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Review

# Quality assurance of biological monitoring in occupational and environmental medicine

K.H. Schaller*, J. Angerer, H. Drexler

*Institute of Occupational, Social and Environmental Medicine, University Erlangen–Nuremberg, Schillerstrasse 25 und 29, 91054 Erlangen, Germany*

**Abstract**

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. In environmental medicine biological monitoring plays also an important role in the assessment of excessive, acute or chronic exposure to chemical agents. To guarantee that the results obtained in biological monitoring are comparable with threshold limit values and results from other laboratories, the analysis must be carried out with tested and reliable analytical methods and accompanied by a quality assurance scheme. Confounding influences and interferences during the pre-analytical phase can be minimised by recommendations from experienced laboratories. For internal quality control commercially available control samples with an assigned concentration are used. External quality control programs for biological monitoring are offered by several institutions. The external quality control program of the German Society of Occupational and Environmental Medicine has been organised since 1982. In the meantime the 27th program has been carried out offering 96 analytes in urine, blood and plasma for 47 substances. This program covers most of the parameters relevant to occupational and environmental medicine. About 350 laboratories take part in these intercomparison programs. At present, ten German and 14 international laboratories are commissioned to determine the assigned values. The data evaluated from the results of the intercomparison programs give a good overview of the current quality of the determination of analytes assessed in occupational and environmental toxicological laboratories. For the analysis of inorganic substances in blood and urine the tolerable variation ranges from 7.5 to 43.5%. For organic substances in urine the tolerable variation ranges from 12 to 48%. The highest variations (36–60%) were found for the analysis of organochlorine compounds in plasma. The tolerable variations for the determination of solvents in blood by head space gas chromatography range from 26 to 57%. If the recommendations for the pre-analytical phase, the selection of reliable analytical methods by the laboratory and the carrying out of adequate quality control are observed, the pre-requisites for reliable findings during biological monitoring are fulfilled


*Keywords:* Reviews; Quality control; Biological monitoring

## Contents



*Corresponding author. Tel.: +49-913-1852-6127; fax: +49-913-1852-2317.
*E-mail address:* kh.schaller@rzmail.uni-erlangen.de (K.H. Schaller).

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## 1. Introduction

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. In environmental medicine biological monitoring plays also an important role in the assessment of excessive, acute or chronic exposure to chemical agents.

One of the essential elements of biological monitoring is the analytical determination of parameters in biological material, mainly blood, plasma and urine. Not only the levels of hazardous substances and their metabolites can be considered as parameters, but also effects of the substances on the human organism.

Assessment of the parameters includes the preanalytical phase, i.e. sample collection, storage and transport, through to the analytical determination. To guarantee that the results obtained in biological monitoring are comparable with threshold limit values and results from other laboratories the analysis must be carried out with tested and reliable analytical methods and accompanied by a quality assurance scheme. This paper deals mainly with quality assurance, but some aspects of the preanalytical and analytical phase should be mentioned very briefly.

## 2. Preanalytical phase

The pre-analytical phase, which is the responsibility of the works medical department, plays an important role for the quality of the results revealed in biological monitoring. In all steps before the actual analysis, from the time of sampling the material until removal of a sample from the matrix, confounding influences and interference factors can falsify the results and make interpretation more difficult or even impossible. The main in vivo factors that influence the results are the time of sampling and the great variations in the composition of spontaneous urine samples as a result of diuresis. The most important in vitro interference factors are:

- Exogenous contamination of the material (at the site of sampling)
- Contamination of the material from the sampling utensils or the vessels used
- Evaporation of volatile components from the material
- Absorption of the components to be analysed onto the walls of the vessels used
- Changes in the samples during storage and transport (e.g. coagulation of blood samples, sedimentation of urinary components, chemical changes in the parameters).

This means that interference can occur during sampling, transport of the samples—which is usually by post or courier—and their subsequent storage in the laboratory. The pre-analytical phase therefore encompasses the time of sampling, correct sampling, the choice of vessel, and transport and storage of the samples. Usually the works medical department oversees that these steps in the procedure towards obtaining toxicological–medical findings are carried out correctly. Possible confounding influences or interference factors cannot be recognized by quality control. The pre-analytical phase must therefore be co-ordinated with the laboratory before the beginning of an investigation. It is recommended that the services generally offered by the analytical laboratories should be taken advantage of to provide sampling utensils, transport vessels and information on sampling. This is particulary important if specially cleaned vessels or special containers are necessary [1]. Recommendations from experienced laboratories and close cooperation with the laboratories entrusted

with carrying out the analyses can guarantee that confounding influences and interferences during the pre-analytical phase can be minimised [2].

## 3. Analytical phase

Specific and sensitive analytical methods with defined and tested reliability criteria are indispensable for carrying out the analyses necessary for biological monitoring [2]. They must be reproducible, be leading to reliable and comparable results, and also be practicable, so they can be used in routine investigations. For the terminology of the analytical reliability criteria, refer to the International Organisation for Standardization [3,4] and endorsed by the International Union of Pure and Applied Chemistry (IUPAC) and the Association of Official Analytical Chemists (AOAC) [5]. When planning the use of analytical methods, one must consider its practicability, i.e. the speed of the method, the equipment, the technical skill required, the precautions and procedures required for safety, the workload, specimen handling, the cost, and the space needed [6].

The complex matrix of biological media and the extremely low concentrations of the parameters usually demand a complicated instrumental analysis. The state of the art is represented by the determination of polychlorinated dibenzodioxins as examples of organic substances and platinum as an example of inorganic substances. These chemicals can be determined in body fluids at concentrations as low as 1 pg/l [7] and 1 ng/l [8], respectively. Mass spectrometric analysis, combined with gas chromatography, liquid chromatography, and ICP–MS will gain further use in biological monitoring. There are also indications that immunologic methods will become routine in biological monitoring [9].

Therefore, biological monitoring can be carried out only in specialized laboratories, which are responsible for the analytical reliability of the results.

The working group ''Analyses of Hazardous Substances in Biological Materials'' of the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area of the Deutsche Forschungsgemeinschaft (DFG) has published a collection of tested methods and analytical procedures for toxicological analysis of human biological materials.

