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Strategies and Methods for Investigation of Airborne Biological Agents From Work Environments in Germany

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In 2004/2005, a European Twinning Project was carried out to support Polish occupational safety and health institutions in putting into practice Directive 2000/54/EC regarding the protection of workers from risks related to exposure to biological agents at work.

Information on and training in sampling and analysing biological agents of people responsible for bioaerosol measurements and the assessment of measuring results from the workplace atmosphere were part of the project.

This paper is an extract of the authors' activities within the project and can be used as a tool for comparable activities in future projects with other European Union candidates. It gives information on working standards for bioaerosol measurements worked out and commonly used in Germany within the frame of European guidelines for bioaerosol measurements in the workplace atmosphere.

Additionally it summarizes the authors' long practical experience in carrying out bioaerosol measurements in the atmosphere of various workplaces.

workplace atmosphere biological agents measuring strategies analytical methods
German specification EU regulation

1. INTRODUCTION

Article 2 of Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work contains some definitions which are necessary for the understanding of the purpose of the directive [1]. Biological agents are defined there as "micro-organisms, including those which have been genetically modified, cell cultures and human endoparasites, which may be able to provoke any infection, allergy or toxicity" (p. 22). In this context "'micro-organism' shall mean a microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material" (p. 22). Biological agents may be incorporated in different ways, e.g., by ingestion or by infection of

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damaged skin or mucous membranes. The most important way of infection at a great number of working places is the inhalation of airborne biological agents by the uptake of aerosols or dust containing biological agents and/or substances of biological origin which are called bioaerosols.

Air is not a natural environment for micro-organisms because their reproduction is limited without water. Furthermore microbes must be released from biofilms, solid or liquid sources to become suspended in the air. Release may be the result of several mechanisms such as turbulence of the air caused by the activity of machines, animals or humans as well as by treatment, dispersal or any other resultant movement of contaminated material [2]. The occurrence of airborne micro-organisms at working places depends therefore on the evidence of good conditions for microbial life and of a mechanism for release. If one of these two factors is missing a contamination of the air with bioaerosols is very unlikely.

If both of them are fulfilled, however, according to Directive 2000/54/EC, it is the employer's duty to qualify nature, degree and duration of the workers' exposure to biological agents in order to make it possible to assess any risk to the workers' health or safety caused by biological agents and to lay down the measures that should be taken to avoid such risk [1]. In Directive 2000/54/EC neither measurements of biological agents are demanded nor limits given.

Nevertheless, it might be necessary to determine the amount and composition of bioaerosols by measurements, e.g., to carry out risk assessment, to compare different workplace situations, to qualify the effect of protective measures or to investigate and to assess the workplace situation if there had been an occupational hazard.

In Germany, a group of national experts, therefore, published detailed instructions for measuring and analysing biological agents like bacteria and fungi at workplaces instructed by the Advisory Committee on Biological Agents (ABAS)[1].

2. GENERAL ASPECTS OF BIOLOGICAL AGENTS AND THEIR INVESTGATION

The most important characteristic of micro-organisms is that they are living organisms which are able to reproduce. The ability to multiply is the essential difference between biological agents and other hazards related to work. For this reason one microbe may theoretically be sufficient to cause considerable contamination of a workplace or an infection. Otherwise, infection does not inevitably mean disease because the growing of a micro-organism in a host, even if it is potentially virulent, does not have to lead to harmful effects. The relationship between micro-organisms and humans is known to be dynamic, because each of them influences the activity and function of the other. Human responses to the same mixture of airborne biological agents may range from innocuous effects to serious or fatal diseases, according to the susceptibility of the exposed person. Because of this variability it is not possible to define a general dose–effect relationship for a certain microbial species and information about infectious doses has to be interpreted in a very careful way.

