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Boar spermatozoa as a biosensor for detecting toxic substances in indoor dust and aerosols

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ABSTRACT

The presence, quantity and origins of potentially toxic airborne substances were searched in moisture damaged indoor environments, where building related ill health symptoms were suspected and reference sites with no health complaints. Boar spermatozoa were used as the toxicity sensor. Indoor aerosols and dusts were collected from kindergartens, schools, offices and residences ($n = 25$) by electrostatic filtering, vacuuming, wiping from elevated surfaces and from the interior of personal computers. Toxicity was measured from the ethanol or methanol extracts of the dusts and aerosols. EC_{50} was expressed as the lowest concentration of the airborne substance that inhibited motility of >50% of the exposed sperm cells compared to vehicle control, within 30 min, 1 day or 3–4 days of exposure. Remarkably toxic aerosols ($EC_{50} \leqslant 6$ μg ml^{-1}) were found from 11 sites, all of these were sites with known or suspected for building related ill health. Toxic microbial cultures were obtained from subsamples of the toxic aerosols/dusts. From these cereulide, amylosin, valinomycin and a novel indoor toxin, stephacidin B were identified and toxicities measured. Airborn dispersal of valinomycin from *Streptomyces griseus* cultures was evaluated using a flow-through chamber. Significant amounts of valinomycin (LC–MS assay) and toxicity (boar sperm motility assay) were carried by air and were after 14 days mainly recovered from the interior surfaces of the flow chamber.

1. Introduction

Indoor air related adverse health effects are frequent in countries where people spend most of their time indoors. The complaints often involve moisture damaged buildings (WHO, 2009) but no correlation of health complaints has been found with microbial densities or species representation (Curtis et al., 2004) except for high concentrations of *Stachybotrys* molds (Kuhn and Ghannoum, 2003) which almost always correlate with occupant health problems. Correlation with endotoxins, known mycotoxins, microbial volatile organic emissions (MVOCs) or other microbial metabolites have been sought for but none has been found (Bornehag et al., 2004; Brasel et al., 2005; Tuomi et al., 2000).

Adverse health effects experienced by persons working or living in moisture troubled buildings range from upper and lower respiratory tract symptoms, eye irritation, headache and tiredness to asthma, chronic fatigue, arthritis, cardiovascular and other serious health damages (Curtis et al., 2004; Husman, 1996; Storey et al., 2004). Yet in many cases the health authorities are left with no tool to monitor the living or working environment for agents to predict or estimate risks for health in a suspected building. The recent study of Polizzi et al. (2009) indicated that the fungal metabolites detected in indoor air of moldy interiors were other than those usually analysed by the mycotoxin targeted techniques. This is similar to the situation for monitoring environmental exposure to endocrine disrupters, of which some are known but many unknown substances, each at a low concentration. A solution for this was recently offered by developing an effect-based bioassay instead of measuring concentrations of known individual

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pseudoestrogenic compounds (Kuch et al., 2010). In this paper, the objective was to achieve an effect-based method for evaluating the presence of mammalian cell toxic substances in indoor aerosols.

Previous work has shown that boar spermatozoa were useful as sensor cells for detecting microbes that produce heat stable toxins in moist buildings and in foods implicated with foodborne illness (Andersson et al., 1998b, 2005, 2007, 2009; Mikkola et al., 2004, 2007; Kruglov et al., 2009; Peltola et al., 2004). The sperm cells are highly sensitive in detecting toxins that disrupt the mammalian cell ion homeostasis, energy generation and mitochondrial functioning (Hoornstra et al., 2003; Andersson et al., 2006) and equal in sensitivity to somatic cell lines used for indicating cytotoxicity by other mechanisms, except for genotoxicity (Severin et al., 2005; Jääskeläinen et al., 2003b).

This paper describes the application of boar spermatozoa for an effect-based evaluation of the presence of substances with potential for mammalian cell toxicity in indoor environments where the occupants suffered from building related ill health. We describe here the methods for collecting the toxic aerosols, the use of boar sperm cells for quantitative assessment of the aerosol toxicity and airborne dispersal of the toxins, as well as an experimental setup for non-contact probing for toxicity in solid building materials. We also describe a novel *Aspergillus* toxin not earlier found from indoor environment.