Table 1 shows the analytical procedures contained in this collection of methods, listed according to analytical technique and class of substance. About 120 methods are available for determining about 180 parameters [10,11].

## 4. Quality assurance

Quality assurance is an important feature of all analytical work and it comprises several features that are common to all chemical analysis.

WHO [12,13] has made efforts to define and unify the use of the terminology concerning quality of health and environment-related laboratories. 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 [14]. 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, interferences [15]) and subsequent quality assessment.

Quality assessment refers to the quality of the analytical results. It has two components: 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 the laboratory performance by an external agency or institution [12].

### *4.1. Internal quality control*

Internal laboratory quality control is the systematic monitoring of precision under repeated conditions to

Table 1
Methods and spectrum of parameters in the series ''Analyses of Hazardous Substances in Biological Materials''

| | Inorganic substances | Organic substances (individual components and substance classes) | Parameters of effect |
|---|---|---|---|
| Parameters to be analysed | Metals:<br>Al, As, Ba, Be, Cd, Cr, Co<br>Cu, Hg, In, Mn, Ni<br>Pb, Pt, Sb, Se, Sn, Sr<br>Ti, Tl, V, Zn<br>Anions:<br>$CN^-$, $F^-$, $Br^-$ | Hemoglobin adducts (e.g. hydroxyethylvaline)<br>Aromatic amino and nitro compounds<br>Solvents<br>Solvent metabolites<br>Organochlorine compounds<br>PAH metabolites (e.g. 1-hydroxypyrene)<br>Pesticide metabolites (pyrethroids, carbamates, phenoxy-carbonic acids, organo-phosphoric acid esters)<br>Phenols | Acetylcholinesterase<br>β2-Microglobulin<br>Cholinesterase<br>CO-Hb<br>δ-Aminolevulinic acid<br>δ-Aminolevulinic acid dehydratase<br>Erythrocyte porphyrins<br>Methaemoglobin |
| Analytical techniques | F-AAS, GF-AAS, Hydride-AAS, Cold vapour AAS, Inverse voltammetry, ICP–OES, ICP–MS Ion-selective electrodes | GC–FID, GC–ECD, GC–TID, GC–MS, Head-space gas chromatography, HPLC, Photometry, Fluorimetry | Fluorimetry, Photometry, Gas chromatography, Enzyme-linked immunoassay, Radioimmunoassay |

determine random errors and the accuracy of quantitative laboratory investigations. It includes a check of precision for every series of analyses and accuracy after every fourth series. In practice it is carried out with a control sample system. The material used for internal quality control should have a matrix similar, if not identical, to that of the routine samples. The control material should be commercially available. These are control materials with an assigned analyte content and with a constant analyte content for ''daily use'' during precision and accuracy checks.

Standard reference materials are samples whose quantitative composition of certain components has been determined using various methods and by qualified laboratories. They are accompanied by a certificate stating the concentration of these components. Reference materials with certified values are offered, for example, by the Community Bureau of Reference (CBR) of the Commission of the European Union, by the American National Institute of Standards and Technology (NIST) and by the International Atomic Energy Agency (IAEA). In the programs of these institutions there are only very few materials with parameters relevant to occupational medicine. In addition, this material is very expensive due to the complicated certification procedure. It is therefore not used for routine accuracy control but during validation of an analytical procedure [16].

Usually, therefore, commercially available control samples with an assigned concentration are used for routine internal quality control. Table 2a–c show the current commercially available control samples. These control samples are sold freeze-dried and have to be reconstituted.

For occupational–medical parameters not contained in these commercially available control samples, control materials that have been produced by the laboratory itself can be used for internal quality control. Animal or human blood, serum or urine are spiked with defined concentrations of the analytes. These samples are in liquid form and aliquots are stored deep-frozen until analysis [17].

When preparing such controls all efforts should be made to ensure the non-infectivity of the control materials.

The results of daily internal quality control are registered on control cards and compared with the standard deviation for this method. The standard

Table 2
Spectrum of parameters in commercially available control materials for daily use: (a) whole blood, (b) serum/plasma, and (c) urine

| Trade name | Matrix | *N* | Parameter | Number of concentration settings |
|---|---|---|---|---|
| (a) *Whole blood* | | | | |
| RECIPE Chemicals and Instruments, Munich, Germany | | | | |
| ClinChek® Whole blood control, lyoph. | Animal blood | | | |
| For trace elements (order no. 8840–8843) | | 7 | Cd, Co, Cr, Hg, Mn, Ni, Pb | 3 |
| For organochlorine compounds (order no. 8863–8866) | | 4 | *p,p*-DDE, HCB, γ-HCH, PCBs (28,52,101,138,153,180) | 3 |
| BioRad Lab, Munich, Germany | | | | |
| Lyphocheck®, lyoph. | Blood | 1 | Pb | 3 |
| Nycomed, Oslo, Norway | | | | |
| Seronorm, for trace elements, whole blood, lyoph. | Blood | 8 | Cd, Co, Cr, Hg, Pb, Mn, Ni, Se | |
| (b) *Serum/plasma* | | | | |
| RECIPE Chemicals and Instruments, Munich, Germany | | | | |
| ClinChek® Whole blood control, lyoph. | | | | |
| For trace elements (order no. 8883–8885; 8880–8882) | Human plasma | 12 | Al, As, Cd, Cr, Co, Cu, $F^-$, Fe, Mn, Ni, Se, Zn | 2 |
| For organochlorine compounds (order no. 8860–8862, (8889)) | Animal serum | 6 (7) | *p,p*-DDE, HCB, (α-), β-, γ-HCH, PCP, PCBs (28,52,101,138,153,180) | 3 |
| Nycomed, Oslo, Norway | | | | |
| Seronorm, for trace elements, serum, lyoph. | Serum | 9 | Al, Cr, Co, Cu, $F^-$, Mn, Ni, Se, Zn | 1 |
| (c) *Urine* | | | | |
| RECIPE Chemicals and Instruments, Munich, Germany | | | | |
| ClinChek® Urine control, lyoph. | | | | |
| For trace elements (order no. 8847–8849) | Human urine | 15 | Al, Sb, As, Pb, Cr, Co, Cu, Mn, Ni, Hg, Pt, Tl, Zn, $F^-$ | 2 |
| For organochlorine compounds (order no. 8867–8869) | | 17 | Cotinine, nicotine, 2,5-dichlorophenol, HA, 1-HP, *o*-cresol, MA, MHA, *t,t*-MCA, PCP, phenol, PGA, TCA, pyrethroid metabolites ($Br_2$-CA, *cis*-$Cl_2$-CA, *trans*-$Cl_2$-CA, 3-PBA) | 2 |
| BioRad Lab, Munich, Germany | | | | |
| Lyphocheck®, lyoph. | Human urine | 22 | Al, Sb, As, Pb, Cd, Cr, Co, Cu, $F^-$, Hg, Mn, Ni, Se, Tl, Zn, ALA, HA, MA, PGA, PCP, phenol, TCA | 2 |
| Nycomed, Oslo, Norway | | | | |
| Seronorm For trace elements, urine, lyoph. | Human urine | 20 | Al, Sb, As, Pb, Cd, Cr, Co, Cu Hg, Mn, Ni, Tl, Zn, HA, MA, MHA, *t,t*-MCA, PCP, PGA, TCA | 1 |

