Evaluation of a method to collect mycotoxins within the inhalable particulates fraction of indoor air via an IOM Multi-Fraction sampler

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Inhalable particles are collected via an IOM sampler containing a filter & foam used together

The IOM Multi-Fraction sampler was developed by the Institute of Occupational Medicine (IOM) in Scotland. It is internationally renowned as a suitable device for the collection of inhaled airborne contaminants for subsequent laboratory analysis.

The IOM Multi-Fraction sampler is alternately known as the IOM Dual-Fraction sampler, IOM Inhalable sampler or IOM Multidust sampler. The two key parts of the sampler are: the 25 mm filter membrane that is used for collection of the finest airborne particles, and the Multidust foam backing which is used to collect larger airborne particles that comprise a different mass "fraction", termed the coarse particles. The inhalable particulates can also be called a "fraction" comprising the sum of the two sets of particulates collected by the filter membrane and foam inclusively. Inhalable particulates were previously known, less elegantly, as "total particulates" and can still be thought of in that way. The strict conditions under which the IOM Multi-Fraction sampler collects the inhalable fraction are specifically: that the Multidust foam is analysed in a lab together with the contents of the filter membrane.

Air coming into the IOM Multi-Fraction sampler first encounters the foam, then it reaches the filter membrane after that. Although the Multidust foam backing allows fine particles of under 10 microns to pass through (*Kenny et al. 2001*), it catches particles in the range of about 100 microns down to 10 microns (*Kenny et al. 2001*). The IOM filter membrane then catches the remaining particles of about 10 microns and under that the foam didn't capture (*Kenny et al. 2001*). As such, the foam covers a further range of 90 microns that the filter isn't exposed to. (Table 1, columns 2 and 4).

Table 1. Particle-size criteria* versus appropriate IOM sample collection media for airborne particulate matter. Particle populations that are collected accumulate progressively towards the top of the table. The thoracic fraction contains the respirable plus more, while the inhalable contains the thoracic plus more. Use of the foam and filter together is required to collect the inhalable fraction.

1. Particulate population or "fraction"	2. Aerodynamic diameter ("size" range) of the sampled particulates	3. Hazardous deposition area	4. IOM sample-collection material to be analysed
Inhalable	0 - 100 microns	Anywhere in the respiratory tract, even the Head Airways Region	Multidust foam + filter together
Thoracic	0 - 25 microns	Anywhere within the lung airways and the gas exchange region	Filter
Respirable	0 - 10 microns	The gas exchange region	Filter

*Based on the ACGIH particle size criteria tables given in US Army Public Health Center 2022, supplemented with IOM information from Kenny *et al.* 2001.

Nominal size cut-offs for these media (foam and filter) in microns, are not strict size cut-offs, rather they are based on a 50% population cut-point for reference material collected mid-range such that oversampling and undersampling are avoided. Aerodynamic particle size does not assume sphericity.

The inhalable particle convention neatly graphs the expectation of the sizes of particles to be captured versus proportion of each of those populations to be captured by an efficient sampler. These criteria were agreed by the International Standards organisation in ISO standard 7708 (*ISO 1995*), as well as by the European Standards Organization in their standard EN481 (*CEN 1993*). The principle and approach of collecting the inhalable particles also accords with guidance from the American Conference of Governmental Industrial Hygienists (ACGIH) (*Phalen et al. 1988*). Table 1 details the area of the respiratory tract that inhalable particles deposit in (*Phalen et al. 1988*), that is, anywhere, versus thoracic and respirable particles which are deposited only deeper down by being small enough to penetrate further.

The vast majority of quantifiable airborne particles are from zero to 100 microns in size and are collected by the IOM sampler when both the Multidust foam and the filter are used. Particles of $\leq$ 100 microns (i.e. which fall under about 100 microns in size), comprise the inhalable fraction and are relevant to human physiology, in particular to potential health effects anywhere in the respiratory tract. The exact type of foam used matters in order to achieve correct sampling, and hence the Multidust foam has been standardized and kept constant by IOM since 2012. Foams purchased before 2012 are not suitable for Multi-Fraction sampling. Similarly, to accommodate the Multidust foam and filter combination, only IOM sampler cassettes manufactured after the year 2000 are suitable housings.

