Hazardous Substances in Biological Materials

Volume 1

edited by J. Angerer and K. H. Schaller
Working Group "Analytical Chemistry"

Commission for the Investigation of Health Hazards
of Chemical Compounds in the Work Area
(Chairman: D. Henschler)

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Preface

The Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area of the German Science Foundation carries the mandate of establishing Maximum Concentrations at the Work Place (MAK Values) and Biological Tolerance Values for Working Materials (BAT Values) in the Federal Republic of Germany. From the very beginning of this work, the need has been stressed to elaborate practical, sensitive and specific methods for the analytical control of concentrations of noxious materials in the workplace. In 1969 a special working group "Analytical Chemistry" began to tackle this formidable task. It established new, and standardized known, methods for monitoring air and biological materials. The results of these activities have been, and continue to be, published in a series of monographs of which the one presented here is concerned with methods for the detection of hazardous substances in biological materials. It provided the basis for the establishment of the new category of Biological Tolerance Values for Working Materials which was introduced into the German list of occupational exposure limits in 1981.

The Commission has always given preference to methods for the representative determination of profiles of exposure over time at the workplace before claims for simplicity and economic feasibility. This principle complies with the general attitude of the Commission to favour thorough scientific argumentation over administrative commodity. The selection of methods has always been governed by the general principles of occupational medicine. Recent developments confirm the early postulate of the Commission, in case of doubt to select the more demanding method.

D. Henschler
Chairman of the Commission for the
Investigation of Health Hazards of Chemical
Compounds in the Work Area

Foreword

Almost 30 years ago, the German Science Foundation created the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. With the specification and justification of maximum permissible concentrations of hazardous substances in the work area, this Commission was carrying on a tradition begun by toxicologists in the Federal Republic of Germany of setting limits (MAK values) for the concentration of chemical compounds in the air of a work area. Observance of these values is meant to ensure the health of the employees. Since 1981 the Commission has reinforced the MAK values with the Biological Tolerance Values for Working Materials (BAT values). These values limit the quantity of a chemical compound, its metabolic by-products, or any deviation from the norm of biological parameters induced by it. BAT values, thus, improve the chances of counteracting any potential damage caused by hazardous materials long before impairment of health occurs.

The Commission's early interest in reliable methods for monitoring these limit values led to the establishment in 1969 of the Working Group "Analytical Chemistry". The loose-leaf collections "Luftanalysen" (ambient monitoring) and "Analysen in biologischem Material" (biological monitoring) were first published in 1976 and have since been appended five and eight times, respectively. In the volume "Luftanalysen" methods for the determination of chemical compounds in the air of the work area are described. The volume "Analysen in biologischem Material" contains methods for the determination of chemical compounds, their metabolites, or metabolic parameters influenced by these substances in the human body that can serve as the basis for an estimate of the burden to which an individual has been exposed. Both volumes include only methods that are suitable for routine use, and the reliability of the analytical procedures is defined and checked. For these reasons and because they satisfy the requirements of statistical quality control, the analytical methods published by the Working Group "Analytical Chemistry" are recommended, by pertinent guidelines and regulations, for use in the Federal Republic of Germany.

In response to the worldwide demand for chemical methods for ambient and biological monitoring, the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has decided to make the German contributions in this field available to an international audience by bringing out an English edition.

As biological monitoring is accorded great importance for the prevention of chemically caused occupational diseases in Germany and other countries of the European Community, it was decided to start the English edition with a volume of "Analyses of Hazardous Substances in Biological Materials". Further volumes are planned to follow at short time intervals.

The methods included in this first volume should make clear the major emphasis of the Commission's work. In addition they should represent the methodological spectrum that is available today for the biological monitoring of occupationally exposed persons. More than half of the methods are concerned with the determination of carcinogenic substances and their metabolites. This reflects the point of view that biological monitoring has its greatest value where the risk posed by a hazardous substance is of utmost importance. In correspondence with conditions at the work place where exposure to more than one hazardous

chemical at a time is more often the rule than the exception, an effort has been made to include as many substances in a single analysis as possible, such as in the methods for determining aromatic amines, benzene derivatives, chlorobenzenes, and chlorophenols. The important role played at present by atomic absorption spectrometry as well as gas and liquid chromatography in the fields of occupational hygiene and toxicology comes through clearly. However, well-proven photometric methods such as the determination of phenol in urine or the Fujiwara method have not been excluded.

The description of each method includes an evaluation of the method, a brief listing of the reliability criteria, and general information on the chemical compound to be tested, i.e., its industrial importance, toxicity, metabolism, toxicokinetics, and its normal and limit values in biological material as far as they are known. This is followed by a detailed description of the preparatory and analytical steps, discussion of the reliability, and a reference list.

Besides the descriptions of analytical methods, this volume contains an introductory chapter on the way the Working Group "Analytical Chemistry" functions and a chapter on the particulars of analyses in biological materials.

