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Indoor air —

Part 19: Sampling strategy for moulds

Air intérieur —

Partie 19: Stratégie d'échantillonnage des moisissures

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 16000-19 was prepared by Technical Committee ISO/TC 146, *Air quality*, Subcommittee SC 6, *Indoor air*.

ISO 16000 consists of the following parts, under the general title *Indoor air*:

— *Part 1: General aspects of sampling strategy*

— *Part 2: Sampling strategy for formaldehyde*

— *Part 3: Determination of formaldehyde and other carbonyl compounds in indoor air and test chamber air — Active sampling method*

— *Part 4: Determination of formaldehyde — Diffusive sampling method*

— *Part 5: Sampling strategy for volatile organic compounds (VOCs)*

— *Part 6: Determination of volatile organic compounds in indoor and test chamber air by active sampling on Tenax TA® sorbent, thermal desorption and gas chromatography using MS or MS–FID*

— *Part 7: Sampling strategy for determination of airborne asbestos fibre concentrations*

— *Part 8: Determination of local mean ages of air in buildings for characterizing ventilation conditions*

— *Part 9: Determination of the emission of volatile organic compounds from building products and furnishing — Emission test chamber method*

— *Part 10: Determination of the emission of volatile organic compounds from building products and furnishing — Emission test cell method*

— *Part 11: Determination of the emission of volatile organic compounds from building products and furnishing — Sampling, storage of samples and preparation of test specimens*

— *Part 12: Sampling strategy for polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polycyclic aromatic hydrocarbons (PAHs)*

— *Part 13: Determination of total (gas and particle-phase) polychlorinated dioxin-like biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDDs/PCDFs) — Collection on sorbent-backed filters*

— *Part 14: Determination of total (gas and particle-phase) polychlorinated dioxin-like biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDDs/PCDFs) — Extraction, clean-up and analysis by high-resolution gas chromatography and mass spectrometry*

— *Part 15: Sampling strategy for nitrogen dioxide (NO_2)*

— *Part 16: Detection and enumeration of moulds — Sampling by filtration*

— *Part 17: Detection and enumeration of moulds — Culture-based method*

— *Part 18: Detection and enumeration of moulds — Sampling by impaction*

— *Part 19: Sampling strategy for moulds*

— *Part 23: Performance test for evaluating the reduction of formaldehyde concentrations by sorptive building materials*

— *Part 24: Performance test for evaluating the reduction of volatile organic compound (except formaldehyde) concentrations by sorptive building materials*

— *Part 25: Determination of the emission of semi-volatile organic compounds by building products — Micro-chamber method*

— *Part 26: Sampling strategy for carbon dioxide (CO_2)*

— *Part 28: Determination of odour emissions from building products using test chambers*

The following parts are under preparation:

— *Part 21: Detection and enumeration of moulds — Sampling from materials*

— *Part 27: Determination of settled fibrous dust on surfaces by SEM (scanning electron microscopy) (direct method)*

— *Part 29: Test methods for VOC detectors*

— *Part 30: Sensory testing of indoor air*

— *Part 31: Measurement of flame retardants and plasticizers based on organophosphorus compounds — Phosphoric acid ester*

— *Part 32: Investigation of constructions on pollutants and other injurious factors — Inspections*

Introduction

Mould spores and metabolites can be inhaled via the air and cause allergic and irritating reactions and/or complex symptoms in humans. Moreover, mould growth can be associated with severe odour nuisances. In rare cases, some mould species can cause infections (so-called mycoses) in certain risk groups.[14][18][19]

There is sufficient epidemiological evidence that damp and mouldy buildings increase the risk of respiratory symptoms, respiratory infections and enhances asthma symptoms of the occupants.[8] In addition, there is some evidence for increased risk of development of allergic rhinitis and asthma. Furthermore, there is clinical evidence for rare symptoms like allergic alveolitis, chronic rhinosinusitis and allergic sinusitis. Toxicological studies *in vivo* and *in vitro* show irritating and toxic reactions of microorganisms (including spores, cell components and metabolites) from damp buildings.[8]

Growth of microorganisms in damp buildings can lead to increased concentrations of spores, cell fragments, allergens, mycotoxins, endotoxins, β-glucanes and MVOC (microbial volatile organic compounds). From the studies conducted so far it is not clear which compounds are the causative agents of the health effects observed. Nevertheless, increased concentrations of each of these compounds are considered a potential health risk[8][18] and growth of mould in buildings should, therefore, be avoided.