It is important to process both the Multidust foam and filter that are contained in the IOM Multi-Fraction sampler, in order to extract the contaminants from each, contaminants that were airborne before sampling was done. Together the deposited contents on those two collection-media constitute the inhalable fraction, as discussed above. Provided the foam and filter have been used together, then during subsequent analysis of both, all of the airborne contaminants that were present and associated with particles will be considered, rather than only a subset. In comparison, analysing only the filter contents, or only the foam contents, would yield in each case only a subset of the airborne materials that were present in the sampled environment. The parts of the Multi-Fraction sampler, and diagrams for its assembly using the Multidust foam and filter together are shown in Figures 1 and 2 respectively.

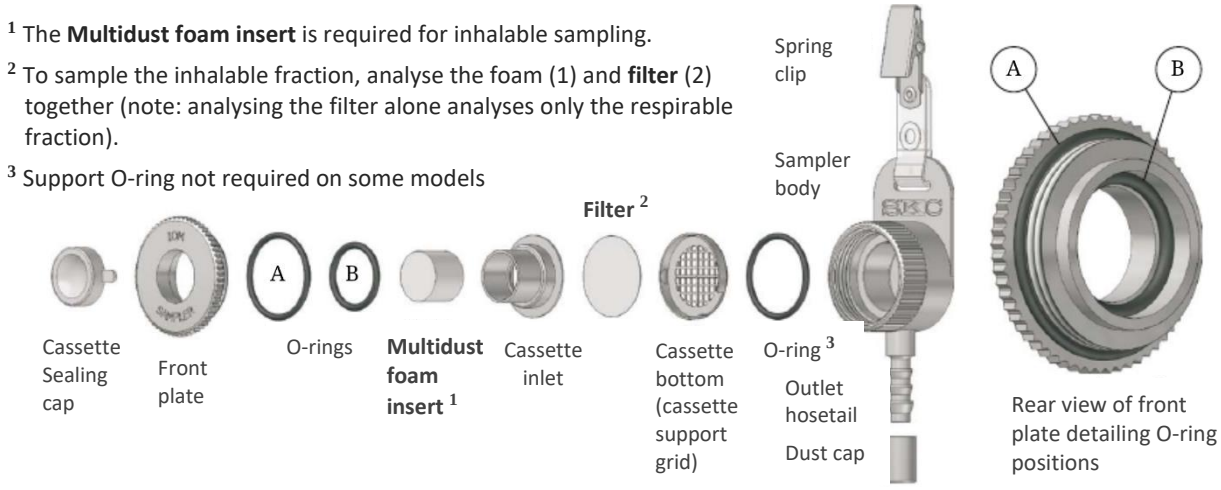

Figure 1. Parts of the IOM Multi-Fraction sampler including Multidust foam and filter.

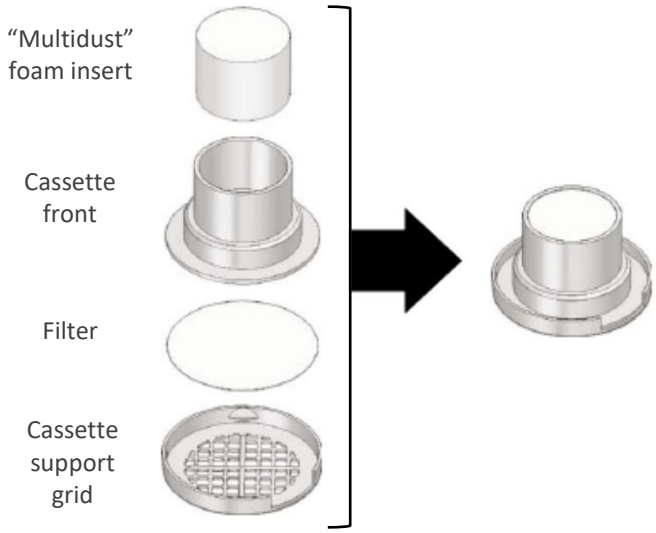

Figure 2. Assembly using the Multidust foam & filter together in the IOM Multi-Fraction sampler.

