WATER VAPOUR MOBILISES BUILDING RELATED NON-VOLATILE CHEMICALS AND MYCOTOXINS AND MAY BE USED TO REMOVE SUBSTANCES OF POTENTIAL HEALTH HAZARD FROM INDOOR SURFACES

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SUMMARY

No inhalation toxicity assessment (H314) is currently required of non-volatile substances (European REACH). However, our study shows that non-volatiles can mobilise into humid air. Aerosolisation of potentially hazardous, medium to large molecular-size (300 – 1500 g/mol) substances relevant to indoor air quality were studied in glass test chambers. The test substances, which are classified non-volatile based on their large molecular structure and lack of vapour pressure data, were: 1) toxins of building colonizing moulds, 2) biocidal cationic antimicrobials, and 3) non-ionic tenside and wetting agent polyoxyethylene isotridecanol ether, widely used in building materials and a major constituent of indoor cleaning formulations.

Each test substance (0.9 – 30 mg) was dosed on a glass tray, placed on the chamber floor. Humidity was controlled by a humidifier and a dehumidifier and intermittent ventilation. Humidity-driven mobilisation of the non-volatile substances transferred the substances from the tray into chamber air, where it was detected with TVOC sensors. The water vapour was condensed in the dehumidifier, where the test substances could be detected using capillary electrophoresis analyser.

Humidification and dehumidification and ventilation removed the test substances from the trays. This protocol could be applied to contaminated indoor spaces during low activity hours to reduce human exposure the adverse substances.

Keywords: indoor air, wetting agent, alcohol polyethoxylate, Genapol, cleaning agent, quaternary ammonium, polyguanide, PHMG, PHMB, antimicrobial, ochratoxin A, mycotoxin, vaporisation, VOC sensors, capillary electrophoresis

1 INTRODUCTION

Building related adverse health symptoms (BRS, SBS) are common in Finland. According to official sources moisture and mould based indoor air problems in schools, kindergartens, care institutions and offices affect daily 235 500 – 361 000 persons (Paavilainen, 2017). A major part of the affected premises are spaces with occasional high human occupancy.

Airborne volatiles are known to connect to adverse health effects, but in a major part of the affected buildings symptoms persist in absence of these agents. Inhalation exposure by non-volatile substances is assumed to be unlikely. In general, non-volatile chemicals are exempted of inhalation toxicity assessment for EU registration. However, water vapour reportedly carries water insoluble (lipophilic) toxic substances. It has been shown that water vapour mobilises hydrophobic toxins in buildings, e.g. valinomycin and toxins of *Penicillium expansum* (Andersson et al., 2010, Salo et al., 2015). Objective of the current study was to measure humidity driven mobilisation of toxic, building related non-volatile chemicals and microbial metabolites.

2 METHODS

2.1 Equipment, field and chamber measurements

The measurements were conducted in enclosed all-glass chambers of 90 l volume and 0.8 l metal lid chambers (Figure 1). The lid was sealed airtight with adhesive aluminium tape. The chambers were equipped with sensors for recording TVOC, temperature and humidity values once a minute. Multiple sensors (2 or 3) were placed at different distances from the emission source, which was located on the chamber floor. The 90 l chambers were equipped with timer-controlled humidifier, switching on for 5 minutes at intervals of 90 minutes. The dehumidifier consisted of a Peltier cooler with heat sinks and two fans to maintain air turbulence inside the chamber and to cool the outside surface of the Peltier element.

Figure 1 Experimental chambers of this study. Small chamber, 0.8 l (left), aquarium chamber, 90 l.

Sensors used were Grove Temperature and Humidity Sensor Pro, TVOC sensors MICS-VZ-89TE by SGX Sensortech, Corcelles-Cormondreche, Switzerland and iAM TVOC sensors by AMS AG, Praemstetten, Austria. Both TVOC sensor types recorded airborne concentration of organic substances as CO_2 equivalents, with baseline (blank, zero emission) set at 400 ppm. Prior to each experiment, the baseline of the TVOC sensors was tested in ambient air (blank) and in humid air (RH 100%), to match the pre-set sensor baseline. Resistance of the MICS -VZ-89TE sensors increased upon aging, we only used sensors with resistance readings lower than 250 kOhm.