Classification of biological agents into risk groups according to Directive 2000/54/EC (article 2) depends on the infectious potential of species for humans, on the possibilities of treatment and prophylaxis as well as on their risk of spreading to the human community [1]. In annex III of the directive species of 152 bacteria, 130 viruses, 69 parasites and 25 fungi were classified as risk groups 2 to 4 (community classification). Additionally, in Germany more detailed national lists of classification were published by ABAS [3, 4, 5, 6] on the basis of a systematic procedure of classification [7]. Details relating to infectious doses are found in literature mostly for micro-organisms belonging to risk groups 3 and 4, which can cause severe human diseases and present a serious hazard to workers. They are rarely mentioned for micro-organisms belonging to risk group 2, which can cause human disease and might be a hazard to workers.

[1] Ausschuss für Biologische Arbeitsstoffe (ABAS)

Especially at workplaces where the occurrence of biological agents is an unintended consequence of the work (e.g., in the waste industry or agriculture) micro-organisms belonging to risk groups 1 and 2 may be present in high doses and have to be considered as potentially hazardous substances because of their irrigating potential.

The situation of risk assessment becomes even more complicated because workers are exposed to a varying mixture of different biological compounds producing adverse medical effects, e.g., infections, hypersensitivity, irrigation or inflammation. The concentration of every component of these bioaerosols may oscillate in a broad range even in short periods of time. It is well known that metabolic substances or microbial spores themselves may cause allergic symptoms. Furthermore, under certain conditions some biological agents may produce chemical substances which can cause pathogenic effects in the host by poisoning (exotoxins, endotoxins and mycotoxins) [8]. These substances of biological origin are not biological agents according to the definition given in Directive 2000/54/EC [1]. Nonetheless, the exposure of an employee to toxins and allergens has to be considered during risk assessment.

All the aforementioned criteria are reasons for the world-wide lack of limits for airborne biological agents. Furthermore, such a limit for microbial species should be valid for the exposure to different bacteria, viruses, fungi, endotoxins and mycotoxins even in dynamic combinations and different concentrations with high variations in short periods of time and in view of the fact that interactions or synergism are possible between all agents.

Currently used standards for the estimation of biological agents at workplaces are based on cultivation. The primary factor associated with inactivation and viability of microbes during sampling, transport and storage is the water content [9]. Generally, cultivation methods underestimate the real numbers of airborne microbes due to many reasons such as death by dehydration during sampling or the inability of some species to grow under laboratory milieu conditions [10]. Without any doubt, any of the so-called general media used for cultivation and colony counting of fungi or bacteria is more or less selective for special groups of micro-organisms. Furthermore, the use of selective media as proof of airborne contaminants at different workplaces is problematic because these media had been mainly developed for the investigation of medical samples and their function as well as their sensitivity are often restricted to this area of application.

3. GERMAN SPECIFICATIONS ON THE BASIS OF EU REGULATIONS

In accordance with the definition of biological agents in Directive 2000/54/EC some European standards for the measurement of biological agents have been published by the European Committee for Standardization in Brussels [1]. All standards describe general aspects on a basic level (Table 1). On this European basis German experts published more detailed instructions for the measurements of bacteria, fungi and endotoxins. They were edited at the BG-Institute for Occupational Safety and Health (BGIA)[2].

TABLE 1. European Standards Relevant for Measurements of Airborne Biological Agents

No.	Current Version	Title
EN 13098	2000	Workplace atmospheres—guidelines for measurement of airborne micro-organisms and endotoxins [11]
EN 14031	2003	Workplace atmospheres—determination of airborne endotoxins [12]
EN 14042	2003	Workplace atmospheres—guide for the application and use of procedures for the assessment of exposure to chemical and biological agents [13]
EN 14583	2004	Workplace atmospheres—volumetric bioaerosol sampling devices—requirements and test methods [14]

[2] Berufsgenossenschaftliches Institut für Arbeitsschutz (BGIA)

TABLE 2. German Standards Relevant for Measurements of Airborne Biological Agents

No. of BGIA Workmap	Title[1]
9411	Anwendung von Messverfahren und technischen Kontrollwerten für luftgetragene Biologische Arbeitsstoffe [15]
9417	Benutzerhinweise für die Auswahl von Messverfahren für Biologische Arbeitsstoffe [16]
9420	Verfahren zur Bestimmung der Schimmelpilzkonzentration in der Luft am Arbeitsplatz [17]
9430	Verfahren zur Bestimmung der Bakterienkonzentration in der Luft am Arbeitsplatz [18]

Notes. 1—authorised English-language versions in progress.

