Assessing indoor air toxicity with condensate collected from air using the mitochondrial activity of human BJ fibroblasts and THP-1 monocytes

Heidi Salonen[1,2*], Tuula Heinonen[3], Marika Mannerström[3], Mark Jackson[2,4], Maria Andesson[1], Raimo Mikkola[1], Jarek Kurnitski[1,5], Shahana Khurshid[6], Atila Novoselac[2], Richard Corsi[2]

[1]Aalto University, Espoo, Finland
[2]The University of Texas, Austin, TX, USA
[3]University of Tampere, Tampere, Finland
[4]Daikin North America LLC, Waller, TX, USA
[5]Tallinn University of Technology, Tallinn, Estonia
[6]Consultant, Austin, TX, USA
[*]*Corresponding email: heidi.salonen@aalto.fi*

SUMMARY
The hypothesis for our study was that air and airborne moisture are carriers of possible toxic compounds and these compounds can be collected as condensate using dry ice. Using human cells, we aimed to evaluate the indoor air toxic risk from the condensed water collected from several office and laboratory rooms in two different buildings, both located at the University of Texas at Austin. Condensate samples were collected by using stainless steel collectors (E-collectors), and the effects of samples on the viability of human BJ fibroblast and activated THP-1-monocytes *in vitro* were investigated by using WST-1 cytotoxicity assays. Toxicological assessment utilizing human BJ fibroblasts and activated THP-1-monocytes of condensate from air reflects the acute toxic effects of the indoor air. The first results suggest that toxicological assessment of condensate collected from air using dry ice in stainless steel condensate collectors and human BJ fibroblasts and activated THP-1-monocytes as test organisms could be useful in detecting indoor air anomalies.

KEYWORDS
Indoor airborne toxicity, air samples, human BJ fibroblast, human THP-1-monocytes

1 INTRODUCTION
Air inside public buildings, such as schools and offices, typically consists of a complex mixture of primary and secondary air pollutants with different chemical and physical properties emitted or formed by a chain of reactions from a variety of sources (Wolkoff and Nielsen, 2001; Salonen, 2009; Nørgaard et al., 2014; D'Amato et al., 2015). Chemical emissions from building materials and cleaning agents as well as microbe-derived toxins (mycotoxins and endotoxins) are the key elements in contaminated indoor air (Kildesø et al., 2003; Jestoi, Ritieni and Rizzo, 2004; Andersson et al., 2009; Andersson et al., 2010; Verdier et al., 2014). In addition to air pollution, environmental characteristics, such as temperature, humidity and outdoor climate confound IAQ responses (Cheung et al., 2017; Liu, Zhong and Wargocki, 2017).

Indoor air quality with different air pollutants is a significant health concern, and there is awakened need to identify unhealthy buildings in order to detect problems and prevent health effects among the occupants. In this identification process, one of the crucial issues is sampling, which should provide meaningful material for analyses and fulfill ease-of-use requirements imposed by practitioners using toxicity tests for health risk assessment (Tirkkonen et al., 2016).

Previous studies including toxicity assays that measure the biological response, e.g. in cell cultures, 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; Huttunen et al., 2010). However, assessment of indoor air quality with the help of toxicity testing has been impeded by the lack of suitable sampling methods specifically tailored for the needs of toxicological assays (Tirkkonen et al., 2016). Generally, the available methods have been developed for chemical or microbiological analysis of the samples, and may include artificats e.g. remnants of filter material or extraction procedure, and should be considered when these methods are used for toxicological characterization (Tirkkonen et al., 2016).

In this study, we tested the cell toxicity of air samples collected as condensed water using dry ice and stainless steel collectors. Human BJ fibroblasts and THP-1 monocytes (differentiated towards macrophages), were exposed to the air samples, and the effects of air samples on the cell viability was investigated using WST-1 assay, an indicator of mitochondrial activity. This study revealed toxicities in some samples taken indoors, and proposes the need for additional investigations concerning the mechanisms behind the detected air toxicity.

2 METHODS

Study design, location, and room characteristics

Air samples (n= 40) from nine different rooms, located in two different university buildings at the University of Texas, USA, were collected by using stainless steel collectors (E-collectors, Elisa Aattela Ltd., Tampere, Finland). Most of the samples were collected in Building 1, which is a laboratory building with a small fraction of office space. All the sampled rooms had mechanical ventilation with 100% fresh air (there was no recirculation and no air mixing between labs or office rooms). In laboratory rooms the ventilation rate was in the range of 6 to 12 ACH per hour of outdoor air, while the office rooms had significantly lower rates (below 6 ACH, and it varied based on weather condition). In addition, samples were collected in Building 2, with mostly office spaces and a small number of classrooms and lab rooms. The sampling rooms were mostly office spaces with one laboratory room; all the sampled rooms had mechanical ventilation systems.