2.2 Chemicals and reagents used in this study

PHMG (polyhexamethylene guanidine chloride, CAS 138261-41-3) and PHMB (polyhexamethylene biguanide, CAS 27083; 32289-58-0) were 0.5% aqueous solutions purchased from A. Seppälä, Espoo, Finland and from Biovasa Oy, Turku, Finland respctively. These and similar other products were marketed in Finland by numerous companies for remediation of and prevention of building contaminating moulds at new and existing premises, and as disinfectants at public spaces. Genapol X080 (Sigma-Aldrich, 98%, v/v) is polyoxyethylene isotridecanol ether (CAS 9043-30-5, 552 g/mol). Didecyl-dimethyl-ammonium chloride (DDMAC) (CAS 7173-51-5; 362,08g/mol), a widely used biocidal detergent ("quat"). It was purchased as 50% (w/v) in isopropanol (Merck Schuchardt). Prior to the chamber experiments, the solvent was evaporated at +60°C in a fume hood until dry. Metabolites of building related moulds were purchased as purified substances: ochratoxin A of *Aspergillus ochraceus* (CAS 303-47-9; 403.81 g/mol, Acros Organics); emodin (>90%, HPLC, 270.24 g/mol, CAS 518- 82-1); enniatin B (CAS 917-13-5, 639.8 g/mol) from Alexis chemicals. 1-octen-3-ol (CAS 391-86-4, 96%, 128.22 g/mol, Sigma) was used as a reference for fungal mVOC. All substances were of analytical or technical products containing no fragrances or other ingredients.

3 RESULTS AND DISCUSSION

3.1 Non-volatile indoor chemical substances generate chemical fog when transiently exposed to humidity

The building associated chemicals, reportedly non-volatile, produced response in the TVOC sensors when relative humidity exceeded >50%. This was observed using air-tight, all-glass test chambers (Figure 1), where air was humidified in controlled manner at constant temperature. Figure 2 shows the plots of TVOC, air humidity and temperature after weighed amounts (0.3 - 30 mg) of Genapol X080, PHMG, PHMB or DDMAC were placed on a glass tray inside a chamber. Following periodic humidity increases, the test substances were emitted into chamber air in a pulsed manner. Airborne TVOC inside the closed chamber decreased each time when humidity of the chamber air decreased, indicating transient sorption of the substance onto the inner surfaces of the chamber. When the chamber lid was opened for ventilation, airborne test substances purged out of the chamber. When the tray held distilled water only or was empty, the TVOC sensors gave the baseline reading 400 ppm irrespective of the humidity. The background value 400 ppm is subtracted from Figures 2 and 3.