In Table 2 these standards are summarised. Those German standards are being translated into English now. Furthermore the standards were translated into the Polish language within the German–Polish Twinning Project of the European Union.

4. SAMPLING STRATEGIES

The concentrations of airborne biological agents are of special interest because of the risk assessment which has to be done for activities at workplaces where these agents are present and where they may be set free into the workplace atmosphere. Although there are neither limits of airborne biological agents nor an obligation to measure them it may sometimes be necessary to generate information on exposure by sampling and analysing. Variations of airborne bacteria concentrations at a defined workplace may be very large. This is caused by the release of biological agents into the workplace atmosphere which depends mainly on the work activity and is often inhomogeneous as far as time and space are concerned. Therefore measurements only reflect instantaneous images of the current conditions. In order to increase the validity of measurements strategies for samplings have to be standardised as far as possible.

An overview of the main steps and different purposes of valid measurement strategies is given in Table 3. The table summarises the content of BGIA Arbeitsmappe code No. 9411, which is identical with the Technical Rule of Biological Agents (TRBA) code No. 405 [15, 19]. TRBA is a concrete term of the German implementation of Directive 2000/54/EC [1] and defines binding minimum requirements. A Technical Control Value (*TCV*) describes the concentration of airborne biological agents for well defined types of plants or special work procedures which are attainable according to state-of-the-art technology. According to this definition a *TCV* is based only on technical feasibility but not on the medical

TABLE 3. Main Aspects of Strategies for Sampling of Biological Agents [15, 19]

Working Steps	Organisation and Preparation of Measurement
1	**Background information** according to technical equipment and operating processes which influence the exposure
	Information regarding expected bacteria and fungi (survey of workplace situation, information relating to comparable sites/installations and work conditions, sample of material/survey measurement if necessary):
	• Type of plant;
	• Work activities;
	• Protective measures (including ventilation);
	• Source of emission;
	• Duration and time of exposition;
	• Materials used;
	• Climatic conditions.

TABLE 3. (*continued*)

Working Steps	Organisation and Preparation of Measurement
2	**Purpose of measurement**
	Efficiency control of protective measures carried out without technical control value (*TCV*)
	• Measurements should also be carried out under worst case conditions. • Protective measures should consider the requirements of state-of-art technology. • Measurements should be taken by utilisation of capacity. • If the concentrations of airborne microbes are influenced by climatic conditions the measurements should be done in periods which favour exposure and growth of micro-organisms. • Efficiency control should be taken by 5 measurements before and 5 measurements after realisation of protective measure; the result is received by comparison of the medians.
	Efficiency control of protective measures carried out with *TCV*
	• A *TCV* is established by the German Advisory Committee on Biological Agents and will be continually brought up to the state-of-the-art technology. • Biological agents shall be replaced with others which are less dangerous by obligation even if a *TCV* is kept. • If a *TCV* is established it is the valid basis for testing the efficiency of protective measures.
3	**General aspects of measurement**
	The standardisation of measuring strategies and methods is obligatory to receive valid and comparable results; living microbes are sensitive to different environmental influences; therefore transport as well as storage of samples of biological agents requires special conditions.
	a. Background measurement Measurement of biological agents on the factory site outside and windward of buildings by using the same strategy and the same equipment as for exposure measurements.
	b. Survey measurement Measurement to obtain the general information whether or not a relevant concentration of airborne biological agents is present at a defined work place; according to the results and to the questions of investigation more specific measurements may be needed. • Total concentration of bacteria and/or fungi or of certain groups of microbes may be sufficient in many cases. • A minimum of 3 measurements in the breathing zone shall be representative for the whole workplace situation; the break between measurements shall be maximal three times of the duration of each solitary measurement; the result for the workplace is calculated as the arithmetical mean value of these measurements. • At work conditions with high and low exposure 3 measurements under each of the conditions are necessary; the result for the workplace is calculated as the arithmetical mean value of these measurements.
	c. Measurement of exposure peaks • Location, abundance and break-time between measurements depend on the operating cycle. • The choice of the measuring system suitable for the measurement depends on the duration of exposition peaks. • A minimum of 3 measurements before, at least 3 measurements during as well as after the exposure peak are necessary to obtain enough information.
	d. Measurement near to an emission source • It is sufficient to get the information whether and in which scale there is relevant exposure. • A minimum of 3 measurements near the emission source and analyses of work material if possible are recommended for an estimation of exposure; the break between measurements shall be at least three times of the duration of each solitary measurement, the result for the workplace is calculated as the arithmetical mean value of these measurements.

