HHS Public Access

Author manuscript

Inhal Toxicol. Author manuscript; available in PMC 2017 September 01.

Published in final edited form as:

Inhal Toxicol. 2016 September ; 28(11): 500–507. doi:10.1080/08958378.2016.1210702.

Evaluation of sampling methods for toxicological testing of indoor air particulate matter

Jenni Tirkkonen[1], **Martin Täubel**[1,2], **Maija-Riitta Hirvonen**[1,2], **Hanna Leppänen**[1,2], **William G. Lindsley**[3], **Bean T. Chen**[3], **Anne Hyvärinen**[2], and **Kati Huttunen**[1]

[1]Department of Environmental Science, University of Eastern Finland, Kuopio, Finland

[2]Department of Health Protection, Living Environment and Health Unit, National Institute for Health and Welfare, Kuopio, Finland [3]National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, WV, USA

Abstract

There is a need for toxicity tests capable of recognizing indoor environments with compromised air quality, especially in the context of moisture damage. One of the key issues is sampling, which should both provide meaningful material for analyses and fulfill requirements imposed by practitioners using toxicity tests for health risk assessment. We aimed to evaluate different existing methods of sampling indoor particulate matter (PM) to develop a suitable sampling strategy for a toxicological assay. During three sampling campaigns in moisture-damaged and non-damaged school buildings, we evaluated one passive and three active sampling methods: the Settled Dust Box (SDB), the Button Aerosol Sampler, the Harvard Impactor and the National Institute for Occupational Safety and Health (NIOSH) Bioaerosol Cyclone Sampler. Mouse RAW264.7 macrophages were exposed to particle suspensions and cell metabolic activity (CMA), production of nitric oxide (NO) and tumor necrosis factor (TNFα) were determined after 24 h of exposure. The repeatability of the toxicological analyses was very good for all tested sampler types. Variability within the schools was found to be high especially between different classrooms in the moisture-damaged school. Passively collected settled dust and PM collected actively with the NIOSH Sampler (Stage 1) caused a clear response in exposed cells. The results suggested the higher relative immunotoxicological activity of dust from the moisture-damaged school. The NIOSH Sampler is a promising candidate for the collection of size-fractionated PM to be used in toxicity testing. The applicability of such sampling strategy in grading moisture damage severity in buildings needs to be developed further in a larger cohort of buildings.

Keywords

Indoor air; *in vitro*; moisture damage; particulate matter; sampling; toxicity testing

---

Address for correspondence: Jenni Tirkkonen, MSc, Inhalation Toxicology Laboratory, Department of Environmental Science, University of Eastern Finland, PO Box 1627, Kuopio FI-70211, Finland. Tel: +358 403553031. jenni.tirkkonen@uef.fi.

Declaration of interest

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Introduction

Due to the adverse health effects linked with exposure to indoor air particularly in moisture-damaged buildings (WHO, 2009) and the high prevalence of moisture observations in building stock (Haverinen-Shaughnessy et al., 2012), it is crucial to be able to identify the buildings that are most likely to cause health issues to the occupants. Since the measurement of microbial markers has been insufficient for identifying moisture damaged buildings linked with ill health, other ways to assess indoor air quality have been explored, including toxicity assays measuring the biological response, e.g. cell cultures. These studies have suggested that the toxicity and inflammatory potential of airborne dust *in vitro* might reflect the biological activity of the exposure (Huttunen et al., 2008, 2010). However, assessment of the indoor air quality with the help of toxicity assays has been hindered by the lack of sampling methods specifically tailored for the needs of toxicological assays; most of the available methods have been developed for microbiological or chemical analysis of the samples. As a result, they may include the source for artifacts such as remnants of filter material or extraction buffer, which need to be considered when applying these methods for toxicological characterization.

Airborne particulate matter (PM) can be collected actively by using devices such as impactors, cyclones, impingers or filters (Frankel et al., 2012; Jantunen et al., 2002; Wang et al., 2015). There are also passive collection methods that represent essentially different ways of collecting airborne dust settling onto surfaces. These approaches include collecting dust in cardboard boxes (Hyvärinen et al., 2006a), in dustfall collectors (Würtz et al., 2005), onto electrostatic cloths (Noss et al., 2008) or in Petri dishes (Adams et al., 2015). Passively settled dust is also part of the house dust reservoir that can be sampled by vacuuming directly from carpets, furniture or floors into filters, tubes or nylon sampling socks (Arbes et al., 2005; Casas et al., 2013; Leppänen et al., 2014; Pitkäranta et al., 2011).

When considering a suitable sampling method for toxicological analysis of the indoor PM, the main aim should be to capture the inhalable particles with the least possible interference caused by the sampling itself and without modifying the biological activity of the collected material. Actively collecting size-fractioned PM from the air is considered to be the closest representation of inhalation exposure, albeit missing volatile compounds contributing to the total exposure. However, actively collecting airborne dust is labor-intensive and the sampling parameters such as choice of filter material, sampling time and impaction velocity may affect the amount and quality of the sample.