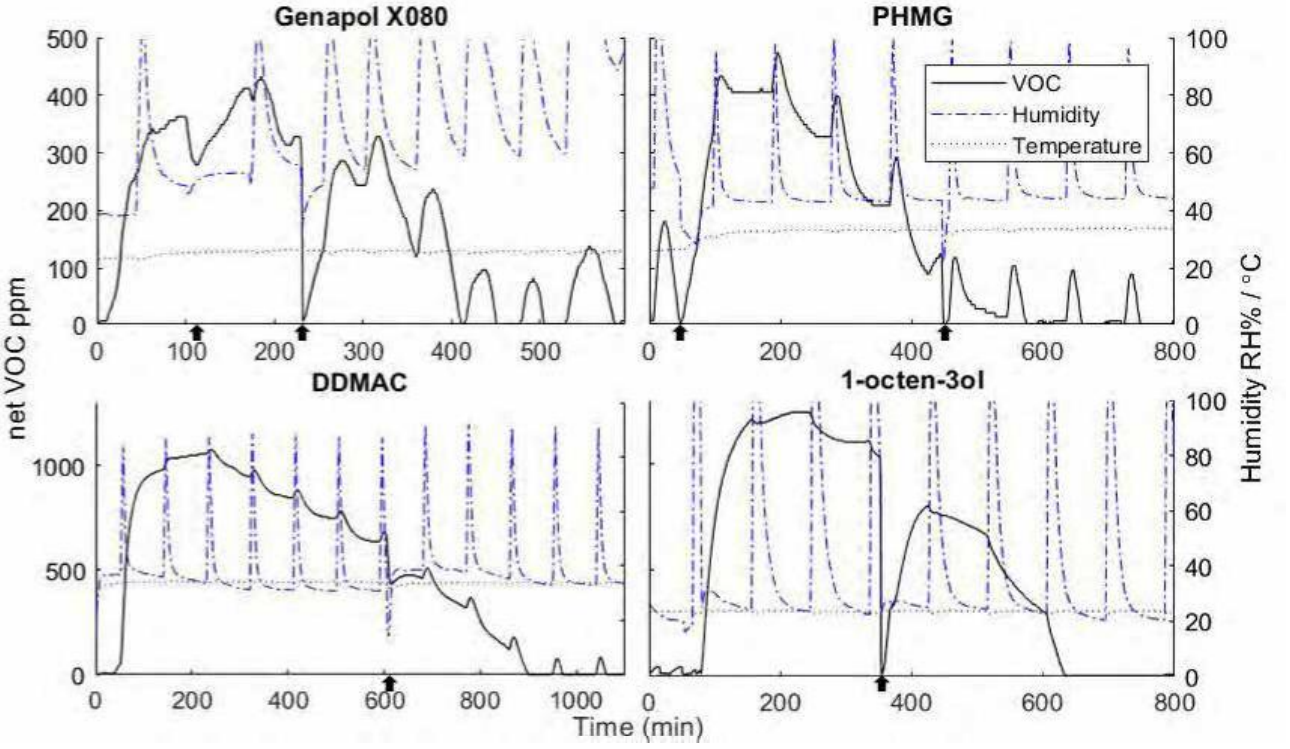

Figure 2. Examples of TVOC responses of three building-related, non-volatile chemicals to air humidity. The ambient RH was 16% to 32%, temperature 21 to 24 ℃. The figure shows TVOC emissions of the wetting agent Genapol X080, and the biocides PHMG, and DDMAC. The common volatile fungal semiochemical, 1-octen-3-ol was tested as reference. The emission source on a glass tray (Θ 38 mm), was placed on the floor of a 90 l all-glass chamber (Figure 1) After air tight- sealing of the chamber, the enclosed air was intermittently humidified (RH 99.9%, 5 min) and dehumidified (to RH 25 – 45% , intervals of 90 min. After 220 min the glass tray containing Genapol was removed. At 67 min PHMG test was started. At 110 min, 440 min, 610 min and 350 min, in each test respectively (arrow heads) the chamber was ventilated and resealed. The input substance minus residual substance was 0.3 – 9 mg per chamber.

Until 2013, PHMG was the most widely used biocide in Finland for prevention and remediation of indoor mould. It was banned in the European Union, when scientific reports proved that 0.1 mg PHMG l^{-1} dispensed in residential humidifiers was responsible for hundreds of cases of fibrotic lung injury, including fatalities among children and pregnant women in South Korea (Lee et al., 2012, Park et al., 2015).

PHMG was replaced with PHMB with similar toxicity and antimicrobial impacts. PHMB generated humidity driven TVOC responses similar to PHMG in our tests. Therefore, it is unlikely to be a safe chemical.