	e. Measurement of an average concentration in a defined period of time
	The aim of this measuring strategy is the scientific examination of a correlation between type, level and duration of an exposure and of resulting medical consequences to employees according to epidemiological aspects.
	• A description of the exposure for more than one period is necessary.
	• The strategy of measuring should give representative results of exposure.
	• Measurements should be done in the breathing zone near the employee and if possible by using personal samplers; stationary samplers can be used if the results are usable for assessing the exposure.
	• In case of doubt it should be measured at locations with higher risks.
	• Especially usable are measuring systems for long time-sampling.
	• The minimum of measurements for 8 hrs is given as an example:
	  • Sampling time: ≤5 min, sampling amount: ≥12, result: median;
	  • Sampling time: 15 min, sampling amount: ≥6, result: median;
	  • Sampling time: 1 hr, sampling amount: ≥2, result: arithmetical mean value.

TABLE 3. (*continued*)

Working Steps	Organisation and Preparation of Measurement
4	**Report**
	The measurements must be reproducible and therefore the report must comprise the following information: • Institution which had carried out the measurement; • Purpose of measurement; • Description of the workplace (procedure, technical protection measure, temperature and humidity of air); • Conditions of work procedure during measurement (capacity utilisation); • Description of the measurement points (sketch); • Date, time, weather conditions, season; • Procedure of measurement (sampler, sampling time, amount of samples); • Conditions during transport (duration, condition up to analysing procedure); • Result.

aspects of workers' health. For the verification of a *TCV* detailed definitions of measurement strategies and methods are obligatory.

Institutions which carry out bioaerosol measurement at workplaces may be organised in different ways. The main structures in Germany are

- decentralised sampling and centralised analysing (e.g., measurement of biological agents within the BGMG, i.e., the measuring system of hazardous substances of the German Berufsgenossenschaften, see www.hvbg.de/ d/bia/ wun/org/orgf1/pdf/bia_bgmg.pdf);
- centralised sampling and decentralised analysing; or
- centralised sampling and analysing (e.g., microbiological laboratories on the market or the microbiological division of the State Institute for Occupational Safety and Health of North Rhine-Westphalia[3]).

Every way of organising the measurement of biological agents at workplaces has its advantages and disadvantages. There might be the fewest possibilities of making mistakes if one does everything on one's own (sampling, transporting, analysing and reporting). However, such measurements require a great deal of manual preparation, manpower and time so a large number of samples or the monitoring of a large area could only be managed with many employees in one institute or with the support of coworkers, decentralised sampling and additional analysing of samples in external laboratories which offer analysis of workplace samples of biological agents on the market.