deviation of the method is determined in a preliminary phase where control samples are analysed on 20 different working days. A result is correct if it does not differ from the actual value by more than three times of the relative standard deviation and if there are no trends towards higher or lower over a long period.

Modern laboratory data acquisition systems 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 acceptibility for control samples indicated and continuously updated.

### *4.2. External quality assessment*

External quality assessment, also called proficiency testing, is also part of the quality management and comprises methods of checking laboratory performance by interlaboratory tests.

External quality assessment on the basis of intercomparison programs serves as objective monitoring of the accuracy of the results of quantitative laboratory investigations under comparable conditions. Its aim is to guarantee the comparability of results from laboratory to laboratory and with the valid occupational–medical threshold values for tolerable exposures (e.g. Biological Tolerance Values at the Workplace (BAT), Exposure Equivalents for Carcinogenic Substances (EKA), Biological Exposure Indices (BEIs) or reference values for biological monitoring in environmental medicine).

The optimal structure of the quality control scheme for any one analyte is as follows [5]: (1) the co-ordinator organises 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.

An important feature of the scheme is the selection and preparation of the test material. The control 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 2,5-hexanedione) [16].

The external quality assessment scheme should provide the participant laboratories with an assigned (target) value for the analysis and criteria for acceptability (tolerance range) only after the analyses have been performed. The assigned values may be determined in general by a consensus from reference laboratories or by a consensus of participant laboratories.

Intercomparison programs for occupational–medical–toxicological investigations are organised by the following institutions:

- German Society for Occupational and Environmental Medicine e.V., organised by the Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, University Erlangen–Nuremberg, Erlangen, Germany.
- Institute of Occupational Health, Helsinki, Finland.
- Danish National Institute of Occupational Health, AME, Copenhagen, Denmark.
- Centre de Toxicologie du Québec, Toxicology Laboratory, Québec, Canada.

With the exception of the intercomparison programs organised by the German Society for Occupational and Environmental Medicine (Deutsche Gesellschaft für Arbeitsmedizin und Umweltmedizin), usually only metals or a limited number of

parameters are studied. Therefore, the German program should be presented in the following.

The external quality control program of the German Society for Occupational and Environmental Medicine has been organised since 1982. In the meantime the 27th intercomparison program has been carried out by this society. About 350 laboratories take part in these intercomparison programs. The high degree of acceptance is understandable when one bears in mind that a range of 96 parameters for 47 substances is offered, the most extensive quality control program in the field of toxicological analysis in biological materials currently offered world-wide. Table 3a,b show the spectrum of parameters offered in the 27th intercomparison program. This range covers most of the parameters relevant to occupational and environmental medicine. These intercomparison programs are held twice a year, in accordance with the recommendations of the German Federal Medical Council. Two concentration settings must be measured per parameter.

The assigned values are determined by reference laboratories. These laboratories are commissioned for toxicological analyses by the supervisor on the basis of their qualifications. These qualifications are apparent, e.g. from the observation that the laboratory or its head has evaluated methods for biological monitoring and published them in the international

Table 3
Spectrum of parameters intercomparison program 27/2001 of the German external quality assessment scheme: (a) occupational and (b) environmental medicine

| Control urine[a] | | Control blood[a] | | Control serum[a] | |
|---|---|---|---|---|---|
| Inorganic components | Organic components | Metals | Solvents | Organo-chlorine compounds | Metals |
| (a) *Occupational medicine* | | | | | |
| Al | δ-Aminolevulinic acid | Cd | *Aromatic* | DDT, *p,p′*-DDE | Al |
| As | Butoxyacetic acid | Co | *hydrocarbons* | HCB | Co |
| As-species | *o*-Cresol | Cr | | α-, β-, γ-HCH | Cr |
| Be | Ethoxyacetic acid | Hg | Benzene | PCBs | Cu |
| Cd | 2,5 Hexandione | Mn | Toluene | (6 congeners) | Fe |
| Co | Hippuric acid | Ni | Xylenes | PCP | Mn |
| Cr | 1-Hydroxypyrene | Pb | Ethylbenzene | | Ni |
| Cu | Mandelic acid | | | | Pt |
| $F^-$ | *N*-methylformamide | | | | Se |
| Hg | Methylhippuric acids | | | | Zn |
| Mn | *t,t*-Muconic acid | | *Chlorinated* | | |
| Ni | Pentachlorophenol | | *hydrocarbons* | | |
| Pb | Phenol | | | | |
| Sb | Phenyl glyoxylic acid | | Dichloromethane | | |
| Tl | *S*-phenylmercapturic acid | | Trichloroethene | | |
| V | 2-Thio-thiazolidine-4-carboxylic acid | | Tetrachloroethene | | |
| Zn | Trichloroacetic acid | | | | |
| | *Alcohols/ketones* | | | | |
| | Methanol, acetone, Methylethylketone | | | | |
| (b) *Environmental medicine* | | | | | |
| Arsenic | 1-Hydroxypyrene | Cadmium | | *p,p*-DDE | See (a) |
| Cadmium | Pentachlorophenol | Lead | | HCB | Occupational |
| Chromium | Four metabolites of pyrethroides ($Br_2$-CA, | Mercury | | α-, β-, γ-HCH | medicine |
| Mercury | *cis*-$Cl_2$-CA, *trans*-$Cl_2$-CA, 3-PBA) | | | PCBs | |
| Nickel | 2,5-Dichlorophenol, 2,4,6-trichlorophenol | | | (6 congeners) | |
| Platinum | Cotinine, nicotine | | | PCP | |

[a] The number of parameters for control urine, blood and serum were 49, 14, and 18 for occupational medicine and 16, 3, and 7 for environmental medicine, respectively.

scientific literature. In addition, the laboratory should have successfully taken part in intercomparison programs organized by other institutions. At present 10 national and 14 international laboratories are commissioned to determine the assigned values.