DDMAC has widely been used for disinfecting purposes. The humidity driven TVOC emissivity of DDMAC is shown in Figure 2. DDMAC is toxic to mammalian cells by being lipophilic (Kow = 2.58) and strongly cationic, thus electrostatically adhering onto the negative charged surfaces of mammalian mucous membranes (Wessels & Ingmer, 2013).

Genapol X080 is a non-ionic detergent and wetting agent. It is widely used preservative in technical materials, water-based paints, 1 – 8% (w/v) in cleaning products used at schools, kindergartens, public and private premises. It has also been used as an additive in gypsum and concrete (bubble former), and softener in vinyl plastics (e.g. flooring). The *ex vivo* measured mammalian cell toxicity of Genapol X080 is high, matching that of known poisons, arsenic trioxide, and potassium cyanide, with an EC_{50} of < 10µg/ml for boar sperm cells measured by inhibition of sperm motility (Andersson et al., 2010; Bencsik et al., 2014).

3.2 Transient pulses of airborne humidity may mobilise mycotoxins of molecular size too high to be detectable as VOC

Figure 3 shows TVOC and humidity response of the non-volatile *Aspergillus* mycotoxin, ochratoxin A (OTA, 403 g/mol), which is a molecule 3-fold bigger than 1-octen-3ol, which was used as the reference fungal VOC (Figure 2). The TVOC output of 1-octen-3-ol changed less than 2-fold, irrespective of the humidity of the chamber air (Figure 2). Humidifying the air in contact with OTA to RH 90% increased its TVOC sensor output (0.3 mg on the tray) from barely visible to over 1000 ppm (Figure 3, measured in 0.8 l chamber).

The two other tested mycotoxins, emodin (270 g/mol) and enniatin B (639 g/mol) gave similar TVOC responses to humidity than OTA (not shown). The three mycotoxins OTA, enniatinB, and emodin represent the typical molecular weight range (250 to 800 g/mol) of toxins emitted by building relevant *Aspergillus, Penicillium* and *Paecilomyces* fungi (Nielsen & Smedsgaard, 2003). These molecular sizes are too high to be detected using the official standard gas chromatography-based testing protocols for VOC (EN 16516:2017). The results in Figures 2 and 3 indicate, that when humidity is high, also the large molecular toxins (enniatin B) may become inhalable even in absence of fungal particulates, such as spores or hyphal fragments.

1-octen-3-ol (128 g/mol) is a semiochemical present in the volatome of numerous fungi. Interestingly, the data in Figure 2 show that humidity did not increase its volatilisation. The result is important, since 1-octen-3-ol is known to be neurotoxic by disrupting dopamine packaging (Inamdar et al., 2013). Exposure to 1-octen-3-ol was shown to connect statistically significantly to human ill health symptoms (OR, mucosal symptoms, 1.91) in a large study made in three North European cities (Sahlberg et al., 2013).

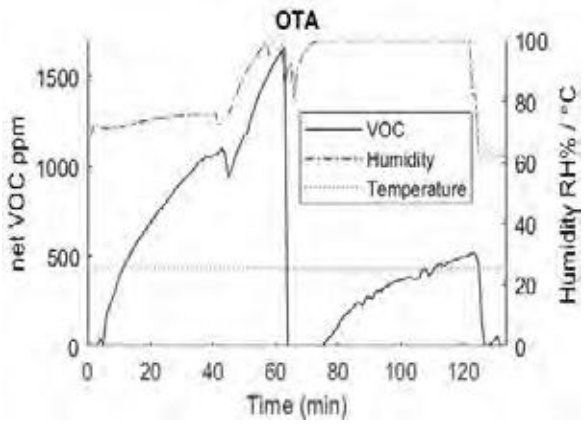

Figure 3. Pure ochratoxin A (OTA), 0.9 mg on a glass tray moistened with 100 µl of water, was enclosed in 0.8 l chamber (Figure 1). Sensors for humidity, temperature and TVOC were located 11 cm above the tray. In humid air (RH>70%) OTA behaved like VOC gases. It was purged from the chamber upon ventilation (at 45min for 1 min, at 65 min for 4 min) but it was restored by desorption from the inside chamber surfaces after the chamber was closed.