2. Materials and methods

2.1. Supply of sperms, reagents and disposables

Commercially supplied boar sperm, ejaculate diluted into an appropriate extender to a density of 27×10^6 sperm cells ml^{-1}, was received at the laboratory within 1–2 days from ejaculation. Before use, the motility of sperms in each purchased lot was inspected and the lot was only used when >80% of the sperms were motile after exposure to 2.5 vol % of ethanol for 30 min (Andersson et al., 2006). In the laboratory the sperm was kept in a Styrofoam box at 21 ± 1 °C and used (= last reading done) with the sperms no more than 5 days old. Each brand of tubes and other disposables intended for coming in contact with sperm, were pretested and only used when found non-toxic to sperm. In the present work polypropene tubes from Mekalasi Oy (Nurmijärvi, Finland) and pipette tips of Biohit Ltd. (Helsinki, Finland) were used. Solvent resistant filters (REZIST 13/0,45 PTFE Green Rim) from Schleicher & Schuell (Dassel, Germany).

All chemicals were of analytical grade. Methanol (100%) (Baker, Deventer, The Netherlands), ethanol 94% (Altia, Nurmijärvi, Finland). The solvents and reference compounds were of analytical quality. 3,5-Dichlorophenol (99.8%, GC) (591-35-5), benzyl butyl phthalate (85-68-7), zinc sulphate heptahydrate (7446-20-0) and potassium dichromate (7778-50-9) were purchased from Riedel-de-Haen; triclosan (3380-34-5, Irgasan DP300) from Merck; dibutyl phthalate (84-74-2), di-*n*-octylphtalate (117-84-0) and bis-(2-ethyl-hexyl) phthalate (117-81-7, Sigma–Aldrich), diisodecyl phthalate (26761-40-0) and diisononyl phthalate (28553-12-0) (Fluka), and valinomycin (90% pure, HPLC) were purchased from Sigma–Aldrich, stephacidin A from AnalytiCon Discover (Potsdam, Germany).

No commercial supplier is available for cereulide, amylosin or stephacidin B; these were purified at our laboratory from the producer organisms as described below (2.8) or published earlier (Andersson et al., 1998a; Jääskeläinen et al., 2003a; Mikkola et al., 2004, 2007). Tryptic soy agar (TSA) was purchased from Scharlau Chemie (Barcelona, Spain), malt extract agar (MEA) and Plate count agar (PCA) from Biokar Diagnostics (Allone, France). Farm barn hay dust (“Hei”) and urban residential dust (“Par”, collected by vacuum suction) were used to monitor motility inhibition by dusts not connected to health symptoms. Their biological properties were described elsewhere (Alenius et al., 2008).

2.2. Collection of aerosols and dusts and preparing the extracts for toxicity assay

The indoor dust and aerosol were collected at ⩾1 m above the floor level by: (1) electrostatic filtration; (2) wiping dust with cotton balls from the interior of personal computers; (3) wiping dust deposited on horizontal surfaces ⩾1 m above the floor level; (4) collecting dust from dust bags and fine-dust collectors of household vacuum cleaners.

The electrostatic filters (FA6, supplied by InspectorSec Ltd., Haukiputaa Finland), with an air flow of 400 m^3 h^{-1}, were placed in Table 1 above the floor surface and run for ca. 30 days. The filter with aerosol was soaked in ethanol (250 ml, 94 wt%), without shaking. Stationary soaking in ethanol at room temperature has been shown efficient for leaching alcohol soluble compounds from paper and recycled paper (Bradley et al., 2010; Honkalampi-Hämäläinen et al., 2010) and it also is the standardised method for leaching toxic substances from plastics (European Committee for Standardisation, 2002).

The ethanol extract was cleared by decanting and centrifugation (20 min 2500 rpm) and/or filtration (solvent resistant filter, 0.45 μm) and evaporated to dryness (+50 °C). The residue was weighed and redissolved into ethanol or methanol at 10–25 mg/ml concentration. The extract was placed in screw capped glass vials, heated for 10 min at 100 °C and stored at −20 °C.

Building material samples were cut to pieces (<20 mm by 20 mm) and extracted by soaking as above.

A portion of the collected dust samples were used for obtaining microbial cultures. Tryptic soy agar (TSA, pH 7.2) and malt extract agar (MEA, pH 5.4) plates were inoculated with the dust and incubated for 30–60 days at 20–22 °C (plates were sealed to prevent drying). For the toxicity assay, the bacterial cultures were collected from the agar plate and extracted by soaking in ethanol as described above.

