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Quality Challenges of the Chemical Analyses in Occupational Health

Michèle Berode, Rosemary Cosca, Chang Kong Huynh, Pierre Olivier Droz, Trinh Vu Duc, and **Michel Guillemin***

Abstract. Much emphasis is put on the precision and accuracy of sampling and analytical procedures in the modern practice of occupation hygiene. This is due to its importance in risk management in various industries, in the occupation health care and in general consumer product safety. Typical examples of current practices include external quality control by analysis of unknown control samples, certification of control samples and materials, interlaboratory comparisons, and, finally, international standardization of sampling and analytical methods. The Institute of Occupational Health Sciences (IOHS) has participated for more than twenty years in several programs of the above-mentioned approaches, and its own methods have been validated by international quality control programs.

Introduction

In order to protect the workers' health against chemical hazards, it is necessary to assess their exposure to determine if the risk is acceptable or not. Chemical analyses performed at the Institute of Occupational Health Sciences (IOHS) of the University of Lausanne concern a lot of mineral and organic pollutants (> 150 different compounds in air and > 50 compounds in biological fluids). Any error or bias in such analyses may have serious consequences for the workers' health and for the management of a company (legal and economic implications). Dramatic errors in the toxicological testing of drugs or other substances [1] have lead to more and more stringent requirements in the way experiments are conducted and in the quality of analytical results. In the early eighties, the GLP (good laboratory practice) principles were introduced in order to ensure mutual acceptance in the study of chemicals toxicity. This trend is universal and known as *Total Quality Management*. The International Standardization Organization (ISO) and the 'Comité Européen de Normalisation' (CEN) have issued series of standards in this field [2][3]. For a laboratory performing toxicochemical analysis, it becomes useful to obtain a formal recognition of competence such as ISO 9000 certification or official accreditation according to European Standard EN 45001. This paper will present the policy and tools set up at the IOHS in Lausanne to cope with the quality challenges related to the chemical analyses carried out in this Institute.

**Correspondence*: Prof. M. Guillemin
Institute of Occupational Health Sciences
Rue du Bugnon 19
CH–1005 Lausanne

Strategy Used at the IOHS, University of Lausanne, Switzerland

Long Tradition

Chemical analyses in the field of Occupational Health started at the University of Lausanne in 1971. As soon as one year later, the early team participated to the first international intercomparison of analytical results concerning the measurement of ALA-D (delta-aminolevulinic-acid dehydratase), which is a very sensitive index of exposure to lead [4][5]. From the beginning, in spite of the lack of an established quality system at IOHS, the education of the staff, the training of the technical personnel in quality matters, and the careful attention paid to each step of the method (preparation of sample, clean-up and enrichment, recovery rate over a large range of concentration, specificity of detection and possible interferences) allowed to produce reliable data. The validation of a method by the use of an other independent analytical method was also realized whenever possible. For each new method proposed for external services, a quality control is now organized. This early concern with quality illustrates that one of the most important priority of the Institute has always been the reliability of analytical results. In this respect, this laboratory was, in Switzerland, one of the pioneers in quality management. As a consequence, the Institute participated to external quality controls as soon as they were available (see below).

External Quality Controls

The programs adopted by IOHS are briefly described here below.

Asbestos and Other Fibers

IOHS has participated in the American Industrial Hygiene Association (AIHA) Proficiency Analytical Testing Program (PAT) for fiber counting on membrane filters since 1978. This program rates 1000 laboratories as proficient or nonproficient in fiber counting based on analytical results obtained by phase-contrast microscopy. 16 Testing samples per year are received, the samples contain chrysotile asbestos, amosite asbestos, and more recently, man-made fibers in order to accommodate changing regulations with respect to glass and ceramic fibers. The analytical method supported by this quality control program is NIOSH 7400 [6], a fiber counting method in which the relative percent difference between reference labs is commonly 30–40%. Since accuracy of this method is not known, it is extremely important for fiber counting laboratories to participate in external proficiency testing of this type in order to confirm that the precision of their analyses lies within an acceptable range from the reference lab results.