A roster of the members and guests of the Working Subgroup "Analyses of Hazardous Substances in Biological Materials" is provided at the end of this volume.

We would like to thank the members and guests of the Working Subgroup "Analyses of Hazardous Substances in Biological Materials" without whose voluntary services this collection of methods would not have been possible. We thank the German Science Foundation, and Mr. Bretschneider in particular, for financial and organizational help in the development of this project. Our thanks go also to our publisher Dr. Giesler of the VCH Verlagsgesellschaft with whom we have enjoyed a long-standing, dependable, and efficient collaboration. We also wish to thank Dr. Heinrich and Mrs. Lübow from the secretariat of the Working Group for painstaking editing and typing of the manuscript of the English edition.

J. Angerer
Chairman of the Working Group "Analytical Chemistry" of the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area

J. Angerer K. H. Schaller
Leaders of the Working Subgroup "Analyses of Hazardous Substances in Biological Materials"

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1 Working Group "Analytical Chemistry" of the Commission of the German Science Foundation for the Investigation of Health Hazards of Chemical Compounds in the Work Area

Organization

The Working Group "Analytical Chemistry" was established in 1969.
Under the chairmanship of Priv.-Doz. Dr. J. Angerer at present it includes three Working Subgroups:

"Air Analyses"
(Leaders: Dr. Th. zur Mühlen and Prof. Dr. A. Kettrup)

"Analyses of Hazardous Substances in Biological Materials"
(Leaders: Priv.-Doz. Dr. J. Angerer and Chem.-Ing. K. H. Schaller)

"Measurement Strategy and Statistics"
(Leader: Dr. E. Drope)

The participants, who have been invited to collaborate on a Working Subgroup by the leaders, are experts in the field of technical and medical protection against chemical hazards at the work place.
A list of the members and guests of "Analyses of Hazardous Substances in Biological Materials" is given in Chapt. 5 of this volume.

Objectives and operational procedure

The two analytical subgroups are charged with the task of preparing methods for the determination of hazardous industrial materials in the air of the work place or to determine these hazardous materials or their metabolic products in biological specimens from the persons working there. Within the framework of the existing laws and regulations, these analytical methods are useful for ambient monitoring at the work place and biological monitoring of the exposed persons.
In addition to working out the analytical procedure, these subgroups are concerned with the problems of the preanalytical phase (specimen collection, storage, transport), the statistical quality control, as well as the interpretation of the results.
The Working Subgroup "Measurement Strategy and Statistics" develops proposals on the manner of specimen collection and on the statistical treatment of the results with the aim of making possible a reliable assessment of the situation at the work place.

Development, examination, release, and quality of the analytical methods

In its selection of suitable analytical methods, the Working Group is guided mainly by the relevant scientific literature and the expertise of the members and guests of the Working Subgroup. If appropriate analytical methods are not available they are worked out within

the Working Group. The leader designates an author, who assumes the task of developing and formulating a method proposal. The proposal is examined experimentally by at least one other member of the project, who then submits a written report of the results of the examination. As a matter of principle the examination must encompass all phases of the proposed analytical procedure.
The examined method is then laid before the members of the subgroup for consideration. After hearing the judgement of the author and the examiner they can approve the method. The method can then be released for publication after a final meeting of the leader of the Working Group "Analytical Chemistry" with the subgroup leaders, authors, and examiners of the method.
Under special circumstances an examined method can be released for publication by the leader of the Working Group after consultation with the subgroup leaders.
Only methods for which criteria of analytical reliability can be explicitly assigned are released for publication. The values for inaccuracy, imprecision, detection limits, sensitivity, and specificity must fulfill the requirements of statistical quality control as well as the specific standards set by occupational health. The above procedure is meant to guarantee that only reliably functioning methods are published, which are not only reproducible within the framework of the given reliability criteria in different laboratories but also can be monitored over the course of time.
In the selection and development of a method for determining a particular substance the Working Group has given the analytical reliability of the method precedence over aspects of simplicity and economy.

Publications of the working group

Methods released by the Working Group are published in the Federal Republic of Germany by the German Science Foundation as a loose-leaf collection entitled "Analytische Methoden zur Prüfung gesundheitsschädlicher Arbeitsstoffe" (Verlag Chemie, Weinheim, FRG). The collection at present consists of two volumes:
Volume I "Luftanalysen"
Volume II "Analysen in biologischem Material"
These methods are also to be published in an English edition. The work at hand represents the first English issue of "Analyses of Hazardous Substances in Biological Materials".

Withdrawal of methods

An analytical method that is made obsolete by new developments or discoveries in the fields of instrumental analysis or occupational health and toxicology can be replaced by a more efficient method. After consultation with the membership of the relevant project and with the consent of the leader of the Working Group, the subgroup leader is empowered to withdraw the old method.

2 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 German Science Foundation, 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 or 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

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 German Science Foundation 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:

$$H: = \frac{\mathrm{d}x}{\mathrm{d}c}$$

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)