Participants in the intercomparison program have fulfilled the analytical requirements when both results for one parameter are within the tolerance range, i.e. in the ±3 SD range determined by the reference laboratories. The participants receive a certificate for this parameter which is valid for 1 year.

Tables 4–6 compare for selected parameters the assigned values with the mean values of the participant laboratories obtained in the external quality assessment scheme 25. In general a good agreement could be found. The tables demonstrate also the coefficient of variation (C.V.) for the reference values as well as for the participant laboratories. As expected the participants of the round robin showed much higher coefficients of variation. The coefficients of variation are influenced by the analyte concentration and the parameter. The influence of the analyte concentration on the coefficients of variation could be confirmed in the evaluation of the results for the reference values of 15 intercomparison programs. Figs. 1 and 2 show the relationship between the analyte level for lead in blood, respectively. 1-Hydroxypyrene in urine and the coefficients of variation.

Low analyte concentration resulted in high coefficients of variation. Depending on the analyte concentration the coefficients of variation ranged for 65 parameters from 2.5% for the fluoride determination in urine to 20% for α-HCH in plasma. Table 7 shows for 65 parameters the precision for the

Table 4
Assigned values and coefficients of variation of selected parameters in the German external quality assessment scheme—occupational range

| Parameter | | Assigned value | C.V. (%) of assigned value | Mean of participants | C.V. (%) | | Deviation (%) of mean for participants/assigned value |
|---|---|---|---|---|---|---|---|
| | | | | | Participants[a] | All participants[b] | |
| Pb–B (μg/l) | A | 313 | 5 | 318 | 9 | 20 | 1.6 |
| | B | 446 | 4 | 452 | 9 | 21 | 1.4 |
| Hg–B (μg/l) | A | 30 | 7 | 30 | 15 | 20 | 0 |
| | B | 55 | 6 | 54 | 14 | 21 | −1.8 |
| Al–U (μg/l) | A | 72 | 8 | 68 | 23 | 49 | −5.6 |
| | B | 133 | 8 | 135 | 21 | 21 | 1.5 |
| As–U (μg/l) | A | 47 | 8 | 45 | 32 | 47 | −4.3 |
| | B | 74 | 7 | 71 | 29 | 38 | −4.1 |
| Hg–U (μg/l) | A | 19 | 8 | 21 | 21 | 31 | 10.5 |
| | B | 88 | 6 | 89 | 16 | 26 | 1.1 |
| 1-HP–U (μg/l) | A | 1 | 13 | 1 | 40 | 58 | 0 |
| | B | 15 | 11 | 15 | 34 | 34 | 0 |
| *t,t*-MCA–U (mg/l) | A | 1 | 10 | 1 | 32 | 38 | 0 |
| | B | 9 | 7 | 9 | 14 | 14 | 0 |
| Phenol–U (mg/l) | A | 12 | 9 | 13 | 26 | 26 | 8.3 |
| | B | 17 | 9 | 18 | 25 | 76 | 5.9 |
| Benzol–B (μg/l) | A | 3 | 13 | 3 | 46 | 162 | 0 |
| | B | 6 | 12 | 7 | 24 | 118 | 16.7 |
| Tetrachlorethene–B (μg/l) | A | 27 | 11 | 25 | 44 | 44 | −7.4 |
| | B | 49 | 11 | 48 | 34 | 34 | −2.0 |
| Methanol–U (mg/l) | A | 17 | 11 | 19 | 23 | 33 | 11.8 |
| | B | 47 | 8 | 47 | 20 | 26 | 0 |
| DDE–P (μg/l) | A | 13 | 10 | 12 | 42 | 42 | 7.7 |
| | B | 26 | 9 | 26 | 39 | 39 | 0 |
| HCH–P (μg/l) | A | 5 | 10 | 5 | 36 | 44 | 0 |
| | B | 14 | 9 | 13 | 32 | 57 | −7.1 |

[a] Laboratories eliminated, when result outside the ±9 SD range.
[b] C.V. (%) for all laboratories.

Table 5
Assigned values and coefficients of variation for selected parameters in the German external quality assessment scheme—environmental range

| Parameter | | Assigned value | C.V. (%) of assigned value | Mean of participants | C.V. (%) | | Deviation (%) of mean for participants/ assigned value |
|---|---|---|---|---|---|---|---|
| | | | | | Participants[a] | All participants[b] | |
| Pb–B (μg/l) | A | 51 | 9 | 54 | 14 | 14 | 5.9 |
| | B | 160 | 6 | 165 | 13 | 13 | 3.1 |
| Hg–B (μg/l) | A | 2 | 13 | 2 | 44 | 67 | 0 |
| | B | 4 | 10 | 4 | 24 | 36 | 0 |
| As–U (μg/l) | A | 16 | 11 | 14 | 45 | 91 | −12.5 |
| | B | 23 | 10 | 22 | 44 | 79 | −4.4 |
| Hg–U (μg/l) | A | 3 | 11 | 2 | 35 | 34 | −33.3 |
| | B | 4 | 10 | 3 | 24 | 30 | −25 |
| 1-HP–U (μg/l) | A | 0.3 | 15 | 0.3 | 22 | 22 | 0 |
| | B | 0.7 | 14 | 0.8 | 18 | 18 | 14.3 |
| Cotinin–U (μg/l) | A | 272 | 7 | 260 | 20 | 28 | −4.4 |
| | B | 558 | 6 | 555 | 19 | 39 | −0.5 |
| *p,p′*-DDE–P (μg/l) | A | 2 | 12 | 2 | 28 | 28 | 0 |
| | B | 4 | 11 | 5 | 25 | 25 | 25 |
| γ-HCH–P (μg/l) | A | 0.5 | 14 | 0.5 | 44 | 80 | 0 |
| | B | 2.1 | 12 | 1.8 | 30 | 48 | −14.3 |