Water sample collection

Condensate samples were collected using dry ice in stainless steel collectors (Figure 1) (Elisa Aattela Ltd., Tampere, Finland; http://sisailmatutkimuspalvelut.fi/). Before sampling, the collector was thoroughly washed using dishwashing liquid and tap water, rinsed with distilled water and thoroughly dried. This collector was set in the object room on a stable surface (floor or table), dry ice grains were inserted into the collector's container, the lid was closed and room temperature and relative air humidity recorded. When the relative humidity and the temperature are recorded during the sample collection the volume of indoor air represented in toxicity assessment can be roughly defined, assuming few air currents.

The temperature near the collector's surface decreases due to the effect of dry ice (-79°C) and when the dew point is reached the air condenses to liquid water and freezes on the collector surface.

After 30 – 60 min, when the collector's surface was covered with frost, the lid was opened. Frost melts down and trickles on to the receiver. The collector box was then lifted away and water from the receiver was poured into Eppendorf tubes. Depending on temperature and relative humidity) from 0,5 to 1 ml of condensed water was collected in each sample. Two paral-

lel samples were collected from each sampling site and these two parallel samples were packed in the same sealed Minigrip plastic bag and frozen (−20°C) until shipped to the lab for toxicological analysis.

The Eppendorf tubes containing the condensate were shipped from Texas to FICAM (Finnish Centre for Alternative Methods) in a cooled container. Some of the Eppendorf tubes opened during the transportation (Room E, building 1,17th Feb 2017; Room F, building 1, 17th Feb 2017; Outdoor (17th Feb 2017 and 21st Feb 2017), and the samples leaked into the Minigrip bags, resulting in a mixture of parallel samples.

Figure 1. Air sampling using stainless steel collectors (E-collectors)

Toxicological analysis

The study was conducted according to the standard operating procedures in force at FICAM and in the spirit of good laboratory practice. Human BJ fibroblasts (Cat. No. CRL-2522) and human THP-1 monocytes (Cat. No. TIB-202) were purchased from ATCC (American Type Culture Collection, LGC Promochem AB, Boras, Sweden, www.atcc.org). The cells were tested for mycoplasma (MycoAlert, Lonza), and found to be mycoplasma-free.

For the cytotoxicity assays, the cells were seeded in 96-well plates as follows: BJ fibroblasts were seeded at a density of~4000 cells/well in 100 µl MEM (Minimum Essential Medium) supplemented with 10% FBS (Foetal Bovine Serum), 1% NEAA (Non-Essential Amino Acids) and 1% L-glutamine. THP-1 cells were seeded at a density of~10000 cells/well in RPMI medium (Roswell Park Memorial Institute) supplemented with 10% FBS and 0.5 µM PMA (Phorbol 12-myristate 13-acetate). All cell culture reagents were from Gibco Invitrogen (Carlsbad, CA, USA). PMA was from Sigma Aldrich, (Steinheim, Germany).

For the exposure, the medium was removed from the cells and replaced by 90 µl/cell with exposure medium, followed by 10 µl of the condensate sample. The exposure media were the same as the normal culture media, except they contained only 5% FBS and 200 U/ml penicillin streptomycin. (The water content in the cell exposure did not exceed 10%). The control cells were treated with 10% dH_2O. Each sample was tested in 6 parallels. The exposure times were 1 and 24 hours.

After 1 or 24 hours exposure to the condensate or to an equal amount of water (blank samples), the cell viability was measured using WST (Water Soluble Tetrazolium Salt)-1-assay. The assay principle is based on the conversion of the tetrazolium salt WST-1 into a colored dye by mitochondrial dehydrogenase enzymes. Hence, the assay measures the net metabolic

activity of cells, which reflects cell number. The absorbance of the reduced reagent was measured at 450 nm with a Varioskan Flash Multimode Reader. The WST-1 reagent was from Roche (Basel, Switzerland). The blank values (water, WST-1 reagent, but no cells) were subtracted from the absorbance data. The percent cell viability of cells treated with condensate samples were compared to respective (water-treated) controls was calculated for each test well (Microsoft Excel®). The statistical significance of the differences in viability between condensate treated cells and (water-treated) control cells was tested using t-test if the normality test of the data was passed and Mann-Whitney Rank Sum test if the normality test of the data was not passed. When $p < 0.05$, the difference in viability between condensate treated cells and control cells was considered statistically significant.

3 RESULTS

The test results are presented in Table 1.

Table 1. Test results. The air sample is considered toxic if the difference in the viability between condensate sample-treated cells and control cells is significantly different ($p< 0.05$). The % reduction of cell viability as compared to the control is given.