It has been established that airborne mycotoxins can be collected in polyurethane foam (*Ndaw et al. 2021*). Polyurethane is the type of foam used in the IOM sampler. The demonstration by Ndaw *et al.* was possible even though the sizes of particles that carry mycotoxins in the air have not been established. What is clear, is that mycotoxins within airborne particulates may potentially adsorb to the Multidust foam in the IOM Multi-Fraction sampler. Specifically, for argument's sake, the subset of mycotoxins that may adsorb to the foam may be those that are carried on large particles of 10 to 100 microns. However, it is not just the particle size that may be determinative. The surface area and surface character of the particles, together with the surface area and surface character of the polyurethane foam may also each play a part in binding mycotoxins, given that even dissolved ochratoxin can bind to polyurethane foam (*Ponce et al. 2023*). The polyurethane-foam approach to the collection of airborne mycotoxins is promising because polyurethane foams are already in use for collection of airborne persistent organic pollutants (*Shoeib and Harner 2002*, *Jaward et al. 2004*). The likely involvement of the Multidust foam in efficient sampling of airborne mycotoxins, does not nullify the need for the filter to be processed as well, because many filter types are known to collect and effectively retain mycotoxins, and also because the objective is to catch as many of the mycotoxin-associated particles as possible.

Typical collection equipment and sampling rate for inhalable aerosols

Sampling guidelines for inhalable aerosols are specified in standards and guidelines that determine how exposure testing should be carried out. These standards and guidelines include:

- ACGIH, Particle size-selective sampling in the workplace: 1985 (USA)
- NIOSH, Particles not otherwise regulated, Method 0500: 1994-2016 (USA)
- Health and Safety Executive, method MDHS 14/4 - General methods for sampling and gravimetric analysis of respirable, thoracic and inhalable aerosols: 2014 (UK)
- CEN, Technical Report 15230 - Workplace atmospheres - Guidance for sampling of inhalable, thoracic and respirable aerosol fractions: 2005 (Europe)
- CEN, Technical Report 13205 - Workplace exposure - Assessment of sampler performance for measurement of airborne particle concentrations: 2014 (Europe)

- Australian Standard 3640 - Workplace atmospheres - Method for sampling and gravimetric determination of inhalable dust: 2009 (Australia)

The IOM Multi-Fraction sampler is a suitable collection device for the inhalable fraction within these guidelines, provided that the wind velocity indoors is not expected to be excessive. The IOM sampler head (with Multidust foam and filter) is used in conjunction with a vacuum pump which is specific for the air-sampling industry. Each pump is calibrated by the user before being put to work, and in practice the sampling rate of the pump is generally constrained to exactly 2 liters of air per minute.

The sampling rate of 2 liters of air per minute is the flow rate originally suggested by IOM (*Mark and Vincent 1986*) for the Multi-Fraction Sampler (*Kenny et al. 2001*) while operating to collect inhalable aerosol (<100 microns inclusive) and is still regarded as the most valid flow rate.

The rate of sampling affects the efficiency of adsorption of airborne particulates onto the 25 mm IOM filter and onto the Multidust foam. Higher flow rates would require a larger diameter filter. Overall, the type of material comprising the filter and the foam, the pressure produced inside the sampler at the recommended flow rate, and the quantity of surface area available for collection of material on the filter and foam all affect the efficiency of the capture process, hence accuracy of the subsequent measurement or analysis process. For instance, at the most basic level it is important not to use too high a flow rate, so that sample-breakthrough is avoided. Similarly, if the flow rate is too low then aerosols may not be drawn into the sampler efficiently, and hence not quantitatively.

The rate of air sampling for IOM personal inhalable air sampler has been set at 2 liters per minute ever since the IOM device was first launched in 1986 (*Mark and Vincent 1986*) and has been permitted at that rate in many concordant standards and methods published since then, relating to the sampling of airborne particulates (for example see Table 2, 3rd column).