5. SAMPLING AND ANALYSING BACTERIA AND FUNGI

Airborne bacteria may be collected with devices based on different sampling principles such as filtration, impingement and impaction. All these principles are well described in the literature [20, 21]. All samplers should be checked for sampling the inhalable fraction of an aerosol according to Standard No. EN 481:1993 [22]. In general, information relating to the sampled particle size is generated by the manufacturer. Table 4 shows a list of samplers which are often used for measurements of airborne bacteria in Germany as well as data about the airflow rate they run with and about the cut-off size for bioaerosol particles.

In general, all samplers are suitable for measuring gram-positive and gram-negative bacteria as well as for spores. However, it is well known that only results based on the

[3] Landesanstalt für Arbeitsschutz des Landes Nordrhein-Westfalen (LAfA, NRW)

TABLE 4. Sampling Devices for Measurements of Airborne Bacteria in the Atmosphere at Workplaces and Technical Information According to [18]

Sampler	Manufacturer	Principle	Cut-off (µm)*	Airflow Rate (L/min)
One-stage Andersen impactor [22]	ESM Andersen Instruments, Germany	Impaction	<2	28.3
Lowest stage of the six-stage Andersen impactor [23]	ESM Andersen Instruments, Germany	Impaction	0.65	28.3
Surface air sampler (SAS)	Pool Bioanalysis Italiana, Italy	Impaction	2	180
Merck air sampler (MAS)	VWR International, Germany	Impaction	>1	100
RCS-Plus	Biotest, Germany	Impaction	1.0–1.3	50
FH 5 (Loreco impactor)	Loreco Reckert, Germany	Impaction Filtration	1	100 impaction 50 filtration
All-Glass impinger (AGI-30) [24]	ACEGLASS, USA	Impingement	0.31	12.5
SKC biosampler	SKC, UK	Impingement	not specified	12.5
Sartorius MD8	Sartorius, Germany	Filtration	not specified	41.7–133.3
Gravikon PM 4	Sartorius, Germany	Filtration	not specified	66.7
PGP	GSM Gesellschaft für Schadstoffmesstechnik, Germany	Filtration	not specified	3.5

Notes. *—above this cut-diameter or aerodynamic diameter more than 50% of particles were collected, smaller ones were only sampled insufficiently.

same sampling and analysing procedure are comparable. Therefore the most suitable sampling system must be selected according to the cause and the specific conditions of each examination.

The desiccation of sensitive bacteria is one of the most important factors which influence the quantitative result, because only viable microbes are detectable by cultivation. At workplaces with continuous high air humidity (>80%) a release of microbes from liquid media and a high percentage of gram-negative bacteria is suspected (e.g., cooling lubricants in the metal industries or recycled water in car washing plants). In such workplace conditions, a sampling system is necessary to prevent the irreversible desiccation of these micro-organisms during the sampling procedure. Impingement and impaction are recommended for such conditions. Prevention against desiccation is also the reason for the maximum sampling time of 10 min when using filtration and impaction systems. For sampling spores, which are not sensitive to desiccation, longer sampling periods are possible. A minimum sampling time of at least 1 min is obligatory because this period of time is necessary to produce a constant airflow. Personal sampling is established only for the use of a filtration system.

Many customary bioaerosol samplers operate on the principle of impaction. Those samplers were originally constructed for measurements in the pharmaceutical and food industry or in hospitals. The concentrations of airborne bacteria at such locations are very low compared to many other work places. The problem of overloading (more than 5 colony forming units per square centimetre) has to be considered in all cases. Impaction may be useful for analysing susceptible vegetative bacteria because the organisms were separated on a moist agar surface. A continuous airflow of more than 10 min, however, leads to desiccation of the agar surface. Consequently, separation is changed (rebounce effect). Filtration is recommended only for the analysis of bacteria which are not sensitive to desiccation.