The prime objective of this part of ISO 16000 is to provide assistance in identifying mould sources in indoor environments.

Indoor air —

Part 19: Sampling strategy for moulds

1 Scope

This part of ISO 16000 describes the measurement strategy for the detection of fungi in indoor environments.

It describes suitable sampling and analysis methods together with a description of the applicability and the interpretation of the measurement results to maximize the comparability of the measured data obtained for a given measurement objective. It does not include details on recording building characteristics or field inspections by qualified professionals which have to take place prior to any microbiological measurement.

This part of ISO 16000 is not applicable to a detailed description of the building physics- and building-engineering-related procedures applicable to field inspections. The methods and procedures presented do not allow quantitative exposure assessment with regard to the room occupants.

The application of this part of ISO 16000 presupposes the knowledge of ISO 16000-1.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 16000-16, *Indoor air — Part 16: Detection and enumeration of moulds — Sampling by filtration*

ISO 16000-18, *Indoor air — Part 18: Detection and enumeration of moulds — Sampling by impaction*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
pre-existing mouldy condition
desiccated “old” mould growth, where additional biomass growth no longer occurs and the indoor air mould spore concentration gradually decreases with time

3.2
biological preservation efficiency
capacity of the sampler to maintain the viability of the airborne microorganisms during collection and also to keep the microbial products intact

[SOURCE: EN 13098:2000[6]]

NOTE The biological collection efficiency considers the sampling stress occurring during sampling and analysis in addition to the physical collection efficiency.

3.3
identification of moulds
assignment of moulds to spore types or groups on the basis of defined properties (e.g. morphological, biochemical, molecular-biological properties)

NOTE The term “differentiation” is frequently used instead of identification. The term “differentiation” is, however, misleading because the intention is not to merely differentiate the moulds but to identify them, i.e. to assign them, e.g. to genera or species.

3.4
filamentous fungus
fungus growing in the form of filaments of cells known as hyphae

NOTE 1 Hyphae aggregated in bundles are called mycelia.

NOTE 2 The term “filamentous fungi” differentiates fungi with hyphal growth from yeasts.

3.5
filtration
collection of particles suspended in a gas or liquid by flow through a porous medium

[SOURCE: EN 13098:2000[6]]

NOTE In this part of ISO 16000, filtration is understood as the separation of microorganisms or moulds from a defined volume of air by means of filters.

3.6
total spore count
number of (culturable and non-culturable) spores that are collected and enumerated under the microscope

NOTE For the term “spores”, see 3.19, Note 2.

3.7
yeast
unicellular fungus that does not normally produce a mycelium and reproduce by budding (budding fungi) as against moulds, which reproduce by sporulation

3.8
impaction
sampling of particles suspended in air by inertial separation on a solid surface (culture medium or adhesive-coated slides)

NOTE 1 See 16000-18.

NOTE 2 Sampling is carried out using either round-hole or slit impactors, for instance. As the air passes through the orifices, it is accelerated and the particles are impacted on the medium located directly behind the nozzles as a result of their inertia, while the air flows around the culture medium and exits the sampler. Impaction samples are only suitable for direct analysis without further resuspension of the sample.

3.9
colony forming unit
cfu
⟨air quality⟩ unit by which the culturable number of microorganisms is expressed

[SOURCE: EN 13098:2000[6]]

NOTE 1 One colony can originate from one single microorganism, from aggregates of many microorganisms as well as from one or many microorganisms attached to a particle.