For a fuller insight into how the 2 liters per minute sampling rate was arrived at, consider that even by the year 2012 (*Sleeth and Vincent 2012*), the testing of inhalable samplers still involved placing each sampler onto a breathing dummy, one that “breathes” at a naturalistic rate of 6 liters to 20 liters per minute. The principle of that study and of similar studies, is that in many industries where occupational exposure is of concern, the sampler is placed just below the mouth and nose, specifically at the chest lapel, because at this location and flow rate, the sampler pump does not overpower the natural forces of the nearby breathing. That is, if the sampling rate is gentle enough, then any measured contaminants will closely resemble in every way those contaminants that are being breathed (*Vaughan et al. 1990*), this is because the sampling procedure is not disrupting the accurate estimation of the amounts of contaminants that otherwise would be breathed, say in a situation where the sampler is not present. Put in another way, it is not the objective of inhalable sampling to sequester any of the material that would have been breathed in: the sampler is not a filter or mask, rather it is intended to accurately sample the contents of the air.

Additionally, researchers need to first agree on baselines or assumptions, in order to model further from those, and once a baseline methodology is set then it is very inconvenient to change it, because such a change would be disruptive to industry. The prescribed flow rates and prescribed volumes collected are just such baselines. If flow rates, total volumes, sampling times, methodologies, and filter media for measuring airborne contaminants have all been correctly taken into account, then individual researchers are able to relate the results of each other’s research to their own experiments. Less direct comparisons than that, come with risks of misinterpretation. Therefore, careful researchers who are unwilling to reinvent the wheel, may tend to stick to prescribed parameters when sampling airborne contaminants.

Pumps that operate at 2 liters per minute, and that can be calibrated as such are readily engineered. Nevertheless, other pumps are also available that can achieve higher flow rates of 5, 10 or even 15 liters per

minute, and yet the gold standard remains 2 liters per minute for inhalable samplers and 2.2 liters per minute for cyclone samplers (specialized respirable samplers). All good-quality pumps need to be able to maintain a consistent flow rate and to cope with the pressure drop imposed by the cassette and the media.

Studies that utilise high flow rates of around 10 liters per minute tend to use larger diameters of collection filters (hence larger surface areas), such as a 37 mm filter (*Anthony et al. 2016*, *L'Orange et al. 2015*, *Stewart et al. 2017*). The larger diameters of filters used in such studies can mean that the results obtained are not directly comparable to studies that use the 25 mm diameter filter.

It is also relevant to consider that sampling reliability requires that a sufficient volume of air be filtered to enable the collection of enough material to analyse, and hence to give sufficient scope for quantitative accuracy. This is especially so considering the limit of detection and error margin of the analysis method that is chosen following collection. The range of air volumes typically recommended for collection and subsequent analysis of airborne particulates is approximately in the range 5 to 1000 liters. The IOM Multi-Fraction sampler running at 2 liters per minute, with a collection time of 1 hour, collects 120 liters of air, an order of magnitude which falls squarely within typically recommended limits.

Table 2. Table of selected NIOSH collection methods[#] that specify mutually similar collection times and volumes to collect samples for analysis of elements and compounds which are either present on airborne particulates or which comprise airborne particulates.

NIOSH method number	Method title	Sampling rate required (liters per minute)	to collect the required volume of air (liters)
0500	Particles not otherwise regulated	1 to 2	17 to 333
7906	Particulate fluorides	1 to 2	15 to 1000
5026	Oil mist, Mineral	1 to 3	20 to 500
7502	Zinc oxide	1 to 3	10 to 400
7013	Aluminium and compounds	1 to 3	10 to 400
7030	Zinc and zinc compounds	1 to 3	2 to 400
5007	Rotenone (a pesticide)	1 to 4	8 to 400
5001	2,4-D (a herbicide)	1 to 3	15 to 200
5515	Polynuclear aromatics	2	20 to 1000
2560	1-Nitropyrene in diesel particulates	1 to 2	480 to 960
5800	Total polycyclic aromatic compounds	1 to 2	5 to 1000

[#] (*NIOSH 1994-2016*). Analysis techniques specified within these NIOSH methods are not discussed in this table nor in the accompanying text, rather only collection parameters are discussed.