Currently, the assessment of concentrations of airborne bacteria sampled at workplaces is based on cultivation on agar media (see Table 5 for details). Therefore the samples must

TABLE 5. Procedure of Sampling and Analysing Airborne Bacteria [28]

Part of Procedure	Impaction[1]	Filtration	Impingement
Sampler[2]	Andersen impactor Surface air sampler (SAS) Merck air sampler (MAS) RCS-Plus, FH 5	Sartorius MD8 PGP, FH 5, Gravikon PM 4	AGI-30 SKC-BioSampler
Sampling medium	agar plates agar strips (RCS-Plus)	membrane-filters: gelatine (pore size: 3.0 µm) preferred polycarbonate (pore size: 0.8 µm) only for direct processing: cellulose nitrate or acetate (pore size: 0.8 µm) or gelatine (pore size: 3.0 µm)	saline solution (0.9% NaCl) AGI-30: 50 ml SKC-BioSampler: 20 ml
Sampling time	1–10 min		not defined
Preparation at the sampling location	safe packing	direct processing: filters placed on agar plates indirect processing: filters are transferred in a liquid	cooling immediately and chain of cold storage units is obligatory
Transport	within <24 hrs by using cooling devices (4–8 ˚C), documentation is obligatory		
Working up of samples	only direct	direct and indirect	indirect (and direct by filtration)[3]
Dissolution/ extraction	—	solution in 10 ml of 0.9% NaCl by soft heating (≤40 ˚C), stirring with maximal energy in a lab mixer for 4 min (the filter surface with the bacterial deposits shall not stick to the container wall). Serial dilution is possible	—
Analytical method	cultivation of viable bacteria on CASO agar[4] (plates or strips) at 30 ˚C for 72 hrs, aerobe, 3 replicates counting of macroscopically visible colony forming units (CFU), max. 5 cm^{-2} agar surface		
Cultivation agar	CASO (containing cycloheximide (0.3 gl^{-1})[4]		
Calibration of samplers	according to recommendations of the manufacturer		

Notes. 1—for names of manufacturers, see Table 4; 2—inhalable particle size according to Standard No. EN 481:1993 [22] must be sampled; 3—fluid is filtered through membrane filters (pore size 0.45 µm), which are placed in an agar plate; 4—CASO (Tryptic Soy Agar, TSA) with cycloheximide (gl^{-1}): peptone from casein 15.0, peptone from soymeal 5.0, sodium chloride 5.0, cycloheximide (actidione) 0.3, agar 16.0.

be transported to the analysing laboratory and must be processed within 24 hrs at the longest. Liquid samples have to be transported cooled at 4–8 ºC. Samples for direct processing will be incubated at defined temperatures after arrival in the lab. Filters used for sampling bacteria are dissolved or suspended in a 0.9% NaCl solution, depending on the kind of filter used (e.g., gelatine membrane filter, polycarbonate filter). Impinger solutions can be used for the inoculation of agar plates without further treatment. For indirect processing fluid samples are serially diluted with a 0.9% NaCl solution and then used for the inoculation of agar plates. The concentration of an impinger solution is also possible by filtration.

The concentration of airborne bacteria is calculated by dividing the volume of the air sampled into the number of macroscopically visible colonies of micro-organisms. Usually, the result of total bacteria counting is reported as colony forming units per cubic metre of air (CFU m^3). Table 5 summarises the most common in Germany procedure of sampling and analysing airborne bacteria at workplaces. Assessment of results is done by comparison with data from an outdoor reference measurement. Sterile controls must be handled in the same procedures for quality control.