Sampling dust reservoirs such as floors or beds is a poor candidate for toxicological testing due to a significant contribution from particles that are not considered to be relevant for inhalation exposure. These include coarse particles carried indoors by shoes or clothing, or particles originating from occupants themselves that do not get airborne. Dust samples vacuumed from furniture or wiped from surfaces are likely to contain contaminants from surface materials, introducing artifacts into toxicological assays. Lack of standardization of the sampling surface makes comparisons between indoor environments problematic. Moreover, the “age” of dust in reservoirs is typically undefined, making it difficult to link the sample with exposure during a specific time period. Instead, collecting airborne settled

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

dust onto a standard surface with little chemical reactivity for a defined period of time overcomes these issues. This method is arguably a relatively easy and affordable way to collect sufficient amounts of sample material (Adams et al., 2015; Frankel et al., 2012; Täubel et al., 2011).

In this study, our goal was to find a practical sampling method for performing toxicological analysis of the indoor PM, which would ultimately allow differentiating moisture-damaged from non-damaged buildings. Altogether four sampling methods were tested in three sampling campaigns conducted in moisture-damaged and non-damaged school buildings. The applicability of the sampling methods was evaluated by assessing the immunotoxicological potential of the collected sample material and the reproducibility of the toxicity assay.

Materials and methods

Sampling

Two primary schools located in the close geographical vicinity in Central Finland were selected: one with reported water damages (index school) and another school with no reported moisture problems (reference school). The school buildings were similar in age, size and construction, but the number of pupils differed between the index and reference schools (176 and 343 pupils, respectively). In addition to moisture damage or mold problems reported by the school representatives, the buildings were inspected by a trained civil engineer to confirm the status of the school. Both schools were visited during active use of the school and within the same season. During three sampling campaigns, three active sampling methods were tested and compared to one passive sampling method. The duration of sampling was adjusted according to pilot testing within a maximum time of 2 weeks. Four samplers were selected for testing as potential candidates according to literature and previous experience. The tested active collectors were the Button Aerosol Sampler (Button Sampler), the Harvard Impactor, the National Institute for Occupational Safety and Health Bioaerosol Cyclone Sampler (NIOSH Sampler). The passive method was the Settled Dust Box (SDB) sampler.

Sampling campaign 1—A Button Aerosol Sampler (SKC Inc., Eighty Four, PA) collected inhalable particles on polytetrafluoroethylene (PTFE) filters (pore size 0.45 μm). Samples were collected from three classrooms for 35 h (4 l/min) during 5 workdays from both schools.

Sampling campaign 2—A Harvard Impactor (Air Diagnostics and Engineering, INC., Naples, ME) collected $PM_{2,5}$ particles on PTFE filters (pore size 3 μm). Samples were collected from three classrooms for 74 h (10 l/min) during 10 workdays from both schools.

Sampling campaign 3—The NIOSH Bioaerosol Cyclone Sampler (CDC/NIOSH/HELD, Morgantown, WV) divides the particles into three fractions according to their size: Stage 1 (>1.9 μm, 1.5 ml tube), Stage 2 (1–1.9 μm, 1.5 ml tube) and Stage 3 (<1 μm, PTFE filter, pore size 0.45 μm). Samples were collected from two classrooms for 66 h (3.5 l/min) during 9 workdays from both schools.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Sampling campaign 2–3—The SDBs collect settling dust passively in cardboard boxes. Four boxes (450 × 200 × 60 mm) were placed into classrooms at a height of ~1.5 m on a shelf or mounted on the wall. The samples were collected from six classrooms in both schools. The classrooms were classified as damaged or non-damaged according to visible signs of moisture and/or mold odor. After 2 weeks, the settled dust was vacuumed onto fluoropore membrane PTFE filters (pore size 0.45 μm).

All samples were stored frozen (−20 °C) until toxicological analysis.

Cell culture

RAW 264.7 mouse macrophages (ATCC, Rockville, MD) were cultured in a humidified atmosphere with 5% CO_2 at 37 °C, using Roswell Park Memorial Institute 1640 (Gibco, Paisley, UK) cell culture medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine and 100 U/ml penicillin-streptomycin (all from Sigma, St. Louis, MO). The cells were seeded in 24 well plates at a density of 0.2×10^6 cells/well 1 day before the exposure.

Sample preparation

The filter samples were extracted into complete cell culture medium (2 ml per sample). The filter was gently washed with medium, sonicated for 15 min and shaken for additional 15 min. The medium was warmed up to 37 °C and the filter was removed. For the NIOSH samples collected directly into sampling tubes, the cell culture medium (1 ml per sample) was added to the tube, mixed thoroughly and warmed to 37 °C. A dilution series (1:2–1:32) of the sample was prepared with complete cell culture medium.