Sample 1:10 dilution	Toxicity to THP-1-cells No/Yes		Toxicity to BJ fibroblasts No/Yes	
Incubation time	*1 h*	*24 h*	*1 h*	*24 h*
		Building 1		
Room A (office space) (8th Feb 2017)	NO	YES 22.4 ± 7.4% (p<0.001)	YES 14.8 ± 10.7 % (p = 0.007)	YES 15.3 ±7.3% (p<0.001)
Room A (office space) (21th Feb 2017)	NO	YES 12.5 ± 11.0% (p=0.002)	NO	YES 6.8 ±10.1% (p=0.032)
Laboratory B (8th Feb 2017)	NO	YES 5.9 ±3.1% (p=0.016)	NO	YES 13.7 ±2.1% (p<0.001)
Room C (office space) (8th Feb 2017)	NO	YES 10.0 ±4.1% (p=0.001)		YES 13.0 ±4.4% (p< 0.001)
Laboratory D (8th Feb 2017)	NO	YES 27.5 ±14.7% (p<0.001)	NO	NO
Room E (office space) (17th Feb 2017)	YES 20.1 ±16.0% (p=0.021)	YES, 14.1 ± 0.7% (p<0.001)	NO	NO
Room F (office space) (17th Feb 2017)	NO	YES 7.6 ±3.1% (p=0.004)	NO	NO
Room G (office space) (17th Feb 2017)	NO	YES 5.5 ± 2.9% (p=0.021)	NO	NO
Room G (office space) (21st Feb 2017)	NO	YES 10.0 ± 6.2% (p=0.002)	NO	NO
Room H (office space) (17th Feb 2017)	NO	YES 9.7 ± 3,3% (p<0.001)	NO	NO
Room H (office	NO	NO	NO	YES

space) (21st Feb 2017)				9.5±8.7% (p=0.04)
Room I (office space) (21st Feb 2017)	NO	YES 5.9 ± 4.3% (p=0.026)	NO	NO
Outdoor* (8th Feb 2017)	NO	YES 8.6 ±1.8% (p=0.006)	NO	NO
Outdoor* (17th Feb 2017)	NO	NO	NO	NO
Outdoor* (21st Feb 2017)	NO	NO	NO	NO
		Building 2		
Room J (office space) (21st Feb 2017)	NO	YES 19.2 ±10.2% (p=0.001)	NO	NO
Room K (office space) (21st Feb 2017)	NO	NO	NO	NO
Room L (office space) (21st Feb 2017)	NO	NO	NO	NO
Laboratory M (21st Feb 2017)	NO	NO	NO	NO
Outdoor* (21st Feb 2017)	NO	NO	NO	NO

*outdoor air sampling was conducted next to the studied building

In general, human macrophages were more sensitive to the toxic effects of air samples than human fibroblasts.

Six of the samples, i.e. samples collected outdoors (next to the building 1, 17th Feb 2017 and 21st Feb 2017, and next to the building 2), rooms K (building 2), L (building 2) and M (building 2), did not affect cell (THP-1 and BJ fibroblasts) viability.

Six of the samples, i.e. samples collected from outdoors (next to the building 1, 8th Feb 2017), Room F (building 1), Room G (building 1), and Room H (building 1) reduced cell (THP-a or BJ fibroblasts) viability by a maximum of 10% as compared to the untreated controls.

Five of the samples, i.e. samples collected from Laboratory B (building 1), Room C (building 1), Room A (building 1, 21th Feb 2017) and Room J (building 2) caused 11-20% reduction in cell (THP-1 or BJ fibroblasts) viability as compared to the untreated control.

Three of the samples, i.e. samples collected from Room A (building 1, 8th Feb 2017), Laboratory D (building 1) and Room E (building 1), caused 21-30% reduction in cell (THP-1 and/or BJ fibroblasts) viability as compared to the untreated control.

4 DISCUSSION

The most common types of air pollutants encountered indoors are particulate matter (PM), gases such as ozone (O_3), nitrogen dioxide (NO_2), carbon monoxide (CO), and sulfur dioxide (SO_2); microbial and chemical volatile organic compounds (VOCs); and passive smoke

(Bernstein et al., 2008). Indoor exposure to complex mixtures of these agents may cause significant health effects among occupants (Nevalainen, Taubel and Hyvärinen, 2015) and targeting individual components of this exposure cocktail might underestimate the potential cellular and health impact of mixture exposures (Tirkkonen et al., 2016). Toxicological testing of condensed water therefore promises to be useful in indoor air quality assessment and may be an economic, high capacity and relevant tool among others to differentiate "healthy" air from "non-healthy" air.