Generally, the same bioaerosol samplers may be used for the analysis of airborne fungi and

TABLE 6. Procedure for Sampling and Analysing Airborne Fungi [26]

Part of Procedure	Requirements of Material and Methods
Sampler	Sartorius MD8, PGP, Gravikon PM 4
Sampling medium	membrane-filters: gelatine (pore size: 3.0 µm), polycarbonate, cellulose nitrate or –acetate (pore size: 0.8 µm)[1]
Sampling time	not defined, but ≥1 min, hours are possible
Preparation at the sampling location	direct processing: filters placed on agar plates indirect processing: filters are transferred in a liquid
Transport	as soon as possible. Conditions: dry (humidity <60 %), ambient temperature (<incubation temperature)
Working up of samples	direct and indirect
Dissolutione/extraction	solution in 10 ml of 0.9% NaCl with 0.01% tween 80 by soft heating (35–40 ˚C) for 15 min and stirring with maximal energy in a Vortex mixer for 4 min. Processing within 2 hrs after extraction. Preparing a serial dilution
Analytical method	cultivation of viable fungi on dichloran-glycerol (DG 18) agar at 25 ± 1 ˚C for 7 days in 3 replicates. Counting of macroscopically visible colony forming units (CFU). First counting after 24 (direct method) or 48 hrs (indirect method), afterwards each following or second day
Cultivation agar	dichloran-glycerol (DG 18) agar with chloramphenicol (0.1 gl^{-1})[2]
Calibration of samplers	not defined

Notes. 1—both cellulose-filters only for direct processing; 2—dichloran-glycerol (DG 18) with chloramphenicol (gl^{-1}): peptone 5.0, glucose 10.0, KH_2PO_4 1.0, $MgSO_4 \cdot 7\ H_2O$ 0.5, dichloran 0.002, chloramphenicol 0.1, agar 15.0, glycerol 18 vol.-%, pH 5.6 ± 0.2.

airborne bacteria (Table 4). The standard for analysing airborne fungi is based on sampling by filtration, cultivation on agar media and colony counting after incubation. The procedure is summarised in Table 6. In contrast to airborne bacteria, fungal particles are normally transferred into the air as spores which seem to be resistant to desiccation. Therefore airborne fungi may be sampled by filtration up to several hours in contrast to airborne bacteria.

For selective cultivation of xerophile fungi dichloran-glycerol (DG 18) agar (a_w = 0.95) containing chloramphenicol as an inhibitior of bacterial growth is used. Dichloran restricts the spread of hyphomycetes.

6. EXPERIENCE

There are no limits in Germany for the assessment of concentrations of biological agents in the workplace atmosphere. To assess the risk of the occurrence of biological agents at workplaces as it is stipulated in the German version of Directive 2000/54/EC [1], the so-called Biostoff-Verordnung [25], it is not necessary to carry out bioaerosol measurements. This is one reason for the lack of detailed standards measurements.

In 1997 a standard for the determination of the concentration of fungi in the workplace atmosphere was published in Germany as a technical rule for biological agents [26]. The content of this technical rule is identical with the standard for the determination of fungi concentration in the workplace atmosphere published in the BGIA Arbeitsmappe [17]. As a technical rule, this standard quickly became well-known and applied in most of the institutions carrying out measurements of fungi in the workplace atmosphere, e.g., laboratories on the market offering such measurements as well as governmental institutes for hygiene or for occupational safety and health. Therefore, there are a lot of comparable results for the concentration of fungi in the workplace atmosphere for some branches (e.g., waste handling). Based on these data, a criterion for assessing the effectiveness of technical measures to reduce the concentration of fungi in the workplace atmosphere called *TCV*, could be established [19]. With a *TCV* the concentration of biological agents in the air is established

with the use of state-of-the-art technology. This concentration is valid for a special working process or for a special type of installation. It may be defined as a sum of biological agents (e.g., bacteria, fungi) or referred to special groups of micro-organisms or species (e.g., all fungi or selected fungal species, e.g., fungi of the *Aspergillus* and *Penicillium* species). It shall be emphasised that a *TCV* is not based on any data referring to human health effects. Currently there is one *TCV* in Germany, which is valid for the handling of biological waste [27].