The Institute participated in the AIHA bulk asbestos proficiency analytical testing program since 1989. This program rates 270 laboratories as proficient or nonproficient in the identification and quantification of asbestos in building materials based on analytical results obtained from polarized-light microscopy [7]. 16 Testing samples per year are received, these samples contain 0–100% asbestos as well as industrial fibers which may be confused with asbestos. The analyst is the 'detector' and is required to make many decisions based on visual observations. This external quality control program provides ex-

ternal calibration of the 'analyst-detector' and is crucial in order to document that the analyst provides acceptable results.

Metals and Solvents and Silica

The same AIHA PAT program was also used to check the analysis of metals (since 1978) and silica (since 1993) on membrane filters and solvents on charcoal tubes (since 1982). Another program called WASP (Workplace Analysis Scheme for Proficiency) is used for metals and solvents since 1992. Samples are prepared either by air sampling or by direct liquid sampling of known concentrations with levels of contaminants in the range of what is expected from workplace samples. They are mailed 4 times a year. Each round consists of 4 unknown samples plus one blank for each category of contaminants. The metals tested are: Cd, Cr, Pb, and Zn and the solvents differ according the round and are the useful industrial ones: aromatics, halogenated hydrocarbons, and other oxygenated solvents. Results are statistically analyzed by the organizer. Target values are defined either by the organizing laboratory or by a subset of reference laboratories. The number of participating laboratories ranges from 40 up to 400.

Biomonitoring

About ten international external quality control programs are actually proposed for the measurements of toxic chemicals or metabolites in biological fluids. The IOHS laboratory participates to two of them: the intercomparison program of the German Society of Occupational Medicine and of the 'Centre de Toxicologie du Québec', Canada [8]. The German program was created for the inter-comparison of lead in blood in 1982. Actually it comprises the determination of a series of important occupational-medical parameter in blood, serum, and urine samples (13 metals in blood/serum, and urine, 5 volatile organic solvents in blood, several organic chemical metabolites in urine, 6 PCB isomers and other chlorinated environmental contaminants in serum). This well-followed program (150 laboratories for the last run) consists of two annual rounds, two concentration levels for each parameter. The coordinator collects the results, performs the statistical analysis, and informs the participants of the results. A certificate is awarded by the German Society for Occupational Medicine for successful participation. The Canadian program is entirely devoted to the intercomparison for metals in blood and urine samples and for fluoride in urine and is followed by *ca.* 300 laboratories.

Certification Materials

The 'Bureau Communautaire des Références' (BCR) of the European Union is one of the international agency for the production and distribution of certified reference materials (CRM). The purchased CRMs always come with an official certificates reporting target values with the dispersion range, details on methods and informations on storage and expiration date. IOHS participated in several of BCR programs on applied metrology and chemical analysis as described below. CRMs can serve calibration purposes, help in the development of methods and finally allow the comparability of analytical results from various laboratories.

Certification of PAH Materials (Polynuclear Aromatic Hydrocarbons)

The determination of PAHs in environmental samples has been an ongoing challenge, since 20 years IOHS was interested to PAH compounds. Since 1990, IOHS was invited to participate to the certification study of PAHs in sewage sludge (CRM 088) [9][10]. Eight PAHs have been selected as target compounds for certification on the basis of their abundance in the environment, their biological activity (carcinogenicity and/or mutagenicity) and their related national recommendations and regulations (*i.e.* benzo[*a*]pyrene). The certified values of the eight PAHs in CRM 088, expressed as mass fraction in (g/g and corrected for the water content and recovery were calculated on the basis of results of eleven selected laboratories, including IOHS results (out of 25 participating laboratories) within < 8% of overall standard deviation. The second certification study of PAHs was carried out in 1994, in a dried contaminated industrial soil (CRM 524) [11], based on results of twelve selected laboratories (18 participating laboratories), and finally a third certification study of PAHs in freshwater sediment (CRM 536) in 1995 (final report accepted, 18 participating laboratories).