Mass and number of particles

Filters—All filters were weighed before and after the sampling by an XP105DR analyzing scale (Metler Toledo, Switzerland, readability: 0.01 mg; 0.1 mg). The samples were stabilized at a room temperature at least for 48 h and a static charge neutralizer (Ion-Care Stat-Pen, Sweden) was used before weighing. The range of the temperature in the weighing room was 20–23 °C (max ± 2 °C during 24 h) and the humidity range was 30–40% (max ± 5% during 24 h). The amount of dust collected with SDB is expressed as mg per sampler area (m^2), whereas the amount of dust collected with Button Sampler, Harvard Impactor and NIOSH Sampler (Stage 3) is expressed as μg per volume (m^3) of sampled air.

Tubes—The total number of particles collected with the NIOSH Sampler (Stages 1 and 2) and suspended in the medium were determined with a PAMAS SVSS particle counter (PAMAS GmbH, Rutesheim, Germany) using a SLS-25/25 sensor (size range 0.5–20 μm, maximum particle concentration 13 000 particles/ml) and PMA analyzing software. Fifty microliters aliquots from Stage 1 and 2 samples collected with the NIOSH Sampler were diluted 1:5000 in ultraclean water and the analysis of particle numbers were run in triplicate. The particle concentration in blank medium samples was subtracted from the results. The number of particles is expressed per volume (m^3) of sampled air.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Exposure of mouse macrophages

A duplicate set of cells were exposed to a dilution series of each dust suspension for 24 h. After the incubation, the exposure was terminated by resuspending the cells by scraping. A sample for assessing the metabolic activity of the cells was taken from the cell suspension and the rest was centrifuged (5 min at 6082 × *g*, 4 °C) to separate the cells from the medium. The inflammatory mediator nitric oxide (NO) was analyzed from the fresh medium and the remaining medium was stored frozen (−80 °C) until analysis of the proinflammatory cytokine tumor necrosis factor α (TNFα).

Toxicological analyses

Cell metabolic activity—The CMA describing the viability of the cells was measured with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which is based on the ability of the mitochondria in the cells to change MTT's color from yellow to purple. Procedures described earlier by Hansen et al. (1989) were modified by shortening the incubation with sodium dodecyl sulfate buffer to 4 h at 37 °C. The absorbance at 570 nm was analyzed with a multilabel plate reader ($Victor^3$, PerkinElmer, Finland) and compared to control samples.

Nitric oxide production—The inflammatory mediator NO produced by cells was analyzed with an assay based on Griess reaction (Green et al., 1982), where NO oxidized to nitrate is reduced to nitrite and reacts with arylamine, creating an aniline-colored azo chromophore. Following the addition of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2% phosphoric acid), the absorbance at 540 nm was measured with a multilabel plate reader ($Victor^3$) and compared with a standard curve for sodium nitrite.

Cytokine production—The concentration of the proinflammatory cytokine TNFα was determined with enzyme-linked immunosorbent assay according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). The absorbance at 450 nm was measured with a multilabel plate reader ($Victor^3$) and compared with a standard curve.

Statistical analysis

The normality of the data was tested with a Shapiro–Wilk test. The correlation between duplicate analyses was tested with a Pearson product-moment correlation test. The statistical significance of the difference between blank and exposed samples was tested with a non-parametric Wilcoxon Matched-pairs signed rank test, and the difference between repeated campaigns with a Mann–Whitney *U*-test (SigmaPlot™ version 12.3., Systat Software Inc., San Jose, CA).

Results

Repeatability of the toxicological analyses

A strong and highly significant correlation between the duplicate analyses was seen in all measured endpoints and sampler types (Table 1). The coefficient of variation (CV) of the duplicate analyses was typically less than 10% (average CV for all sampling methods 8.5%),

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

indicating good repeatability of the analyses. The repeatability of replicate samples collected from different classrooms within the same schools was clearly lower, average CV for different methods ranging from 16% to 50% (Button Sampler and SDB, respectively). The variability was typically higher in the index school regardless of the sampler type (Table 2).

The amount of dust in the schools

The mass of collected PM tended to be higher in the reference school than in the index school for Button Sampler, Harvard Impactor and SDB. The total particle count in suspension of the NIOSH samples (Stages 1 and 2) was higher in the samples from the reference school than in the index school for Stage 2 (1–1.9 μm particles), and lower in Stage 1 (>1.9 μm particles) (Table 3). The filter samples from Stage 3 (<1 μm particles) were weighed, but the mass was too small to produce reliable estimates (reference school 3 ± 2 $\mu g/m^3$, index school 2 ± 0.2 $\mu g/m^3$, $N = 2$).

Comparison of immunotoxicity of samples collected with different methods

Passive method (settled dust box)—Overall, the exposure of mouse macrophages to settled dust caused a dose-dependent and statistically significant decrease of metabolic activity of the cells and an increase in the production of the inflammatory mediators NO compared to blank samples (Table 4). The results of analyses of settled dust from the same classrooms from two subsequent sampling campaigns did not correlate strongly with each other (Supplemental material, Table S1).

