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Analyses of Hazardous Substances in Biological Materials

Volume 6

edited by Jürgen Angerer and Karl-Heinz Schaller
Working Group Analytical Chemistry

Commission for the Investigation of Health Hazards
of Chemical Compounds in the Work Area
(Chairman: Helmut Greim)

WILEY-VCH

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Preface

The arrangement of Volume 6 of Analyses of Hazardous Substances in Biological Materials is similar to that of the previous volumes in this series. It contains a contribution on the use of a special method of instrumental analysis (ICP/MS) and 11 analytical methods for the determination of working substances in human body fluids.

These analytical methods used within the scope of 'biological monitoring' facilitate the estimation of the exposure and the inner stress caused to humans by chemical substances. The special merit of the analytical methods described here lies in the fact that their analytical reliability and their reproducibility have been checked. Thus, these procedures contribute to the goal of achieving comparable measurement results from one laboratory to another, even across international borders. The comparability of measurement results is a prerequisite for the evaluation of the results with regard to their significance for health. Keeping this in mind and in view of the fact that the European countries are growing closer together, it is of great importance that this series is not only published in the German language but also in English. This is all the more significant as the member states of the European Union attach an ever growing importance to biological monitoring.

The Deutsche Forschungsgemeinschaft's Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, of which I am the chairman, recognized the importance of biological monitoring at a very early stage. This applies not only to the range of interest to occupational medicine, but also the range of interest to environmental medicine. Today we view with satisfaction the considerable progress we have made on the way to our goal. In this connection my special thanks are due to the Deutsche Forschungsgemeinschaft, which has lent its support to this collection of methods for almost three decades.

I would also like to express my gratitude to Professor Angerer, who has headed the "Analytical Chemistry" Working Group for many years, for his successful work. Similarly, I extend my thanks to the members and guests of the working group who have contributed to this work in a multitude of different ways.

H. Greim
The Chairman of the Deutsche Forschungsgemeinschaft's
Commission for the Investigation of Health Hazards
of Chemical Compounds in the Work Area

Foreword

This volume contains an introductory chapter on the use of a special method of instrumental analysis in the field of biological monitoring: ICP/MS (inductively coupled plasma/mass spectrometry). This chapter, which has been prepared by scientists with extensive experience in this field, is of particular relevance due to its consideration of the practical aspects of biological monitoring.

In addition, this volume contains 12 analytical methods for the detection of organic and inorganic substances in human body fluids. As always, these methods have been thoroughly checked for their analytical reliability and reproducibility, and the form in which they are presented enables them to be directly duplicated. Wherever the state of instrumental analysis permits, these methods are set out so that it is possible to detect the concentration range of interest to environmental medicine in addition to the range of interest to occupational medicine. Typical examples are the methods for the determination of metals by means of ICP/MS and the determination of the PAH metabolites in urine by means of HPLC. The latter method has proved to be of such sensitivity and reproducibility that it can be used to estimate the health risk to the general population posed by these important carcinogens. It is also important to highlight the fact that this volume contains three methods for the determination of plant protection agents and their metabolites in body fluids (amitrole, 2,4-diamino-6-chloro-s-triazine, and pyrethroid metabolites). The latter substances can also be detected at the concentrations of interest to environmental medicine. Thus, we are gradually nearing our goal: to make biomonitoring available for the screening of plant protection agents.

In the meantime, this collection of methods has gained recognition as a standard publication for occupational and environmental-toxicological analysis in Germany. As the use of biological monitoring grows, we hope that the English version will also become more widely distributed.

Since publication of the last volume several colleagues who made important contributions to this work over a period of many years have left the Analysis in Biological Materials working group. I wish to thank Dr. Bencze (Munich), Dr. Fleischer (Saarbrücken), Prof. Dr. Goenechea (Bonn), Dr. Hörmann (Basel), Prof. Dr. Machata (Vienna) and Prof. Dr. Seiler (Basel) for their many contributions and for their commitment and friendly co-operation. My thanks are also due to all the members and guests of the working group who have contributed to this volume. I am deeply indebted to the Deutsche Forschungsgemeinschaft which has supported our work for almost three decades and has provided the basis which has enabled us to quantify and assess exposure to chemical substances. This support cannot be esteemed highly enough at a time in which the actual or putative risk posed to health from chemical substances is in the forefront of public attention. I would like to express my special thanks to Dr. Konze-Thomas who is always ready to provide her support within the Deutsche Forschungsgemeinschaft. I am very grateful to Dr. Gündel for his help in coordinating the working group and preparing this volume for publication. Finally,

and thus in the most elevated position, my sincere thanks are due to Prof. Greim, the chairman of our Commission, who has always regarded analysis as an integral part of the Commission's work and who has ensured that we have been able to work in a committee which has been characterised by exemplary interdisciplinary co-operation for four decades.

J. Angerer
Chairman of the "Analytical Chemistry" working group
of the Deutsche Forschungsgemeinschaft's Commission
for the Investigation of Health Hazards
of Chemical Compounds in the Work Area

Working Group Analytical Chemistry of the Commission of the Deutsche Forschungsgemeinschaft for the Investigation of Health Hazards of Chemical Compounds in the Work Area

Organization

The Working Group Analytical Chemistry (Chairman: J. Angerer) was established in 1969. It includes two Working Subgroups: Air Analyses (Leader: A. Kettrup), Analyses of Hazardous Substances in Biological Materials (Leaders: J. Angerer, K. H. Schaller). 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 workplace.
A list of the members and guest of Analyses of Hazardous Substances in Biological Materials is given at the end 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.