Certification of Isocyanate Derivatives

In general, isocyanates are measured as their (2-methoxyphenyl)piperazine (2-MP) derivatives using reversed-phase HPLC coupled to UV detector. Within the project 'Preparation and certification of a reference material for the determination of diisocyanates in workplace air' initiated by the BCR a certification exercise was organized in 1993–1995. Test materials for four diisocyanates as their 2-MP derivatives (HDI, 2,4-TDI, 2,6-TDI, and MDI) were produced, consisting of the solid materials and evaporated residue of solution mixtures simulating the mixture obtained after sampling by impinger technique. All IOHS results were selected for calculation of the certification values (18 participating laboratories) and certified materials will be available soon by BCR.

Certification of Aldehyde Derivatives

Development of reliable measurement methods for low-molecular aldehydes, as well as certification of reference material for analytical quality control has been given high priority by the EC member states due to the toxicity of these compounds. Three types of reference materials related to the monitoring of aldehydes in air by derivatization with 2,4-dinitrophenylhydrazine (DNPH) and subsequent determination of the formed hydrazones by HPLC have been prepared by coordinator laboratory (TNO, The Netherlands) since 1992: *a*) 13 mm glass-fiber filters spiked with formaldehyde hydrazone and excess DNPH, *b*) ampoules containing a solution of hydrazone of formaldehyde, acetaldehyde, acrolein, and acetone in acetonitrile with excess DNPH, and *c*) the pure hydrazones of formaldehyde, acetaldehyde, acrolein, acetone, and glutaraldehyde. All IOHS results were selected for the calculation of the certified values (21 participating laboratories) and certified reference materials are now available by BCR (CRM 546 – CRM 554).

International Standardization

The 'workplace air' has been the topic of interest for standardization regarding the performances of instruments, apparatus, and measuring procedures. The ISO technical committee 146, subcommittee 2 (TC146/SC2) is currently working on the analysis of chemical pollutants workers are exposed to. The standardized method is not based on the latest development achieved but on consensus on the state of the art. At the European Committee for Normalization, CEN/TC 137 equivalent to ISO TC 146/SC2, IOHS is actively participating in the production of standards, *e.g.*, on 'guidance for the assessment of exposure to chemical agents' SN-EN 689, on 'performances requirements of measuring procedures' SN-EN 482, *etc.* which have become Swiss standards superseding the national norm if any. IOHS is also involved with the French commission X43C of AFNOR as invited expert. At the national level, IOHS promotes the occupational health informations through the Swiss Association for Standardization SNV/ASN/ TK 115 relevant to the topic.

Intercomparison Studies (Round Robin Tests)

These types of studies are used when a developed method or a reference material has to be validated. A few identical samples are sent to different laboratories and the results are compared and evaluated in term of accuracy, if the exact value of the samples is known, and in term of precision, (reproducibility within and between labs) if several determinations of the same samples are done. Another way to make intercomparison studies is to offer to participating laboratories access to chambers or tunnels containing known concentrations of airborne contaminants. This second method allows the sampling methods to be included in the global testing of the method. Two examples of Round Robin Test with a sampling exercise and one example with the distribution of identical samples are given here below.

Experimental Chamber of IOHS

The Institute has a 12 m^3 exposure chamber for generation of gases and vapors of known composition. Designed mainly for human subject exposure, this tool has been of very much use also in analytical method testing and validation. On several occasions, intercomparison were made with other laboratories. For example, the Swiss Society of Occupational Hygiene (SSOH) has mandated the Institute for a round robin testing of chlorinated hydrocarbon measurements in air by several methods. Laboratories came with their own usual instrumentation to sample the chamber atmosphere. The Institute was responsible for generation of solvents in air, results analysis and dissemination.