Active collection (Button Sampler, Harvard Impactor and NIOSH Sampler)— The PM collected with active methods caused a decrease in metabolic activity and increase in production of inflammatory mediators in mouse macrophages compared to blank samples. The strongest response was seen for NIOSH Sampler Stage 1 (>1.9 μm particles), whereas the samples collected with NIOSH Sampler Stage 2 (1–1.9 μm particles) induced only minor effects on the exposed cells. Responses to blank (filter) samples from both Button Sampler and Harvard Impactor were slightly higher compared to blank (tube) samples from NIOSH sampler (Figure 1). The amount of sample in NIOSH Sampler (Stage 3) was insufficient for reliable toxicological analysis.

Comparison of immunotoxicity of samples from index versus reference building

We observed a trend for the higher immunotoxicological activity of the samples from index schools for some of the methods while acknowledging that sample numbers in these assessments were too low to produce reliable statistical estimates. In samples collected with the SDB method, we observed higher NO production induced by samples from the index school (Table 4). For the NIOSH Sampler, the trend toward higher immunotoxicological activity was seen both for Stage 1 (>1.9 μm) and Stage 2 (1–1.9 μm) particles from index schools, causing a slightly lower metabolic activity and higher production of inflammatory mediators NO and TNF α compared to the reference school (Figure 2).

Considering that the amount of dust was significantly different in the studied schools, we also adjusted the toxicity results for sample mass (for SDB, Button sampler and Harvard Impactor) or total particle count (for NIOSH sampler) to establish the relative

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

immunotoxicological activity of the collected material. The relative immunotoxicological activity of the dust from index schools tended to be higher than the dust from reference schools with all methods (Supplement material, Figures S1–S4). The greatest relative difference between the two schools was seen in samples collected with the NIOSH Sampler Stage 2 (1–1.9 μm particles) (Figure 3).

Discussion

Indoor exposures are notoriously complex, in particular, in the context of moisture damage and indoor dampness problems (Nevalainen et al., 2015). Targeting individual components of this exposure cocktail – such as microbes, toxins, volatile organic compounds, or PM itself – might underestimate the potential cellular and health impact of the combined exposures. Toxicological testing of indoor PM holds, therefore, promises to be useful in indoor assessments and in particular also in differentiating moisture-damaged from non-damaged buildings. A sampling method suitable for toxicological testing should collect a sufficient amount of airborne dust within reasonably short sampling period with easy-to-use equipment. Importantly, when assessing the health relevance of the exposure, the collected sample should represent the inhalable exposure agents present in moisture-damaged environments. In this study, we evaluated three active and one passive sampling approaches for their suitability in toxicological studies of the indoor PM. Our findings indicate that size fractionated sampling as performed here with the NIOSH Cyclone Aerosol Sampler could be a suitable sampling approach for toxicological testing of indoor PM, even though issues of yielding sufficient sample amounts and the potential to differentiate moisture damaged from non-damaged indoor environments are still to be resolved.

Biologically active material released due to the microbial growth in buildings could contribute to any of the size fractions within inhalable particles as spores, hyphae and bacteria can be fragmented and their metabolites carried along in small particles (ukiewicz-Sobczak et al., 2013). The difference in the immunotoxicological properties of the samples collected with the Button Sampler and the Harvad Impactor presumably reflects the difference in the mass and size of collected particles (<100 μm and $PM_{2.5}$, respectively). The measured responses to both of these sample types were relatively low and a slight increase in the baseline samples was seen, most likely due to the effect of filter material itself. Within the active methods, the PM collected with the NIOSH Sampler (Stage 1,>1.9 μm) induced the highest responses in the exposed cells. The immunotoxicological activity of the sample material collected in Stage 2 (1–1.9 μm) of the sampler was clearly lower, but the comparison of the school buildings suggested that the difference between the moisture-damaged and reference buildings might be more pronounced in this size fraction. Smaller particles are interesting also for their ability to stay airborne longer and penetrate deeper into the respiratory system, making it more likely to be exposed to the active components carried by small fragments. Compared to filter-based active collection methods, the advantage of the cyclone-based NIOSH Sampler was the possibility to increase the sampling time without fear of clogging the filter membrane, and the collection of sample material directly into the sampling tube, which avoids the possible interference caused by the filter material.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

We noted that the amount of dust collected with different sampling methods differed between the schools, being higher in the non-damaged school compared to moisture-damaged school in almost all cases. This complicates the comparison between samples from different schools because a high amount of dust is toxic to the cells regardless of the moisture damage status of the sampled building. In our study, the buildings were sampled within the same season, all sampled rooms were in active use and the number of pupils per classroom was similar in both schools. However, different aspects, such as ventilation conditions, exact sampling location or activity level of the pupils during school hours could affect the settling and accumulation of particles.