[a] Laboratories eliminated, when result outside the ±9 SD range.
[b] C.V. (%) for all laboratories.

determinations of reference laboratories and the concentration range of the assigned values from 15 intercomparison programs. For seven parameters (1-hydroxpyrene in urine, benzene in blood, α- and γ-HCH and PCB-28,52,101) a coefficient of variation of 15% was exceeded. The data from Table 7 give a good overview of the current quality on the determinations of analytes assessed in occupational and environmental toxicological laboratories.

The tolerance range in the German external quality assessment scheme is defined as ±3 times the coefficient of variation calculated from the data of the reference laboratories. The percentage of variation of the assigned value is also presented in Table

Table 6
Assigned values and coefficients of variation for selected parameters in the German quality assessment scheme—metals in plasma

| Parameter | | Assigned value | C.V. (%) assigned value | Mean of participants | C.V. (%) | | Deviation (%) of mean for participants/ assigned value |
|---|---|---|---|---|---|---|---|
| | | | | | Participants[a] | All participants[b] | |
| Al–P (μg/l | A | 38 | 9 | 37 | 25 | 90 | −2.6 |
| | B | 46 | 9 | 45 | 22 | 39 | −2.2 |
| Cr–P (μg/l) | A | 3 | 9 | 3 | 20 | 135 | 0 |
| | B | 17 | 8 | 18 | 17 | 32 | 5.9 |
| Co–P (μg/l) | A | 2 | 11 | 2 | 25 | 24 | 0 |
| | B | 6 | 9 | 6 | 15 | 15 | 0 |
| Cu–P (μg/l) | A | 922 | 5 | 920 | 8 | 8 | −0.2 |
| | B | 1220 | 4 | 1244 | 9 | 9 | 1.8 |
| Se–P (μg/l) | A | 117 | 8 | 119 | 15 | 15 | 1.7 |
| | B | 146 | 7 | 145 | 16 | 16 | −0.7 |

[a] Laboratories eliminated,when result outside the ±9 SD range.
[b] C.V. (%) for all laboratories.

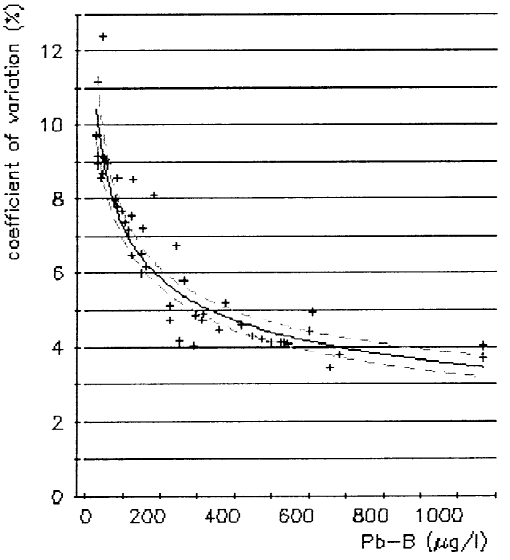


Fig. 1. Correlation between blood lead concentration and coefficient of analytical variation for the runs in the German External Quality Assessment Scheme of the reference laboratories.

7. The results of the laboratories participating at the round robin are tolerable when they are within this range. The variations define the current analytical requirements for determinations of hazardous substances in biological materials [18,19].

For the analysis of inorganic substances in blood and urine the tolerable variation ranged from 7.5 to 43.5%. The determinations of cadmium and mercury in blood and urine in the ecological concentration range were characterised by variations of about ±40%. The data for the analysis of metals in plasma showed variations lower than 30%.

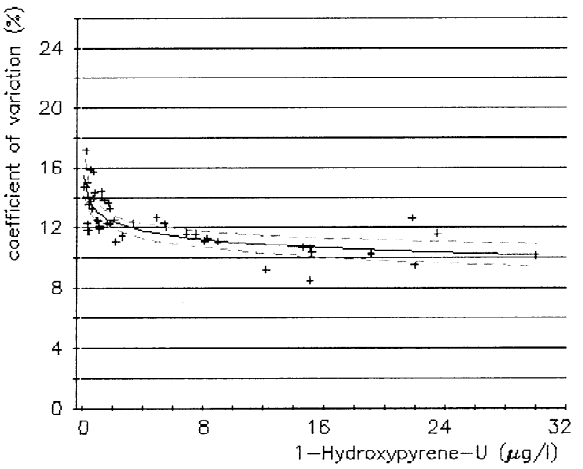


Fig. 2. Correlation between urinary 1-hydroxypyrene concentration and coefficient of analytical variation for the runs in the German External Quality Assessment Scheme of the reference laboratories.

Regarding the determination of organic substances in urine the tolerable variation ranged from 12 to 48%. The highest variation were observed for the analytical determination of 1-hydroxypyrene, PCP, alkoxycarbonic acids and pyrethroid metabolites.

In general the highest variations were found for the analysis of organochlorine compounds in plasma. For the analyte concentrations observed in plasma of the general population the variations ranged from 36 to 60%. An improvement of the analytical method for the determination of organochlorine compounds is necessary.

The determination of solvents in blood and urine samples by head space gas chromatography is also characterised by high variations in the lower exposure range and needs analytical improvement. The tolerable variations for the solvents in whole blood ranged from 26.1 to 57%.

The participant laboratories receive a certificate for the successful participation in the intercomparison program if, in accordance with the guidelines of the German Medical Council (Bundesärztekammer), both results for one parameter are within the tolerance ranges. Table 8 gives an overview about the success rates for all parameter and differentiated to the occupational and environmental concentration range and organic and inorganic parameters. The success rate ranged from 68 to 75%. There were differences for the comparison of the results for the analyses of organic and inorganic parameters. The results for the determinations in the environmental concentration range (mean success rate 75%) were better than for the occupational concentration range (mean success rate 68%).