The US National Institute of Occupational Safety and Health (NIOSH) sampling methods (*NIOSH 1994-2016*) specify parameters to be used to collect airborne contaminants such as the contaminants listed in Table 2, a tabulation which includes many particulates (substances that form particles, as well as substances that may reside on particles). The selected set of methods shown gives some sense of how a key method - Particles Not Otherwise Regulated (PNOR) - may have been arrived at. That is, the other methods in such a table can be used as physical and chemical examples. Key method PNOR in turn may be used to guide collection parameters for additional substances that are of a particulate nature. For instance, technically speaking, airborne mycotoxins fall within Particles Not Otherwise Regulated.

The second half of Table 2 contains examples of substances that, while they are airborne on particulates, are actually chemical in nature such as pesticides and herbicides. Such substances typically have an "amphiphilic" characteristic that enables them to cross biological membranes, or enables them to target proteins that are present on biological membranes. These physical properties are shared by many mycotoxins, hence the sampling parameters that are necessary, are also comparable to the sampling parameters used for the herbicides and pesticides. In practice, when sampling aerosols in an environmental-hygiene context, a flow rate of 2 liters per minute allows detection of ochratoxin A with a suitable sampler (*Skaug et al. 2000*, *Pottier et al. 2014*).

Among a set of eight personal inhalable samplers tested by the European Commission and reviewed in their early classic study (*Kenny et al. 1997*), six of these samplers operate at 2 liters per minute (generally with a 25 mm filter but up to a 50 mm filter), while the remaining two samplers operate at 3.5 and 10 liters per minute (with a 37 mm filter, and a rotating foam in the shape of an annular prism, respectively). Even among samplers that operate at 2 liters per minute, not all managed to match the inhalable convention curve established by ISO/CEN, meanwhile the IOM sampler (operating at 2 liters per minute and fitted with the 25mm foam and 25mm filter) did match that convention well with greater accuracy and precision than other samplers tested (*Mark and Vincent 1986*, *Kenny et al. 1997*).

Active sampling is suitable for inhalable sampling in enclosed spaces

An "active" sampling methodology is suitable for sampling airborne contaminants in enclosed spaces. This is because the vast majority of homes and offices have calm air, are relatively airtight, and the air movement which occurs is largely due to occupation, but that air movement is not equivalent to outdoor air movement speeds, hence particles settle readily.

Active sampling means that the level of air movement present is controlled and is non-zero. Further, it means that as far as practical, every part of the air in the room or workspace gets agitated, and so has non-zero air movement, just prior to sampling. Control of the pre-sampling method in this way is a necessity because, in the indoor environment, particles from around ~30 microns to ~100 microns may settle quickly and so are undersampled in the air column unless an active sampling method is used.

By comparison, if the air movement in a home is achieved by either of the more common agitation means, that is by occupation, or by Heating Ventilation Air-Conditioning and Cooling (HVAC), which both generate a consistent if gentle internal airflow, and if either of those modes of air movement are normally plentiful, then those forms of agitation could mimic active sampling, just not reproducibly. Active sampling is therefore the preferred method, because it generates reproducible conditions (*Efthymiopoulos et al. 2023* and references therein, *Rylander et al. 1999* and references 11-18 therein) and correctly samples the contaminants that are consistently airborne, together with the contaminants that could become airborne (*Brasel et al. 2005*). Among the contaminants that are airborne and can become airborne, are the mycotoxins (reviewed in *Hope and Hope 2012*).

The effect of wind velocity (strong unidirectional or shifting air movement) on sampling has been assessed over nearly four decades and is an issue in need of discussion when technicians are sampling to test for potential occupational exposure to particulate matter outdoors (where wind is present), but drafts are much less of an issue for indoor sampling of particulate matter. Professionals in the industrial hygiene industry have found that the IOM sampler performs competitively well with other samplers at draft velocities that may be encountered indoors: velocities as low as 0.1 meters per second (*Sleeth and Vincent 2011*). The IOM inhalable sampler also performs well when an artificially imposed draft velocity is up to 1 meter per second (*Kenny et al. 1997*). As such, even if HVAC is present indoors, then the air movement

created by the HVAC is generally not an obstacle to IOM sampling, especially if locations nearby to a vent are avoided when selecting sampling sites.