A sampling method suitable for toxicological testing should collect in a standardized manner a sufficient amount of sample within a reasonably short sampling time with easy-to-use equipment. Importantly, when assessing the health risk, the collected sample should represent the inhalable exposure agents present in "unhealthy" environments. Condensed water samples used in this study presents the state of the indoor air at that moment. Recently, a study by Atosuo et al (2016) used similar stainless steel collectors (E-collectors), but the toxicity evaluation was performed by a different method; Atosuo et al (2016) tested the toxicity of the condensate water samples with the Escherichia coli-lux method and the toxicity was assessed by decreased bacterial viability. According to their study, the condensed water allows repeated and quantitative sampling for determination of possible toxicity of indoor air samples.

Although the condensed water collected with the stainless steel collectors describes the "acute" indoor states, the settled dust, on the other hand, is a cumulative product presenting the history, i.e., the chronic state of the indoor air (Atosuo et al., 2016). Tirkkonen et al (2016) tested the immunotoxicological activity of dust collected from moisture damaged and non-damaged buildings by using different active and passive sampling methods. The results by Tirkkonen et al (2016) suggested that dust collected from a moisture-damaged school had higher relative immunotoxicological activity than other sampled buildings. The usefulness of the stainless steel collectors as a tool for moisture damage identification requires further testing. Atosuo et al (2016) reported, that due to some unknown factor or substance, which they were not able to identify yet, samples from non-damaged houses usually give some cytotoxic response in their bacterial test, too. This unidentified toxic response may be due to cytotoxic wetting agents or cytotoxic antibacterials such as triclosan used in cleaning chemicals (Ajao et al., 2015; Castagnoli et al., 2018). This response is usually weaker compared to samples from damaged buildings.

The WST-1 cytotoxicity assay used in this study measures acute cytotoxicity providing information on how many viable cells are remaining after exposure to a foreign substance. Human BJ cells are human diploid foreskin fibroblasts (Bodnar et al. 1998), and they have been shown to predict acute toxicity of chemicals to human better than the mouse BALB/c 3T3 fibroblasts, (Mannerström et al. 2017) which are used in the cytotoxicity assay included in the OECD guidelines (OECD, 2010). However, when used alone BJ fibroblasts can over- or underestimate cytotoxicity as they lack many specialized cellular functions typical e.g. to immune cells. Immune cells of alveolar system have a key role in the toxicity of the airborne foreign substances, as respiratory system is one of their most important entry points (Hiraiwa and van Eeden 2013). Therefore we also used the THP-1 cell line in this study which is a human monocytic cell line derived from an acute monocytic leukemia patient (Tsuchiya et al 1980). After treatment with phorbol esters in culture, THP-1 cells differentiate into macrophage-like cells, which mimic native monocyte-derived macrophages in morphological and functional properties, including differentiation markers (Auwerx, 1991; Hjort et al. 2003; Kramer and Wray, 2002; Sakamoto et al. 2001; Tsuchiya et al. 1982; Ueki et al. 2002). In this study, the differentiated macrophages were exposed to air samples to mimic the actual situa-

tion where a person is exposed to foreign substances by inhalation. Macrophages were shown to be more sensitive to toxicity of indoor air samples than fibroblasts. This was expected as the role of macrophages is to respond to external stimuli initiating the inflammatory response (Sica and Mantovani 2012). Next, we will extend the test battery to focus further on the target organ i.e. lungs and to develop specific tests to find out the most prevalent mechanisms of toxicity. We will add human NHBE lung epithelial cell test and tests using cells transfected with different reporter genes for identifying the specific mechanisms of toxicity. This, in turn, would also help to identify the source of toxicity of the indoor air samples. For further identification of the harmful substances in indoor air samples we will fraction the samples by HPLC in order to identify the toxic fraction if possible with chromatographic methods (LC-MS).

Toxicological assessments should not replace the microbiological or chemical methods for indoor air quality investigations, but they can be one relevant tool among others to survey buildings and help to identify "healthier" and "unhealthy" buildings. Especially with cellular methods, one can reveal the mixture effect.

5 CONCLUSIONS

Our study demonstrated that laboratory tests with human BJ fibroblast and THP-1-monocytes in vitro using WST-1 assay are promising tools to identify potential toxicity in indoor air, which may cause health effects. The condensed water allows repeated and quantitative sampling reflecting the quality and possible toxicity of the indoor air and allows comparison to similarly sampled outdoor air. However, further studies with higher number of samples from different buildings and indoor spaces are needed. Comparing that data with the health status of the occupants will produce more evidence about the relevance and sensitivity of human cellular tests to predict toxicity of air samples, and finally give support to the suitability of including this new test method in the standard battery of tests for indoor air.

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