Table 9 demonstrates the latter observation for the comparison of the coefficients of variation for the single parameters. Except for mercury in urine all success rates agreed upon or revealed better than the rates for the occupational concentration range. This might not be expected. But one have to bear in mind that the tolerance ranges (see C.V.s in Figs. 1 and 2 and Table 7) in the environmental concentration

Table 7
Concentration-dependent coefficients of variation for biomonitoring parameters based on the results of 15 round robins of the German Society of Occupational and Environmental Medicine

| Matrix | Analytes | Unit | Concentration range of assigned values | Precision of reference laboratories, C.V. [%] | Tolerable variations of the reference values [±%] |
|---|---|---|---|---|---|
| *Inorganic substances in blood* | | | | | |
| Blood | Pb | μg/l | 50–700 | 10.0–4.0 | 30.0–12.0 |
| | Cd | μg/l | 0.5–15 | 14.0–4.5 | 42.0–13.5 |
| | Cr | μg/l | 3–16 | 7.0–4.0 | 21.0–12.0 |
| | Co | μg/l | 4–28 | 8.5–4.5 | 25.5–13.5 |
| | Mn | μg/l | 24–60 | 7.0–5.5 | 21.0–16.5 |
| | Ni | μg/l | 5–32 | 9.0–5.0 | 27.0–15.0 |
| | Hg | μg/l | 2–75 | 14.0–6.0 | 42.0–18.0 |
| *Inorganic substances in urine* | | | | | |
| Urine | Al | μg/l | 30–200 | 9–7.8 | 27.0–23.4 |
| | As | μg/l | 10–120 | 12–7.0 | 36.0–21.0 |
| | Be | μg/l | 0.1–0.3 | 13–10.5 | 39.0–31.5 |
| | Pb | μg/l | 20–100 | 7.5–5.5 | 22.5–16.5 |
| | Cd | μg/l | 1–15 | 14.5–5.5 | 43.5–16.5 |
| | Cr | μg/l | 1–40 | 11.0–5.0 | 33.0–15.0 |
| | Co | μg/l | 4–32 | 8.5–6.5 | 25.5–19.5 |
| | $F^-$ | mg/l | 1–16 | 3.5–2.5 | 10.5–7.5 |
| | Cu | μg/l | 20–150 | 7.0–5.0 | 21.0–15.0 |
| | Mn | μg/l | 3–24 | 9.0–6.0 | 27.0–18.0 |
| | Ni | μg/l | 3–50 | 12.0–6.0 | 36.0–18.0 |
| | Hg | μg/l | 2–100 | 13.0–6.0 | 39.0–18.0 |
| | Tl | μg/l | 3–50 | 10.0–8.0 | 30.0–24.0 |
| | V | μg/l | 5–65 | 10.5–7.0 | 31.5–21.0 |
| | Zn | μg/l | 300–1600 | 5.0–3.0 | 15.0–9.0 |
| *Metals in plasma* | | | | | |
| Plasma | Al | μg/l | 15–220 | 10–6.8 | 30.0–20.4 |
| | Cr | μg/l | 4–38 | 9–7.5 | 27.0–22.5 |
| | Co | μg/l | 2–28 | 10.5–6.8 | 31.5–20.4 |
| | Cu | μg/l | 600–2400 | 5.5–4.8 | 16.5–14.4 |
| | Fe | μg/l | 1000–3000 | 7.8–5.5 | 23.4–16.5 |
| | Mn | μg/l | 5–35 | 9.0–7.2 | 27.0–21.6 |
| | Ni | μg/l | 5–55 | 9.5–7.2 | 28.5–21.6 |
| | Pt | μg/l | 0.1–0.5 | 9.5–8.0 | 28.5–24.0 |
| | Se | μg/l | 50–145 | 9.0–7.0 | 27.0–21.0 |
| | Zn | μg/l | 1000–2400 | 5.0–4.0 | 15.0–12.0 |
| *Organic substances in urine* | | | | | |
| Urine | Hippuric acid (HA) | g/l | 0.3–1.9 | 4.5–4.0 | 13.5–12.0 |
| | 1-Hydroxypyrene (1-HP) | μg/l | 1–30.0 | 16–10 | 48–30 |
| | Mandelic acid (MA) | mg/l | 100–525 | 5.8–5.4 | 17.4–16.2 |
| | Methylhippuric acids (MHA) | mg/l | 75–800 | 7.5–6.0 | 22.5–18.0 |
| | *t,t*-Muconic acid (*t,t*-MCA) | mg/l | 1.0–16.0 | 11.5–6.2 | 34.5–18.6 |
| | Pentachlorophenol (PCP) | μg/l | 2.5–55 | 14.0–7.8 | 42.0–23.4 |
| | Phenol | mg/l | 5–60 | 11.0–7.6 | 33–22.8 |
| | Phenylglyoxylic acid (PGA) | mg/l | 50–500 | 10.0–5.5 | 30.0–11.5 |
| | *S*-Phenylmercapturic acid (*S*-PMA) | μg/l | 30–160 | 10.5–7.0 | 31.5–21.0 |
| | Trichloroacetic acid (TCA) | mg/l | 20–160 | 5.4–5.0 | 16.2–15.0 |
| | *o*-Cresol | mg/l | 2–11 | 12.0–8.0 | 36.0–24.0 |
| | 2-Thio-4-thiazolidine carboxylic acid (TTCA) | mg/l | 2–13 | 12.0–5.5 | 36.0–16.5 |
| | Ethoxyacetic acid | mg/l | 10–80 | 12.0–6.0 | 36.0–18.0 |
| | Butoxyacetic acid | mg/l | 10–80 | 14.0–8.5 | 42.0–25.5 |
| | *N*-Methylformamide (NMF) | mg/l | 4–22 | 12.0–6.0 | 36.0–18.0 |