3.3 Capillary electrophoresis as a tool to verify humid air carriage of chemical fogs

The experimental evidence, discussed above, suggests that humid air may act as a vehicle for adverse substances resulting in human exposure by inhalation. Figure 4 shows two options for preliminary quantitation and identification of the air humidity carriage of adverse substances: time of migration (for polydisperse substances) and wavelength selection (Riekkola et al., 1997; Adler & Siren, 2014). When the substances need to be identified, mass spectral analysis may be used. Salo et al. (2015) reported earlier the presence of toxic substances in water condensate, harvested from indoor water vapour of a building affected by toxigenic moulds, *Aspergillus versicolor*, *A. calidoustus*, and *Penicillium expansum*. The *Penicillium expansum* isolates of that building emitted liquid droplets containing the mycotoxins communesins and chaetoglobosins, identified by LC/MS.

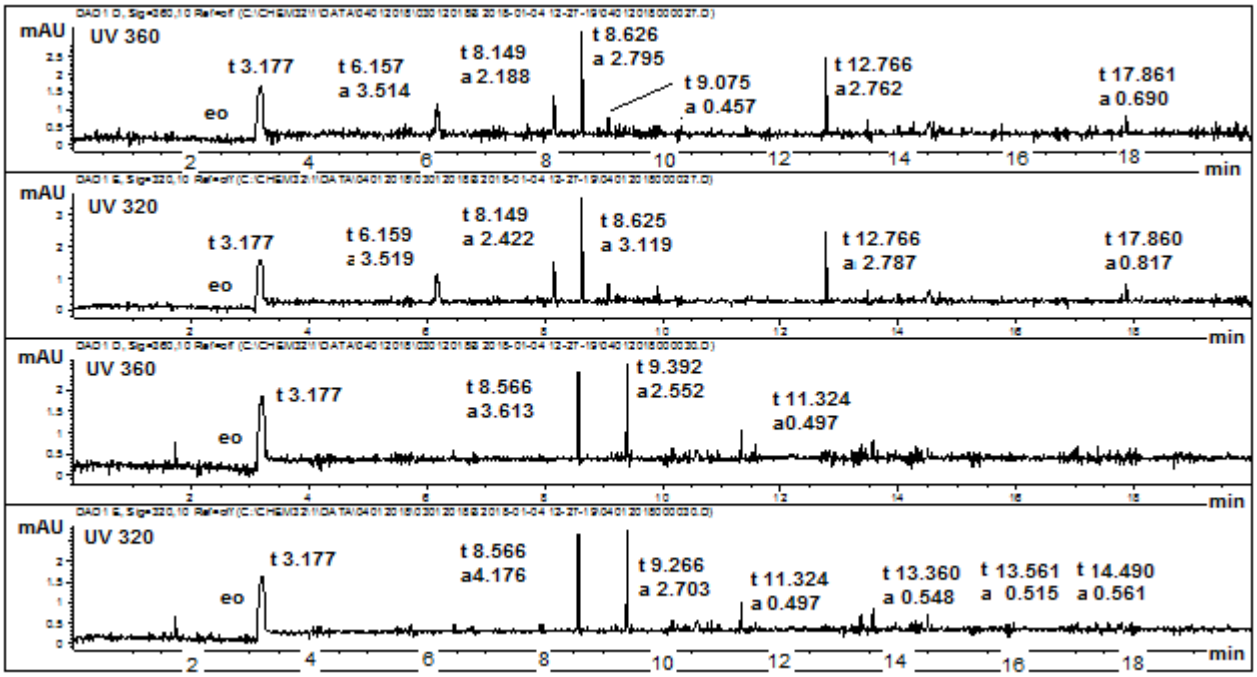

Figure 4. Capillary electropherograms of the input PHMG (top traces) and of the vapour condensate harvested from the chamber spiked with PHMG (bottom traces). The detector was set to collect absorbance at 320 nm and 360 nm wavelengths. The diagrams show original tracings of the water solutions with no pre-treatment. PHMG is a polydisperse mixture of oligomers, some of which appear to have migrated with airborne vapour into the condensate during the 800 min run.