During the last decade of the 20th century a national working group of experts from different institutions dealing with occupational safety and health calculated some standards for the sampling of bioaersols at workplaces. Meanwhile all existing standards for measurements of biological agents at workplaces are not longer published as technical rules but only in the BGIA Arbeitsmappe [15, 17, 18]. They are available in print and as downloads[4]. However, information regarding the existence of such standards requires some publicising. This is a huge problem concerning the quality and comparability of the results of such measurements. Often neither the customers ask for the measurement procedure nor is this field of analytical service covered by any authority.

The interpretation of results generated by measuring and analysing biological agents is very difficult because generally valid dose–effect relationships are not known at present and it may be possible that they do not exist at all. Consequently, limits were not defined and the interpretation of exposure was based on the comparison of concentrations from the workplace on the one hand and from a reference place, free of contamination, on the other. Microbes are ubiquitous in nature and therefore airborne microbes are also present in the outdoor atmosphere, which is assumed to be free of contamination and therefore used as a reference. Bioaerosol components and concentrations vary widely within each environmental as well as occupational settings, especially in the environment are they also influenced by factors such as weather, climate, vegetation or season. The aim of the protection of workers is that the composition of the workplace atmosphere, including the concentration of biological agents, is no worse than local outdoor/environmental conditions. To receive comparable results the measurements at the workplace and at the reference point have to be under surveillance at the same time. Because of the aforementioned factors of variation it is accepted that replicate sampling at several days is not customary in bioaerosol assessment. In addition to comparing the results from the outside air with data from the workplace atmosphere, it is also necessary to compare the composition of the microbiological population in quality and quantity. A low concentration of airborne micro-organisms does not indicate a healthy workplace environment alone. Often only the total numbers of bacteria or fungi were enumerated by plate counts and it is obviously not possible to identify all species which form a colony, especially in bacteriology. Therefore only some representative colonies on inoculated plates may be classified. These colonies must be chosen carefully and the results of identification must be checked at least by a plausibility test in view of the environmental conditions of the sampling point and the biology of the micro-organisms. Consequently, for an interpretation of the results profound knowledge and experience of environmental microbiology in general and of microbial aerobiology in particular are absolutely indispensable. Currently these personal prerequisites for valid measurements are lacking in European as well as German instructions.

Another critical point, which makes it difficult to compare results of bioaerosol measurements, is that the currently published strategies and methods are not standardised in detail. This seems to be one of the main problems because all currently published analytical procedures depend on cultivation of airborne microbes in

[4] http://www.hvbg.de/d/BGIA/pub/mappe.html

the laboratory after sampling. Consequently, all factors influencing viability during sampling, transport and storage up to analysing as well as all factors influencing colony-forming by cultivation in the laboratory are of deciding importance for the quantification and characterisation of airborne bacteria and fungi. Generally, none of the well-known bioaerosol sampling methods (filtration, impaction or impingement) is suitable for measuring all types of microbes because important factors like the collection efficiency of the samplers, the media used for collection (filters, agar media or liquids), the collection time and the airflow rate differ for every measuring principle.

All these problems are well known and have been discussed elsewhere (e.g., [28]). Data from measurements may only be comparable if exactly the same sampler and analysing methods are used to give information about the same objectives.

Another problem is that detailed prerequisites of quality management are missing in current protocols. For the comparability of measurements quality-related measures for internal and external quality control (e.g., a quality management manual containing all standard working instructions, the organisational structure, responsibilities, etc., as well as a quality plan which describes the specific quality-related working procedures, auxiliary articles and activities, and participation in collaborative studies) in combination with quality surveillance and quality audit are also obligatory. Up to now, requirements for quality have not been defined and problems with quantification of microbes in collaborative studies have not been finally settled yet.

Notwithstanding, the problem has been recognised and a national group of experts have started to develop a concept on how to establish and to transfer quality criteria for risk assessment according to the protection of workers from risks related to exposure to biological agents at work.

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