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 Deutsche Forschungsgemeinschaft as a loose-leaf collection entitled "Analytische Methoden zur Prüfung gesundheitsschädlicher Arbeitsstoffe" (WILEY-VCH, Weinheim, FRG). The collection at present consists of two volumes:
Volume I: Luftanalysen
Volume II: Analysen in biologischem Material.
These methods are published in an English edition. The work at hand represents the sixth 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.

Terms and symbols used

Terminology

Accuracy – the agreement between the best estimate of a quantity and its true value. It has no numerical value.
Analyte – the component to be measured.
Assigned value – the concentration of the analyte in the control specimen assigned either arbitrarily, e.g., convention, or from preliminary evidence, e.g., in the absence of a recognized reference method.

Biological Tolerance Value for a Working Material (BAT) – 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 exposed humans. According to current knowledge these conditions generally do not impair the health of the employee, even if exposure is repeated and of long duration. As with MAK values, the maximum period of exposure to a working material is generally given as eight hours daily and 40 hours weekly.
Blank value – the analytical result obtained when the complete procedure is carried out on ultrapure water containing no analyte instead of biological specimens.

Calibration standards – specimens with known concentrations of the analyte that are used for calibration.
Certified value – the concentration of the analyte in control specimens certified by an official body subject to conditions established by that body.
Control material – a material that is used solely for quality control purposes and not for calibration.

Definitive method – the analytical method of all the methods for determining the analyte that is capable of providing the highest accuracy. Its accuracy must be adequate for its stated purposes.
Definitive value – the concentration of analyte in control specimens determined by a definitive method. It is the best available estimate of the true value.
Detection limit – the minimum analytical result that is still clearly detectable and distinguishable from the background noise; defined as three standard deviations of the appropriate blank value.

External quality control – a procedure of utilizing for quality control purposes the results of analyses performed on the same specimen or specimens by several laboratories.

Imprecision – the standard deviation or coefficient of variation of the results in a set of replicate measurements. A distinction is made between within-series, between-day, and interlaboratory imprecision.
Inaccuracy – the numerical difference between the mean of a set of replicate measurements and the true value.

Influence factors – lead to changes in vivo in the clinical chemical parameter. Their influences are independent of the specificity of the analytical method.
Interference – the effect on the accuracy of measurement of one component caused by another component that does not itself produce a reading.
Interference factors – all factors that alter the result in vitro, i.e., after the specimen has been collected from the patient.
Internal quality control – the procedure of utilizing the results of only one laboratory for quality control purposes. It includes the control of imprecision as well as inaccuracy.

Maximum Concentration Value at the Workplace (MAK) – is defined as the maximum permissible concentration of a chemical compound present in the air within a working area (as gas, vapor particulate matter) which, according to current knowledge, generally does not impair the health of the employee nor cause undue annoyance. Under these conditions, exposure can be repeated and of long duration over a daily period of eight hours, constituting an average work week of 40 hours (42 hours per week as averaged over four successive weeks for firms having four work shifts).
Measure – the measured change of one parameter of the analyte in the physical or chemical system used for analysis.

Preanalytical phase – the period from the specimen collection to the aliquotation (sampling) of the biological specimens.
Precision – the agreement between replicate measurements. It has no numerical value.
Prognostic range – an interval that with a given probability includes the analytical result from an identical specimen.

Quality control – the study of those errors that are the responsibility of the laboratory and the procedures used to recognize and minimize them. This study includes all errors arising within the laboratory during the time from aliquotation of the specimen to dispatch of the report.

Recovery rate – in recovery experiments the amount of recovered analyte divided by the amount of added analyte expressed as a percentage.
Reference material – a material or substance for which one or more properties are sufficiently well established to be used for calibration of an apparatus or for verification of an analytical method.
Reference method – an analytical method whose inaccuracy and imprecision are small enough as demonstrated by direct comparison with the definitive method and show low incidence of susceptibility to known interferences is so well documented that the stated aims of the reference method may be achieved.
Reference method value – the most probable value derived from a set of results obtained by the most reliable reference methods available.
Reliability criteria – defined quantifiable parameters for the assessment of the quality of an analytical method, e.g., imprecision, inaccuracy, detection limit.

Sample – that appropriate representation portion of a specimen used in the analysis.
Sensitivity – the differential quotient of the calibration function.
Selected method – routine method with known systematic error.
Specificity – the ability of an analytical method to determine solely the component or components it purports to measure. It has no numerical value.
Specimen – the material available for analysis.

Symbols

c	substance concentration of analyte
$\bar{c}$	mean substance concentration of analyte
E	*extinction*
k'	reciprocal calibration factor
m	mass
M	molar mass
n	number
p	pressure
P	probability
r	recovery rate
s	relative standard deviation
$s_{\mathrm{bl,abs}}$	standard deviation of the blank value
s_{d}	relative standard deviation derived from duplicate analyses
s_{w}	relative standard deviation derived replicate analyses of the same specimen
t_{p}	Student's t factor
T	thermodynamic temperature
u	prognostic range
V	volume
V_{m}	molar volume
x	observed measure of analyte
$\bar{x}$	mean
x_{bl}	observed measure of blank
z	number of duplicate analyses
H	sensitivity
ϱ	mass concentration
σ	volume concentration

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