4 CONCLUSIONS

A set of toxic non-volatile chemicals and fungal metabolites was selected for testing water vapour mediated carriage: non-volatile building related chemicals and non-volatile mycotoxins. In humid air (RH ~50 – 70%) several non-volatile, harmful chemicals became airborne and may thus expose humans by inhalation to chemicals and toxins that behave in humid air as volatiles. Humid air allowed exodus of non-volatile chemicals and toxins to become airborne in the test chambers within minutes. When air humidity decreased (< ~ 40% RH), major part of the substances inside the test chambers immobilized onto interior surfaces, but returned airborne when high humidity was restored. The effective vapour carriage of non-volatile, poorly water-soluble substances may be explained by the hydrophobic nature of the air-water interface and the strong hydrogen bonds attracting the water molecules to one another. The observations suggest that it may be possible to design programmes of pulsating air humidity,

combined with intermittent ventilations, for cleaning up surfaces and spaces contaminated by substances of adverse chemical or microbial origins.

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REFERENCES

Andersson, M.A. et al (2010). Boar spermatozoa as a biosensor for detecting toxic substances in indoor dust and aerosols. Toxicology In Vitro 24, 2041-2052

Adler, H & Siren, H (2014), Study on Dicarboxylic Acids in Aerosol Samples with Capillary Electrophoresis, Journal of Analytical Methods in Chemistry , vol 2014 , 498168 . DOI: 10.1155/2014/498168

Bencsik, T. et al., (2014) Ophiobolin A from Bipolaris oryzae perturbs motility and membrane integrities of porcine sperm and induces cell death on mammalian somatic cell lines. Toxins 6, 2857-2871.

Inamdar, A. A. and Bennett J.W., (2013) Volatile organic compounds from fungi isolated after Hurricane Katrina induce developmental Defects and Apoptosis in a Drosophila melanogaster model. Envir. Toxicol., DOI:10.1002/tox.21933

Inamdar, A. A., et al., 2013. Fungal-derived semiochemical 1-octen-3-ol disrupts dopamine packaging and causes neurodegeneration PNAS 110 (48) 19561-19566

Lee, J.-H. et al., (2012). Fatal misuse of humidifier disinfectants in Korea. Importance of chemicals in consumer products. Envir. Sci. Technol., 46, 2498-2500.

Nielsen, K. F., Smedsgaard J. (2003). Fungal Metabolite screening: data of 474 mycotoxins and fungal metabolites for dereplication by standardised liquid chromatography mass spectrometry methodology. J Chromtogr. A, 1002, 111-136.

Paavilainen, M. (2017) Governmental information (Healthy buildings program for 2018-2028).

Park, D.U., et al., (2015) Estimating retrospective exposure of household humidifier disinfectants. Indoor Air 25, 631-640

Riekkola, M-L, et al., (1997). Capillary electrophoresis theory: Selectivity in capillary electrophoresis in presence of micelles, chiral selectors and non-aqueous media, J. Chrom. A 792 (1997) 13-35,

Sahlberg, B. et al. (2013) Airborne molds and bacteria, microbial volatile organic compounds (MVOC), plasticizers and formaldehyde in dwellings in three North European cities in relation to sick building syndrome (SBS), Science of The Total Environment, Vol 444, 433-440, ISSN 0048-9697

Salo J, et al. (2015) Vapor as a carrier of toxicity in a health troubled building. ISIAQ Healthy Building paper ID 346, 8 pp.

Wessels S., and Ingmer H. (2013), Modes of action of three disinfectant active substances: a Review. Regul Toxicol Pharmacol 67, 456-467.