Issues of indoor drafts and their potential effect on sampling are largely countered by the active sampling principle. Active sampling conditions are relatively easy to achieve in any indoor situation, while they are either less easy to achieve, or even unable to be achieved, in outdoor situations.

It can be important to use a Multi-Fraction sampler together with active sampling if wishing to quantify the full amount of any given contaminant that is present in the environment and that can be airborne. For instance, that way, when the same environment is subsequently tested at a second date by that same method, some certainty will be present to guide the subsequent comparisons. Namely, because the air column contents were sampled under the same conditions (actively) at both time points, and if a remediation has occurred, or any other condition has been deliberately changed between those time points, then no material that could become airborne at either time point has been missed, and so a quantification of airborne contaminants by comparing across the two time-points will be maximally meaningful.

Balancing the recognition of a need for the inhalable fraction to be sampled and actively, it must also be clearly understood by hygienists and toxicologists that the inhalable fraction is defined as what can be taken in by mouth and nose, and that the inhalable fraction represents a greater quantity, and at greater particle sizes, than the quantity that can actually reach the alveoli of the lungs. However, the ACGIH specifies a definition of inhalable to be the fraction that is able to be “deposited” anywhere in the respiratory tract (Table 1, column 3), a definition which has important implications. Deposition implies some capacity of material to adsorb or adhere to the respiratory tract and accordingly it implies at least some capacity for assimilation of the carried organic/hazardous materials by the body, given that such assimilation into the physiology is not precluded once deposited (*Phalen et al. 1988*).

In one approach to respirable air sampling, hence outside the scope of the method evaluated here but useful as a contrast, some researchers use an IOM sampler to collect the respirable fraction only. These researchers use the foam during sampling, but they correctly refrain from processing the foam of the IOM Multi-Fraction Dust sampler, and by this approach they selectively analyse the contents of the filter only. That selective approach is best practiced only by qualified experts. As an example of why that approach is best left to specialists, consider that some proportion of incoming airborne particles are known to adhere to the cassette surface rather than to the filter, and only practitioners with considerable experience could attest that the important material had not adhered to the cassette surface, or else they could attest that it may be necessary to harvest sample material from the internal surfaces of the cassette as well as from the appropriate capturing media.

By comparison, when performing inhalable sampling with the IOM sampler, the presence of a Multidust polyurethane foam within the IOM sampler may substantially lessen the confounding factor of some incoming material adhering to the sampler cassette, and so the use of that foam to augment the filter is a valuable approach. The foam has extra value in binding (collecting) things that can otherwise bind directly to the cassette surface. When the foam and filter are subsequently processed for analysis together, a range of potential inaccuracies and a range of assumptions are conveniently countered and avoided.

All in all, one appropriate solution to sampling of indoor particulates is to sample the totality, i.e., the inhalable fraction. An inhalable sampling procedure more fully samples the environment than respirable sampling does, and arguably inhalable sampling is also more readily achieved and replicated. Similarly, active sampling (agitating all of the air in an enclosed space) is reproducible and applies well to situations where inhalable particle sampling is being practised.

Sample-collection time, height, and sampler orientation

The sample collection time used in the method presented here is 1 hour, long enough to collect the mycotoxin-associated particles from the actively conditioned air column. Shorter sampling periods may involve a risk of not collecting a representative sample. During the 1-hour sampling, any lamination of the air in a room, or any other non-homogeneity of distribution across the space, is essentially accounted for: as particles of different masses settle at different rates and all particles are dispersive in character (will spread over time).