One challenge in establishing a method that could identify and even grade the severity of moisture damage in buildings is variability between sampling locations within the buildings. This implies that toxicological testing cannot replace the need for a thorough technical investigation of the building because the information about signs of moisture damage and dampness is required to decide on the appropriate sampling locations. Rather should the aim of toxicological testing of indoor PM be to support building investigations and facilitate decisions on prioritization and urgency of renovation actions. In our study, variation in toxicological response between different classrooms was high particularly in the moisture-damaged school, indicating that potential health impacts of moisture damage in a building may follow hot spots of the moisture problems or their manifestation. Variability within the toxicological analyses was small, suggesting good repeatability of the assessment method itself.

In terms of toxicity testing, the main issues, particularly with the active sampling methods, are low amounts of collected particles as well as the possible interference from the filter material itself. According to our results, the amount of the collected sample was high enough to induce a significant and dose-dependent increase in immunotoxicity measures in all tested sampling approaches although the responses were clearly lower for samples collected with the Button Sampler and the Harvard Impactor. For the NIOSH Sampler, the response was undetectable for the very small particle fraction (Stage 3,<1 μm particles), low for the mid-size fraction (Stage 2, 1–1.9 μm particles), and very high for the largest particles (Stage 1,>1.9 particles), presumably due to the higher mass of the larger size fraction. The differences seen between classrooms within the studied school buildings emphasize the importance of a robust sampling strategy including several sampling locations.

The amount of airborne settled dust collected with the passive SDB method over a sample accumulation time of 2 weeks was sufficient for the toxicity testing. However, the combined results of consecutive sampling campaigns did not differ clearly between the schools with different moisture damage status, indicating that the amount and properties of the dust varied between samplings. Interestingly, categorizing the results according to the damage status of the classroom showed a difference in the toxicological properties of the dust within the building, suggesting that the moisture damage in one part of the building may change the toxicological characteristics of the dust rather locally. Out of the tested methods, the SDB has the advantage of being an inexpensive and easy-to-implement method of collecting dust from indoor environments, proven to harvest many of the components typical for moisture-damaged environments and representing airborne dust better than floor dust (Hyvärinen et

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

al., 2006b). This sampling method has also been used earlier for studying toxicological characteristics of dust from moisture-damaged environments (Huttunen et al., 2016).

Conclusion

In conclusion, we identify several important issues when considering the suitability of a sampling approach to fulfill the needs of toxicity testing of the indoor PM. Those relate to the challenge of collecting sufficient sample amounts for the determinations, as well as to the obvious importance of a prudential sampling strategy given the variation in the biological response to indoor PM from different locations in a building. In addition, the ease of use as well as cost and time efforts of the sampling campaigns are relevant factors when it comes to actual field application by practitioners. Out of the tested sampling methods, size fractionating NIOSH Bioaerosol Cyclone Sampler was considered to be a promising approach. The possibility to further increase sample amounts and the potential of this approach to ultimately differentiate moisture damaged from non-damaged indoor environments are questions that will have to be answered by subsequent studies including a larger cohort of buildings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Matti Peltonen and Katja Saarnio for technical assistance and the participating schools for their collaboration.

Funding

This work was supported by Tekes – the Finnish Funding Agency for Innovation, New knowledge and business from research ideas – HOVAVART (Homevaurion vakavuuden arviointi toksisuustestin avulla) Grant agreement no. 2285733-9. Professor Maija-Riitta Hirvonen, Drs Martin Täubel and Kati Huttunen as well as doctoral students Jenni Tirkkonen and Hanna Leppänen reported funding from Tekes – the Finnish Funding Agency for Innovation, New knowledge and business from research ideas – HOVAVART (Homevaurion vakavuuden arviointi toksisuustestin avulla) Grant agreement no. 2285733-9. Dr Bean T Chen has U.S. Patent Nos. 8,205,511 & 7,370,543 issued. Drs Anne Hyvärinen and William G Lindsley reported no declarations of interest. Doctoral student Jenni Tirkkonen was also funded by The Finnish Work Environment Fund Grant agreement no. 115283.

References

Adams RI, Tian Y, Taylor JW, et al. Passive dust collectors for assessing airborne microbial material. Microbiome. 2015; 3:46. [PubMed: 26434807]

Arbes SJ, Sever M, Mehta J, et al. Exposure to indoor allergens in day-care facilities: results from 2 North Carolina counties. J Allergy Clin Immunol. 2005; 116:133–9. [PubMed: 15990786]

Casas L, Tischer C, Wouters IM, et al. Endotoxin, extracellular polysaccharides, and beta(1-3)-glucan concentrations in dust and their determinants in four European birth cohorts: results from the HITEA project. Indoor Air. 2013; 23:208–18. [PubMed: 23176390]

Frankel M, Timm M, Hansen EW, Madsen AM. Comparison of sampling methods for the assessment of indoor microbial exposure. Indoor Air. 2012; 22:405–14. [PubMed: 22299641]

Green LC, Wagner DA, Glogowski J, et al. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem. 1982; 126:131–8. [PubMed: 7181105]

Hansen MB, Nielsen SE, Berg K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J Immunol Meth. 1989; 119:203–10.