Aldehyde Sampling Exercise

In a BCR meeting in 1994, experts identified the need for further investment of measurement methods for aldehydes at occupational hygiene levels including sampling and specially sampling with solid sorbent collection methods for personal monitoring, more convenient than impinger (liquid absorption) methods. Thirty-one participants from different European laboratories, public research organizations, universities, and industry, including IOHS, took part in the exercise. Each participant was invited to apply his usual measurement methodology. Analysis of the samples should be performed at home by each laboratory using its own analytical procedure. Each exercise was organized in 6 rounds, including 5 priority analyses: formaldehyde, acrolein, glutaraldehyde, acetaldehyde, and acetone in the range of 0.1 to 30 ppm. We observed a very good correlation between the 6 methods used in our laboratory and the generated concentration for formaldehyde (< 30% Relative Overall Uncertainty (ROU) calculated according to CEN or EN 482 [12]). In contrast to formaldehyde results, the acrolein quantification was more complicated. In addition to the main peak of acrolein-DNPH derivative, 2 other acrolein isomers were observed on DNPH-based devices during sampling exercise. Similar formation of isomers was observed with glutaraldehyde (2 isomers). For GC methods, only one pure oxazolidine-acrolein derivative peak was observed in XAD-2 sampling device. Since the 2-HMP coated on XAD-2 tubes give only one peak of oxazolidine-acrolein derivatives by GC techniques, this method is finally the preferred one for acrolein in field situation. For other aldehydes, DNPH-based samplers combined with HPLC is the method of choice.

Distribution of Identical Sample.
Diesel *Soots*

The IOHS participated recently to Round Robin Tests on the *Diesel* soots. This contaminant is of great concern both in the Public Health and in the Occupational Health fields because it is considered as a probable carcinogen [13]. Traditional chemical analyses cannot be used for such a complex mixture of fluctuating composition, thus a surrogate has to be found. In some countries the 'elemental' carbon (core of the soot particle) has been selected as the appropriate surrogate. The determination of this so-called 'elemental' carbon is done after volatilization of the organic compounds adsorbed on the particles, by burning the remaining carbon under oxygen and analyzing the CO_2 formed by IR spectroscopy [14]. An agreement on a standardized method has not yet been reached and exchanges of experience is going on.

Particle Sampling

The acquisition of knowledge on the particle size fractionation which takes place during inhalation and their deposition in the respiratory tract resulted in the definition of new convention on size fractions related to health risk. Therefore, the sampling in the workplace should be based on these three biologically relevant fractions namely the inhalable, thoracic, and respirable fractions. There is no instrument enabling the simultaneous collection of the three fractions at present. A new generation of sampling instruments should be developed consistent with the new criteria. Within the Standard, Measurement and Testing program of the European Union (4th framework program on science, research, and development 1994–1998), IOHS is involved in such a project entitled 'Size-selective personal air sampling using porous plastic foam' with five other European partners.

Direct Reading Instruments

Technical developments in the last years has made available numerous direct reading instruments for the measurement of aerosols, gases, and vapors. As a consequence estimation of occupational exposure has become very easy with an orientation towards black boxes hiding the chemical and physical principals of measurement. In order to keep high quality, even for operators without chemical background, it is essential that to clearly state the limits of the measurement instruments (interferences, precision, and accuracy), and also to periodically submit them to independent calibration. This is presently not very much developed in Switzerland. It is mainly in the hands of commercial representatives, or manufacturers. There is, however, a need to have a reference-independent laboratory for these types of calibrations.

Speciation in Trace Metal Analysis

Traditionally toxic metal analyses in the field of environmental and occupational health were restricted to the determination of the total amount of the metal of interest in the sample without considering its different forms (oxidation states, mineralogical species, organic derivatives, aggregation states, *etc.*) Such an approach cannot be accepted in all the cases where different forms have different toxicities and pharmacokinetic behavior. Therefore, metal speciation is becoming a field under rapid development for a better assessment of toxic risks due to metal exposure.

Conclusion

Occupational hygiene is not a basic science, it is a multidisciplinary field of knowledge aimed at the workers' health protection through an efficient management of risks at the workplace. Chemistry and especially analytical chemistry belong to this field and represents a necessary tool for the control of chemical risks. This article has shown that the application of the basic principles of analytical chemistry is not a straightforward process in occupational health, since a lot of factors may bias the analytical results. The only

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way to adequately cope with these difficulties is to build up a quality management program based on a clear policy. This should be true in any laboratory performing chemical analyses.