Table 7. Continued

| Matrix | Analytes | Unit | Concentration range of assigned values | Precision of reference laboratories, C.V. [%] | Tolerable variations of the reference values [±%] |
|---|---|---|---|---|---|
| *Organic substances in urine* | | | | | |
| Urine | $Br_2$-CA | µg/l | 1.0–7.0 | 12.5–9.0 | 37.5–27.0 |
| | *cis*-$Cl_2$-CA | µg/l | 1.0–7.0 | 12.5–9.0 | 37.5–27.0 |
| | *trans*-$Cl_2$-CA | µg/l | 1.0–7.0 | 14.0–8.0 | 42.0–24.0 |
| | 3-PBA | µg/l | 1.0–10.0 | 13.0–9.0 | 39.0–27.0 |
| | Cotinine | µg/l | 50–600 | 8.5–6.5 | 25.5–19.5 |
| | Nicotine | µg/l | 100–1200 | 8.0–7.0 | 24.0–21.0 |
| *Organochlorine compounds in serum* | | | | | |
| Serum | *p,p*-DDE | µg/l | 1.0–44.0 | 14.5–8.8 | 43.5–26.4 |
| | HCB | µg/l | 1.0–30.0 | 14.5–8.5 | 43.5–25.5 |
| | α-HCH | µg/l | 0.3–12.0 | 20.0–12.0 | 60.0–36.0 |
| | β-HCH | µg/l | 0.3–28.0 | 14.0–10.0 | 42.0–30.0 |
| | γ-HCH | µg/l | 0.3–24.0 | 16.0–9.0 | 48.0–27.0 |
| | PCB-28 | µg/l | 0.2–12.0 | 16.0–9.5 | 48.0–28.5 |
| | PCB-52 | µg/l | 0.2–12.0 | 16.0–7.5 | 48.0–22,5 |
| | PCB-101 | µg/l | 0.3–11.0 | 16.0–8.2 | 48.0–24.6 |
| | PCB-138 | µg/l | 1.0–17.0 | 12.0–9.0 | 36.0–27.0 |
| | PCB-153 | µg/l | 1.0–17.0 | 12.0–8.8 | 36.0–26.4 |
| | PCB-180 | µg/l | 1.0–21.0 | 12.0–8.0 | 36.0–24.0 |
| | PCP | µg/l | 5.0–85.0 | 12.0–6.5 | 36.0–19.5 |
| *Solvents in blood and urine* | | | | | |
| Blood | Benzene | µg/l | 1.0–15.0 | 19.0–12.0 | 57.0–36.0 |
| | Toluene | µg/l | 25–800 | 13.0–8.7 | 39.0–26.1 |
| | Xylenes | µg/l | 50–1000 | 13.0–9.8 | 39.0–29.4 |
| | Ethylbenzene | µg/l | 25–550 | 14.0–10.8 | 42.0–32.4 |
| | Dichloromethane | µg/l | 50–400 | 14.5–11.5 | 43.5–34.5 |
| | Trichloroethene | µg/l | 25–200 | 14.5–12.0 | 43.5–36.0 |
| Urine | Acetone | mg/l | 10–110 | 9.2–6.8 | 27.6–20.4 |
| | Methanol | mg/l | 16.0–48.0 | 10.0–7.8 | 30.0–23.4 |
| | Methylethylketone | mg/l | 3.0–13.0 | 9.0–6.5 | 27.0–19.5 |

range show much more variation than in the occupational concentration range.

From an analytical point of view, it is important to know which parameters are analysed insufficiently. In the last column of Table 10 the parameters with the lowest success rates are listed. In the same table the parameters with the highest success rates are reported. The determination of beryllium, methanol in urine and DDT in plasma revealed success rates of below 50%, arsenic (environmental and occupational exposure range), 2-thio-4-thiazolidine carboxylic acid (TTCA) in urine and *p,p′*-DDE in plasma showed success rates of 50%. Sufficient success rates were obtained for platinum, nickel and 1-hydroxypyrene in urine and PCBs in plasma. These results are influenced by the number of participant laboratories, but the experience of the laboratories seems to be primarily important for a successful result.

## 5. Conclusions

Quality assurance is an important feature of all analytical work and it comprises several features that are common to all chemical analysis. In the present text, emphasis is on internal and external quality control.

Even when using reliable and tested standard operational procedures, internal quality control in toxicological laboratories is necessary. Control materials are available from various companies for routine quality control. The spectrum necessary for internal quality control can be supplemented by the laboratory's own control samples. The requirements for adequate internal quality control are thus fulfilled. Table 2a–c give an overview of the current spectrum of commercially available control samples.

The obligatory participation in external intercomparison programs for analyses in biological materials

Table 8
Success rates in the German external quality assessment scheme: overview

| Group of parameters | No. of pairs of determinations | No. of pairs correctly analysed | Success rate (%) |
|---|---|---|---|
| Total parameters | 2172 | 1529 | 70 |
| Occupational range | 1472 | 1005 | 68 |
| Environmental range | 497 | 372 | 75 |
| Metals in plasma | 203 | 152 | 75 |
| Inorganic parameters in urine | 612 | 415 | 68 |
| Organic parameters | 277 | 187 | 68 |

Table 9
Comparison of success rates (%) for determinations in the occupational and environmental range (round robin 25) of the German external quality assessment scheme

| | Occupational range (%) | Environmental range (%) |
|---|---|---|
| *Metals in blood* | | |
| Pb | 77 ($n=68$) | 77 ($n=35$) |
| Hg | 76 ($n=46$) | 71 ($n=24$) |
| Cd | 64 ($n=47$) | 54 ($n=28$) |
| *Metals in urine* | | |
| Ni | 82 ($n=34$) | 90 ($n=10$) |
| Cr | 78 ($n=41$) | 67 ($n=15$) |
| Cd | 73 ($n=44$) | 86 ($n=21$) |
| Hg | 65 ($n=86$) | 74 ($n=27$) |
| As | 50 ($n=26$) | 50 ($n=12$) |
| *Organic compounds in urine* | | |
| PCP | 60 ($n=10$) | 83 ($n=12$) |
| 1-HP | 56 ($n=16$) | 91 ($n=11$) |
| *Organochlorine compounds in urine* | | |
| PCBs | 73 ($n=15$) | 87 ($n=22$) |
| HCHs | 66 ($n=12$) | 61 ($n=20$) |
| HCB | 64 ($n=11$) | 52 ($n=21$) |
| PCP | 58 ($n=12$) | 78 ($n=18$) |
| *p,p*-DDE | 50 ($n=12$) | 82 ($n=22$) |