NOTE 2 The number of colonies can depend on the cultivation conditions.

3.10
colony morphology type
group of colonies which due to their morphological appearance seem to belong to a specific species

3.11
colony count
⟨air quality⟩ number of all microorganism colonies visible on a culture medium after incubation under the selected cultivation conditions

3.12
culturable mould
mould that can be cultured under the selected cultivation conditions

NOTE Parameters governing the culturability are, for instance, the type of culture medium and the incubation temperature.

3.13
cultivation
growing of microorganisms on culture media

3.14
mycotoxin
secondary metabolites of moulds which are toxic to humans and animals

3.15
mycelium
total of fungal hyphae

3.16
non-culturable mould
mould that cannot be cultured under the selected cultivation conditions

3.17
physical sampling efficiency
capacity of the sampler to collect particles with specific aerodynamic diameters suspended in air

[SOURCE: EN 13098:2000,[6] modified — "aerodynamic diameters" has replaced "sizes".]

3.18
sampling stress
damage suffered by the microorganisms during sampling (e.g. through mechanical and chemical effects or through water deprivation)

3.19
mould
filamentous fungi from several taxonomic groups, namely *Ascomycetes*, *Zygomycetes*, and their anamorphic states formerly known as *Deuteromycetes* or fungi imperfecti

NOTE 1 Taxonomically, moulds do not represent a uniform group.

NOTE 2 Moulds form different types of spores depending on the taxonomic group they belong to, namely conidiospores (conidia), sporangiospores or ascospores. In practice, all these reproductive stages are summarized under the term “spores”.

3.20
mould damage
damage caused to building materials and surfaces by mould growth

NOTE Mould damage can result in loss in value, health risks and restrict the occupancy of the affected sites.

3.21
secondary colony
colony that does not originate from the “primary” sampling of airborne spores but from a spore released from a colony growing on the agar plates

3.22
secondary contamination
mould contamination of surfaces not caused by mould growth but originating from a (contaminated) primary source after aerial dispersion

3.23
cut-off value
particle size (aerodynamic diameter) for which the sampling efficiency is 50 %

3.24
total sampling efficiency
product of the physical sampling efficiency and the biological preservation efficiency

[SOURCE: EN 13098:2000[6]]

4 Properties, origin and occurrence of moulds in indoor environments

Moulds are ubiquitous on our planet. They are involved in the decomposition of organic material and, therefore, play an important role in the natural carbon cycle. Their concentration in the ambient air depends, *inter alia*, on location, climate, time of the day and season. Airborne mould concentrations are subject to great variability.[9][10][11] This is due to the following reasons.

The mould concentration in local ambient air is mainly determined by the location relative to the respective mould sources, wind direction and wind force. Mould spores are frequently released by specific sources such as decaying material. Both natural processes and production processes, such as composting, recycling, animal production facilities, grain and food processing plants as well as horticulture facilities, can be sources of mould dispersion.

Sporulation, i.e. the production of mould spores occurs discontinuously. It is governed, *inter alia*, by the mould lifecycle phase, the environmental conditions, stress factors, humidity as well as substrate composition and availability.

Factors governing the dispersion of spores, most of which have aerodynamic diameters in the range of 2 µm to 40 µm, are mechanically or thermally induced air movements, drying phases (leading e.g. to de-agglomeration of deposited dust) and the capability of air dispersal of the mould spores.[12][13][14]

Due to the ubiquitous nature of moulds, it can be assumed that they are always present in indoor air. The presence of moulds in indoor air can be due to spores originating from ambient air on the one hand and to recent active mould growth, pre-existing mouldy conditions or mould deposits (settled spores) on the other. To distinguish between sources, it is, therefore, important to perform ambient air measurements for reference whenever conducting indoor air measurements for moulds.[14][15] In addition, the collection of a control sample from a suitable reference room may be helpful.