Haverinen-Shaughnessy U, Borras-Santos A, Turunen M, et al. Occurrence of moisture problems in schools in three countries from different climatic regions of Europe based on questionnaires and building inspections – the HITEA study. Indoor Air. 2012; 22:457–66. [PubMed: 22404345]

Huttunen K, Kaarakainen P, Meklin T, et al. Immunotoxicological properties of airborne particles at landfill, urban and rural sites and their relation to microbial concentrations. J Environ Monit. 2010; 12:1368–74. [PubMed: 20431840]

Huttunen K, Rintala H, Hirvonen MR, et al. Indoor air particles and bioaerosols before and after renovation of moisture-damaged buildings: the effect on biological activity and microbial flora. Environ Res. 2008; 107:291–8. [PubMed: 18462714]

Huttunen K, Tirkkonen J, Täubel M, et al. Inflammatory potential in relation to the microbial content of settled dust samples collected from moisture-damaged and reference schools: results of HITEA study. Indoor Air. 2016; 26:380–90. [PubMed: 25967114]

Hyvärinen A, Sebastian A, Pekkanen J, et al. Characterizing microbial exposure with ergosterol, 3-hydroxy fatty acids, and viable microbes in house dust: determinants and association with childhood asthma. Arch Environ Occup Health. 2006a; 61:149–57. [PubMed: 17867568]

Hyvärinen A, Roponen M, Tiittanen P, et al. Dust sampling methods for endotoxin – an essential, but underestimated issue. Indoor Air. 2006b; 16:20–7. [PubMed: 16420494]

Jantunen M, Hänninen O, Koistinen K, Hashim JH. Fine PM measurements: personal and indoor air monitoring. Chemosphere. 2002; 49:993–1007. [PubMed: 12492162]

Leppänen HK, Nevalainen A, Vepsälainen A, et al. Determinants, reproducibility, and seasonal variation of ergosterol levels in house dust. Indoor Air. 2014; 24:248–59. [PubMed: 24883434]

Nevalainen A, Täubel M, Hyvärinen A. Indoor fungi: companions and contaminants. Indoor Air. 2015; 25:125–56. [PubMed: 25601374]

Noss I, Wouters IM, Visser M, et al. Evaluation of a low-cost electrostatic dust fall collector for indoor air endotoxin exposure assessment. Appl Environ Microbiol. 2008; 74:5621–7. [PubMed: 18676704]

Pitkäranta M, Meklin T, Hyvärinen A, et al. Molecular profiling of fungal communities in moisture damaged buildings before and after remediation – a comparison of culture-dependent and culture-independent methods. BMC Microbiol. 2011; 11:235. [PubMed: 22017920]

Täubel M, Sulyok M, Vishwanath V, et al. Co-occurrence of toxic bacterial and fungal secondary metabolites in moisture-damaged indoor environments. Indoor Air. 2011; 21:368–75. [PubMed: 21585551]

Wang CH, Chen BT, Han BC, et al. Field evaluation of personal sampling methods for multiple bioaerosols. PLoS One. 2015; 10:e0120308. [PubMed: 25799419]

WHO – World Health Organisation. WHO guidelines for indoor air quality: dampness and mould. Copenhagen: WHO Regional Office for Europe; 2009.

Würtz H, Sigsgaard T, Valbjorn O, et al. The dustfall collector –a simple passive tool for long-term collection of airborne dust: a project under the Danish Mould in Buildings program (DAMIB). Indoor Air. 2005; 15:33–40. [PubMed: 15910527]

ukiewicz-Sobczak W, Sobczak P, Krasowska E, et al. Allergenic potential of moulds isolated from buildings. Ann Agric Environ Med. 2013; 20:500–3. [PubMed: 24069854]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