Table 10
Parameters with highest and lowest success rates in round robin 25 of the German external quality assessment scheme

| Group of parameters | Parameters with highest success rate (%) | Parameters with lowest success rate (%) |
|---|---|---|
| *Occupational range* | | |
| Metals in blood | Mn–B: 84<br>Pb–B: 77<br>Hg–B: 76 | Cd–B: 64<br>Cr–B: 60<br>Co–B: 53 |
| Metals in urine | Ni–U: 82<br>Co–U: 79<br>Cr–U: 78 | Cu–U: 52<br>As–U: 50<br>Be–U: 44 |
| Organic components in urine | *o*-Cresol–U: 87<br>HA–U: 81<br>MHA–U: 74 | 1-HP–U: 56<br>*t,t*-MCA–U: 55<br>TTCA–U: 50 |
| Solvents in blood and urine | Ethybenzene–B: 77<br>Benzene–B: 67<br>Xylenes–B: 64 | Toluene–B: 61<br>Tetrachloroethene: 60<br>Methanol–U: 47 |
| Organochlorine compounds in plasma | PCB: 79<br>α-HCH: 73<br>β-HCH: 67 | γ-HCH: 57<br>DDE: 50<br>DDT: 40 |
| *Environmental range* | | |
| Metals in blood | Pb–B: 77<br>Hg–B: 81 | Cd–B: 54 |
| Metals in urine | Pt–U: 100<br>Ni–U: 90<br>Cd–U: 86 | Hg–U: 74<br>Cr–U: 67<br>As–U: 50 |
| Organic compounds in urine | 1-HP: 91<br>Nicotine: 83<br>*t*-$Cl_2$CA: 83 | $Br_2$-CA: 67<br>*cis*-$Cl_2$-CA: 67<br>Cotinine: 64 |
| Organochlorine compounds in plasma | PCB: 93<br>DDE: 82<br>PCP: 78 | β-HCH: 62<br>γ-HCH: 52<br>HCB: 52 |
| Metals in plasma | Cu: 88<br>Zn: 81<br>Co: 80 | Mn: 65<br>Al: 60<br>Fe: 57 |

completes the possible program of quality control. The German Society for Occupational and Environmental Medicine organises two intercomparison programs a year which include a current range of 96 toxicologically important parameters. The certificates received for successful participation certify the quality of the analytical performance of the participant laboratory, so that occupational physicians using the service of the laboratory can be assured of the quality of this laboratory [20–22].

If the recommendations for the pre-analytical phase, the selection of reliable analytical methods by the laboratory and the carrying out of adequate quality control are observed, the pre-requisites for reliable findings during biological monitoring are fulfilled.

## References

[1] J. Angerer, K.H. Schaller, H. Seiler, Trends Anal. Chem. 2 (1983) 257.
[2] J. Angerer, K.H. Schaller, in: R.A. Meyers (Ed.), Wiley, Chichester, 1998.

[3] International Organization for Standardization (ISO), Draft International Standard ISO/DIS 5725-1. Accuracy (Trueness and Precision) of Measurement Methods and Results, Part 1. General Principles and Definitions, 1990.
[4] International Organization for Standardization (ISO), ISO Guide 30: Terms and Definitions Used in Connection with Reference Materials, 2nd ed, 1992.
[5] M. Thompson, R. Wood, Pure Appl. Chem. 65 (1993) 2123.
[6] P. Hoet, Biological Monitoring of Chemical Exposure in the Workplace, Vol. 1, World Health Organization, Geneva, 1996.
[7] P. Schrey, J. Wittsiepe, U. Ewers, M. Exner, F. Selenka, Bundesgesundheitsblatt 36 (1993) 455.
[8] J. Messerschmidt, F. Alt, G. Tölg, J. Angerer, K.H. Schaller, J. Fresenius, Z. Anal. Chem. 343 (1992) 391.
[9] D. Knopp, S. Glass, Int. Arch. Occup. Environ. Health 63 (1991) 329.
[10] J. Angerer, K.H. Schaller (Eds.), Analyses of Hazardous Substances in Biological Materials, Vols. 1–7, Wiley–VCH, New York, 1985, also 1988, 1991, 1994, 1997, 1999, and 2001.
[11] J. Angerer, K.H. Schaller (Eds.), Analyses of Hazardous Substances in Biological Materials, Vol. Band 2, Deutsche Forschungsgemeinschaft, Weinheim, 1975–2001.
[12] World Health Organization, Euro Reports and Studies 36, World Health Organization, Regional Office Europe (Geneva), 1981.
[13] World Health Organization, EFP/HEAL: 84.4, World Health Organization, Geneva, 1984.
[14] A. Aitio, Laboratory Quality Control, Interim Document 4: Health Aspects of Chemical Safety, WHO, Copenhagen, 1981.
[15] J. Büttner, R. Borth, J.H. Boutwell, P.M.G. Broughton, R.C. Bowyer, J. Clin. Chem. Clin. Biochem. 18 (1980) 78.
[16] A. Aitio, Laboratory Quality Control, Vol. 1, World Health Organization, Geneva, 1996, pp. 22.
[17] J. Angerer, K.H. Schaller, Arbeitsmed. Sozialmed. Präventivmed. 11 (1975) 311;
J. Angerer, K.H. Schaller, Arbeitsmed. Sozialmed. Präventivmed. 12 (1976/77) 33.
[18] J. Angerer, G. Lehnert, Dtsch. Ärzteblatt 94 (1997) A2331.
[19] J. Angerer, Th. Göen, G. Lehnert, Umweltmed. Forsch. Prax. 3 (1998) 307.
[20] K.H. Schaller, J. Angerer, G. Lehnert, Int. Arch. Occup. Environ. Health 62 (1991) 537.
[21] K.H. Schaller, J. Angerer, G. Lehnert, Toxicol. Lett. 77 (1995) 213.
[22] G. Lehnert, K.H. Schaller, J. Angerer, Int. Arch. Occup. Environ. Health 72 (1999) 60.