Possible causes of indoor mould sources are surface moisture on building materials or moisture in the building structure, but also rotting food, potted plants, biowaste collection, source separation of waste, deposited dust due to poor cleaning as well as the keeping of animals in residential settings. Moisture damage can be attributable to building defects, inappropriate ventilation and heating or unfavourable arrangement of furniture as well as water damage (e.g. plumbing leaks or flooding events). Elevated mould levels in indoor environments and the occurrence of certain mould species (see Annex A) are indicative of excessive moisture. When residential environments or occupational settings are infested with moulds, the mould source shall be located to be able to plan remedial measures.

Main factors affecting the intensity of mould growth and the mould species developing are moisture, temperature, nutrient supply and the pH. If environmental conditions are favourable, a great variety of moulds can develop. Once environmental conditions become less favourable, the species best adapted to the given conditions will predominate.[16]

Mould sources can release spores, mycelial fragments, but also cell components and metabolic products such as β-glucans (polysaccharides contained in the cell wall of fungi), ergosterol (steroid compound contained in the cell membrane of fungi), toxins and MVOCs (microbial volatile organic compounds such as certain aldehydes, alcohols, esters, ketones). On cultivation, colonies can grow not only from spores, but also from mycelial fragments.

The number and airborne dissemination of spores released vary with the type of mould damage. For an assessment of indoor mould sources, it is, therefore, important to differentiate the individual mould species by their type of spore dispersal. Experience has shown that even minor mould contamination of materials can result in elevated indoor air mould levels if the species involved have dry spores with good air dispersal capabilities (e.g. *Penicillium* and *Aspergillus).* By contrast, airborne spore concentrations are much lower when materials are colonized, for instance, by moulds of the genera *Acremonium*, *Fusarium* or the species *Stachybotrys chartarum* that have relatively large spores embedded in slimy substances and, therefore, have poor air dispersal capabilities.

Furthermore, it should be taken into account that mould spores are not necessarily present as individual spores in the air or settled dust, but also occur in the form of spore aggregates or are particle-borne. Depending on the analysis method, they are determined individually or as spore aggregate. Materials, indoor air and house dust contain not only culturable but also non-culturable mould spores, some of which can have the same allergenic and toxic effects as culturable spores. For this reason, techniques have been developed that allow the microscopic determination of both culturable and non-culturable moulds.

Mould detection and identification are performed either after cultivation based on morphological criteria, biochemical reactions and/or molecular techniques or by direct microscopic examination. Identification based on the morphological structure (macroscopic examination, stereo-microscopy and microscopy) either after prior cultivation or by direct microscopy is still the most prevalent approach for the detection of moulds.

Besides, there are other analytical methods based on the determination of cell components and metabolites of moulds such as β-glucans, ergosterol, toxins and MVOCs.[17] The determination of these compounds serves, however, only as supplementary information.

The sampling methods employed for detection of moulds are determined by the objective of the investigation. Depending on the sampling method, the moulds suffer a sampling stress during sample collection and preparation, which can lead to their drying-out or dying. Factors affecting the culturability of mould spores are their physiological state as well as the culture medium employed. Some mould species cannot be cultured at all under laboratory conditions.

NOTE The genera *Stachybotrys* and *Chaetomium* hardly grow and sporulate only poorly, if at all, on DG18 agar. The use of this culture medium for culture-based analysis of these genera is therefore not recommended (see ISO 16000-17).

For a literature summary see References [8]–[10], [12], and [14]–[18].

5 Sampling and detection methods

Depending on the objective of the investigation, materials (see ISO 16000-21, in preparation), air (see ISO 16000-16 and ISO 16000-18) and house dust may be sampled and analysed for culturable moulds (see ISO 16000-17). Moulds can also be quantified and, to some extent, differentiated without prior cultivation. For this purpose, airborne mould spores are collected on filters or directly on an adhesive-coated microscope slide, followed by staining and subsequent direct microscopy.

Annex B gives an overview on the most common devices for total spore count measurements as well as for sampling devices for filtration and impaction and the respective analysis methods.