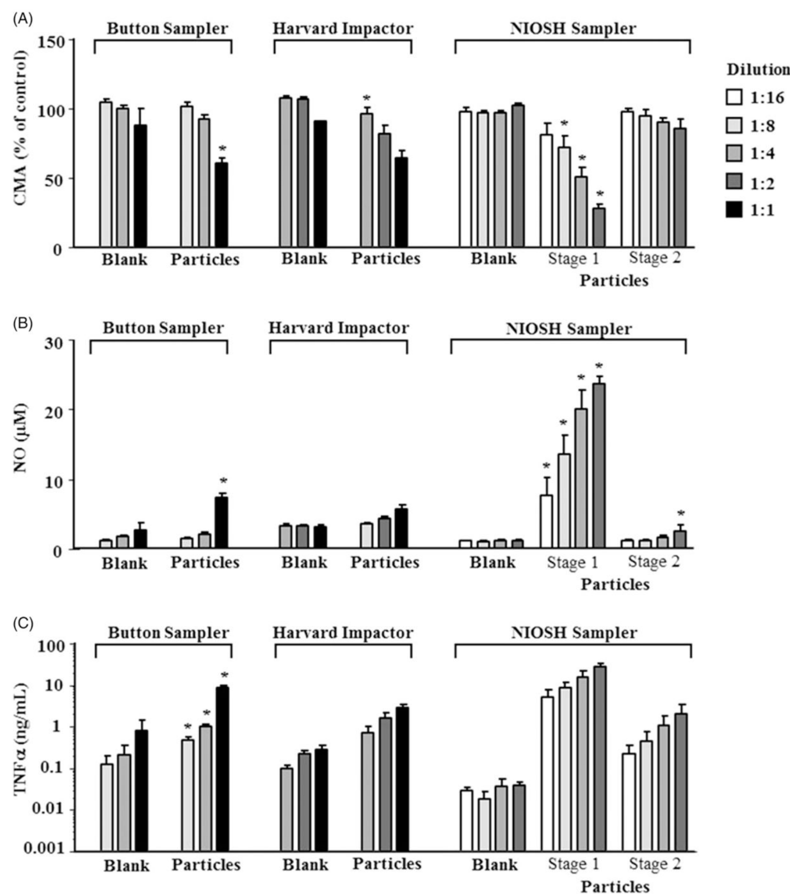

Figure 1.

Average (±SEM). (A) Cell metabolic activity (CMA), (B) NO production and (C) TNFα production of mouse RAW264.7 macrophages after 24 h exposure to increasing doses (dilutions 1:16–1:1) of dust collected with the Button Sampler ($N = 6$), Harvard Impactor ($N = 6$) and NIOSH Sampler ($N = 4$) from two school buildings. The results are compared to blank samples (extract from blank filters/tubes, $N = 2$). Star (*) indicates a statistically significant difference compared to respective blank samples (Wilcoxon matched-pairs signed rank test, $p<0.05$).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

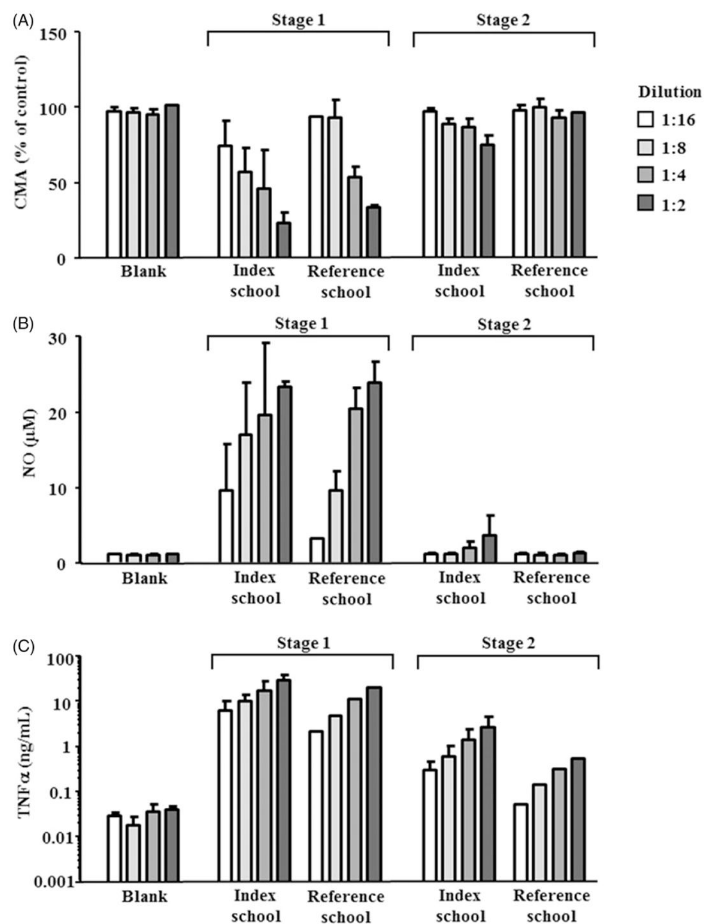

Figure 2.

NIOSH Sampler, Stage 1 (>1.9 µm particles) and Stage 2 (1–1.9 µm particles), sample collection for 66 h: Average (±SEM). (A) Cell metabolic activity (CMA), (B) NO production and (C) TNFα production of mouse RAW264.7 macrophages after 24 h exposure to blank samples (extract from blank tubes, $N = 2$) or increasing doses (dilutions 1:16–1:2) of dust from index school and reference school ($N = 2$, for TNFα in reference school $N = 1$).