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High-Pressure Stopped-Flow Study of Inclusion Reactions with α-Cyclodextrin: Dynamic Aspects in Host-Guest Interactions

Pascal Bugnon, Peter G. Lye, Amira Abou-Hamdan, and **André E. Merbach***

Abstract. The full volume and entropy profiles of the inclusion reaction between α-cyclodextrin and the guest molecules, ethylorange (**1**) and mordant yellow 7 (**2**), have been constructed from variable-pressure and -temperature stopped-flow kinetic experiments.

Introduction

Cyclodextrins are well known as molecular hosts capable of binding a range of guest molecules *via* noncovalent interactions with their hydrophobic cavity. Recently, some progress has been made in the understanding of the complex mechanisms involved in the molecular recognition by α-cyclodextrin (α-CD) [1]. The success of high-pressure studies is related to the fact that it is comparatively simple to interpret volume differences in molecular terms even in the case of complex chemical systems, whereas it is often very difficult to interpret entropy differences on a similar level [2].

Description of the HPSF Apparatus

The third generation of our high-pressure stopped-flow instrument (*Fig. 1*) is able to operate over a temperature range of –40 to +100° and up to 200 MPa [3]. The system has been designed so that it can perform measurements in absorbance or in fluorescence mode or both. The observation cell has path lengths of 10 and 2 mm for absorbance and fluorescence measurements, respectively. The stopped-flow unit can easily be combined with an optical system of a conventional ambient pres-

Table. *Kinetic Parameters for the Inclusion Reaction Between α-CD and a Selection of Azo Dyes, in Aqueous Solution*

Parameters	1	2	3	4
$k_{1,f}^{298}$/M^{-1} s^{-1}	(1.22±0.02) 10^4	(1.52±0.03) 10^4	2.0 10^4	1.2 10^4
$k_{1,r}^{298}$/s^{-1}	1.8 ± 0.1	25.4 ± 0.5	6.0	9.4
K_1^{298}/M^{-1}	6660 ± 470	600 ± 20	3300	1280
$H_{1,f}^{\ddagger}$/kJ mol^{-1}	+20.1 ± 1	+27.3 ± 1	+22.5	–
$H_{1,r}^{\ddagger}$/kJ mol^{-1}	+54.8 ± 3	+38.6 ± 1	+49.8	–
$S_{1,f}^{\ddagger}$/J K^{-1} mol^{-1}	–99.3 ± 3	–73.3 ± 4	–87	–
$S_{1,r}^{\ddagger}$/J K^{-1} mol^{-1}	–55.9 ± 8	–88.6 ± 4	–65	–
$k_{2,f}^{298}$/s^{-1}	0.20 ± 0.02	1.83 ± 0.04	0.87	0.58
$k_{2,r}^{298}$/s^{-1}	(9.3 ± 1.2) 10^{-2}	0.17 ± 0.02	0.55	0.26
K_2^{298}	2.1 ± 0.5	11 ± 1	1.6	2.2
$H_{2,f}^{\ddagger}$/kJ mol^{-1}	+72.0 ± 3	+50.8 ± 1	+54.5	–
$H_{2,r}^{\ddagger}$/kJ mol^{-1}	+43.8 ± 3	+39.5 ± 5	+63.3	–
$S_{2,f}^{\ddagger}$/J K^{-1} mol^{-1}	–16.8 ± 9	–69.4 ± 4	–63	–
$S_{2,r}^{\ddagger}$/J K^{-1} mol^{-1}	–117.7 ± 11	–127.0 ± 17	–37	–
References	this work	this work	[1b]	[1b]

1: R_3=R_5=H, R_4=N(CH_2CH_3)$_2$; **2**: R_3=COO^-, R_4=OH, R_5=CH_3; **3**: R_3=$CH_2CH_2CH_3$, R_4=OH, R_5=H; **4**: R_3=$CH(CH_3)_2$, R_4=OH, R_5=H.

*Correspondence: Prof. A.E. Merbach
Institut de chimie minérale et analytique
Université de Lausanne
Bâtiement de chimie (BCH)
CH–1015 Lausanne