Crucially, during active sampling, periods of longer than 1 hour do not yield contaminant levels that vary in proportion to the time taken. Rather, 1 hour is enough and is also the amount of time required to get a sufficient sample when the flow rate is 2 liters per minute. Longer sampling than 1 hour, from an active start, may yield progressively less contaminant per equivalent unit of time thereafter. The time period of 1 hour is necessary and sufficient for active sampling to be effective. By comparison, researchers undertaking passive sampling may sometimes be constrained to testing for an 8-hour occupational exposure or a 24-hour exposure, by statutory requirements: these typically involve either measuring particulate weights, or the quantification of contaminants specific to that workplace.

The IOM Multi-Fraction sampler is operated at approximately mouth-and-nose height (*HSE 2014*, *Mark and Vincent 1986*, *Sleeth and Vincent 2012*), so as to sample the breathed component of the air in the room. It is important to maintain this height for the full hour. Accordingly, regardless of sampler type, the sampling head should be immobilised at that height during collection (*Fischer et al. 2000*, Chew *et al.* 2006, *Brasel et al. 2005*, *Charpin-Kadouch et al. 2006*).

Convention is, that the sampler be mounted so that its opening (the cassette front plate in Figure 1) faces directly outward (facing the horizon) (*Mark and Vincent 1986*, *Sleeth and Vincent 2012*, *Chew et al. 2006*), not angled up or down. This convention is strictly followed in order to prevent undersampling or oversampling. Movement of particles with diameters greater than 2 microns is generally downward by gravity when in low wind conditions, such as occurs in the majority of homes and offices. Undersampling would result if the cassette front plate faces downward and is unable to collect particles that have vectors which travel towards the ground, while oversampling would result if the cassette front plate faces upward and preferentially collects particles that have vectors which travel towards the ground. In this sense, the cassette front plate can be thought of as a receptacle into which particles could fall, and either the undue involvement of the falling process in collection or neglect of the effects of the falling process, during collection are both best to be excluded as much as possible, in order to make sampling methods comparable across sites.

Application

Application of the IOM Multi-Fraction sampler to establish the presence or absence of airborne mycotoxins indoors was successfully achieved by 21st Global Pty Ltd (Laboratory Division) from 2020-2023 (data not shown), is ongoing, and may be published elsewhere. Parameters used by that group when using the IOM Multi-Fraction sampler are listed in the summary below, adhering to 1-hour sampling at 2 liters per minute along with the other parameters specified here.

The collection method and conditions used and outlined in the summary below are reported to have enabled subsequent sensitive and quantitative analysis of the airborne mycotoxins targeted (21st Global Pty Ltd [Laboratory Division] pers. comm.[4]), so facilitating the monitoring of indoor environments before and after remediation, under comparable conditions between the two time-points.

[4] (personal communication)

Summary

The sampling criteria stipulated here require an adherence to the set of conditions described above, which are summarised as follows. The method utilises, without exception, all of:

- IOM Multi-Fraction sampler with both filter and foam analysed†, for total inhalable particles
- Active sampling
- 1-hour sampling time
- Sampling to be performed at chest or chin height of an adult-human.
- Sampler outlet is to face directly outward, not up or down

Comparison of results obtained in mold-affected homes to results obtained in control homes with no known history of mold, have shown that naturally-occurring background levels of airborne mycotoxins exist (21st Global Pty Ltd [Laboratory Division] pers. comm.). When all comparable samples are collected using the methodology that is the subject of this evaluation, then the background levels of airborne mycotoxins previously established by the same method can be used as a guide by which to compare against mycotoxin levels present in air samples obtained in mold-affected homes. However, it is important to note that no safe or unsafe levels of any airborne mycotoxin has yet been established in any nation.

It is critical that the control samples from unaffected homes need to have been quantified with the very same parameters, media and equipment as the samples from affected homes are quantified. Adherence to the parameters, media and equipment specified in this summary enables the natural background levels of airborne mycotoxins, and the airborne mycotoxin levels found in mold-affected homes, to be compared equally.

† Laboratory-supplied cassettes for indoor airborne mycotoxin sampling are prepared under cleanroom conditions and contain both a 25mm Multidust polyurethane foam and a 25mm PVC filter that are specific to IOM cassettes. Adherence to these same preparation conditions is imperative when self-assembling cassettes.

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