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

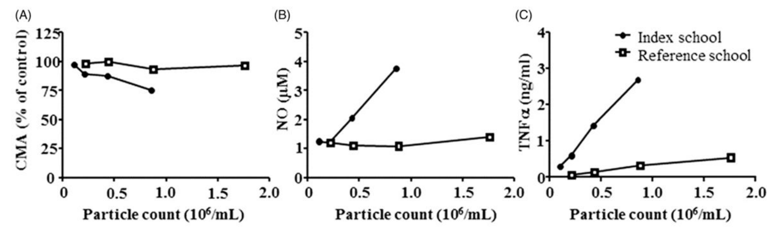

Figure 3.

NIOSH Sampler, Stage 2 (1–1.9 μm particles), sample collection for 66 h: Average. (A) Cell metabolic activity (CMA), (B) NO production and (C) TNFα production of mouse RAW264.7 macrophages after 24 h exposure related to total number of particles in the samples from index school and reference school ($N = 2$, for TNFα in reference school $N = 1$).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

Pearson correlation coefficients of duplicate analyses of samples collected with four different methods. All correlations are statistically significant (*p*<0.001).

	Cell metabolic activity		NO	
Sampling method	***R***	***N*[a]**	***R***	***N*[a]**
Settled Dust Box	0.93	134	0.88	111
Button Sampler	0.91	21	0.92	21
Harvard Impactor	0.94	22	0.97	22
NIOSH Sampler, Stage 1	0.98	18	0.98	22
NIOSH Sampler, Stage 2	0.91	20	0.98	24

[a]Number of duplicate pairs.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 2

Coefficient of variation (%) of replicate samples from different locations within one moisture damaged and one reference school building.

		Cell metabolic activity		NO		TNFα	
Sampling method	***N***	**Reference school**	**Index school**	**Reference school**	**Index school**	**Reference school**	**Index school**
Settled Dust Box, campaign 2	6	23	57	45	55	29	38
Settled Dust Box, campaign 3	6	18	31	85	79	53	91
Button Sampler	3	8.5	11	23	28	17	9.8
Harvard Impactor	3	9.6	19	9.6	21	76	83
NIOSH Sampler, Stage 1	2	15	47	25	55	_[a]	64
NIOSH Sampler, Stage 2	2	6.0	20	16	48	_[a]	99

[a]Only one sample available for analysis.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 3

Average mass or particle number (± SEM) in samples collected with four different methods during three sampling campaigns from one moisture damaged and one reference school building.

Sampling method	Campaign	Reference school (N^a)	Index school (N^a)	Unit
Button Sampler	1	70 ± 25 (3)	19 ± 2.1 (3)	$\mu g/m^3$
Harvard Impactor	2	4.0 ± 0.7 (3)	3.5 ± 0.7 (3)	$\mu g/m^3$
Settled Dust Box	2	18 ± 7.0 (6)	16 ± 6.0 (6)	mg/m^2
Settled Dust Box	3	18 ± 7.3 (5)	6.5 ± 1.1 (6)	mg/m^2
NIOSH Sampler, Stage 1	3	160 ± 63 (2)	225 ± 147 (2)	$10^3/m^3$
NIOSH Sampler, Stage 2	3	270 ± 119 (2)	121 ± 72 (2)	$10^3/m^3$

[a]Number of sampled classrooms.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

Table 4

Average (±SEM) cell metabolic activity and production of inflammatory mediators NO and TNFα of mouse RAW264.7 macrophages after exposure to blank samples or four doses of settled dust. Settled dust was collected using SDBs during two sampling campaigns from in total of 12 classrooms of one index school and 12 classrooms of one reference school.

	Dose	Reference school (*N* = 12)	Index school (*N* = 12)	Blank (*N* = 3)
Cell metabolic activity (%)	1:16	69 ± 2.4	69 ± 6.1	94 ± 4.0
	1:8	52 ± 3.1 *	56 ± 6.9 *	93 ± 10
	1:4	35 ± 3.2 *	40 ± 5.6 *	98 ± 1.4
	1:2	25 ± 2.1 *	28 ± 3.9 *	90 ± 3.2
NO (μM)	1:16	8.0 ± 1.0 *	7 ± 1.8	0.9 ± 0.5
	1:8	12 ± 1.3 *	10 ± 2.3	1.1 ± 0.2
	1:4	13 ± 1.4 *	12 ± 1.8 *	1.2 ± 0.2
	1:2	8.0 ± 0.8 *§	12 ± 1.5 *§	1.2 ± 0.2
TNFα (ng/ml)	1:16	6.1 ± 1.4	5.2 ± 1.8	0.1 ± 0.05
	1:8	9.6 ± 2.2	10.2 ± 3.5	0.1 ± 0.06
	1:4	21.5 ± 5.0	15.6 ± 4.5	0.2 ± 0.07
	1:2	39.7 ± 10.1	19.9 ± 4.6	0.3 ± 0.18

[*] Statistically significant difference compared to blank sample ($p<0.05$).

[§] Statistically significant difference between index and reference schools ($p<0.05$).