2.3. Protocol for the boar sperm motility inhibition bioassay of the extracts prepared from dusts and aerosols

The assay was done as follows. Aliquots (2.5–20 μl) of the cleared extract and of the prepared serial 2-fold dilutions (in ethanol or methanol) were dispensed into 2 ml aliquots of the extended boar semen in screw capped exposure vials. The tested range of exposure concentrations was 0.0002–250 and 0 μg (= vehicle only) of the extracted substance (dry wt.) or of the pure substance per ml of the extended semen (ca. 27×10^6 sperms ml^{-1}). Vehicle (ethanol or methanol, max. 1 vol %) exposures, separately for each exposure concentration and each time point, were prepared simultaneously with the test samples. After the exposure time (30 min, 1 day and 3–4 days, 20–22 °C) the toxicity endpoints were read as follows. Before the read-out, the test tubes, capillaries (75 μl hematocrite), object glasses, cover glasses and the microscopic stage were prewarmed to 37 °C. This is because at cool temperature the boar spermatozoon is not motile. The exposure vial was gently shaken to disperse the sperm cells, 200 μl of the exposed sperm suspension was drawn from the exposure vial into a warmed test tube, allowed to warm up in a heating block for 5 min (use a stop watch) to activate motility. Sperm motility was assessed by dispensing ca.10 μl of the warmed sperm suspension onto a microscopic slide and immediately inspected for motility with a 40× inverted phase contrast objective. For each dilution step, the similarly prepared vehicle exposure was inspected on an adjacent position on the same slide. The lowest exposure

Table 1
Indoor dusts and aerosols collected for *in vitro* study of toxicity by wiping horizontal surfaces and personal computers, vacuuming or by aerosol collection using electrostatic filters. Sampling sites with reported building related health symptoms are marked *. Properties of the reference dust Hei** were described in Alenius et al. (2008).

Code	Sampled space	Sampled site and period (when known) and the harvested dust and aerosol dry wt	
Settled dusts collected from ⩾1 m above floor level			
RT5*	University institute (non-laboratory), Helsinki	On lamp shades (daylight lamps), accumulated over several years	63 mg
RTK*	University research laboratory, Oulu	Deposit in the ventilation system filter element	97 mg
Tav*	University laboratory office, connected to serious illness of the occupant, Oulu	Lamp shades, top of book shelves and cabinets	75 mg
PKV-4*	One-family house, Viiala	Dust on top of a household refrigerator	330 mg
Dusts accumulated in the interior of a computers			
TATK*	Office and rest facility of a taxi station, Kankaanpää	Dust accumulated in the interior of an office computer (multiple users)	16 mg
C727*	Small office in a large office building, Helsinki	Dust accumulated in the interior of a computer (since 1½ years placed in a ventilated storage space)	30 mg
C710*	Small office in a large office building, Helsinki	Dust accumulated in the interior of a computer (<1 year)	12 mg
HRTK*	Apartment of a single male with serious building related (?) illness, Pori	Dust accumulated inside a personal computer at home	28 mg
Nas26	Upstairs office in a one-family house, Helsinki	Personal computer, over 3 years	60 mg
Aerosols collected by electrostatic filtration			
Uni*	Kindergarten, children's lunch room, 26 m^2, Tampere	Electrostatic filter collection time 31 × 24 h, May	48 mg
Pip*	Kindergarten, children's play room, 26 m^2, Tampere	Electrostatic filter collection time 31 × 24 h, May	74 mg
Mei*	Kindergarten, children's sleeping room 40 m^2, Tampere	Electrostatic filter collection time 31 × 24 h, May	74 mg
Nei*	Kindergarten, children's lunchroom, 32.5 m^2, Tampere	Electrostatic filter collection time 30 × 24 h, May	26 mg
HSko	Kindergarten, children's playroom, 28 m^2, Helsinki	Electrostatic filter collection time 28 × 24 h, February	19 mg
HMtu	Kindergarten, children's lunch- and playroom, 33 m^2, Helsinki	Electrostatic filter collection time 28 × 24 h, February	35 mg
HKi	Kindergarten, children's playroom and sleeping room, 52 m^2, Helsinki	Electrostatic filter collection time 31 × 24 h, April	88 mg
LK10*	Classroom, 62 m^2, elementary school, Lahti (out-of-service)	Electrostatic filter collection time 32 × 24 h, March	16 mg
LA21	Classroom, 60 m^2, elementary school, Lahti	Electrostatic filter collection time 32 × 24 h, March	42 mg
LA22	Classroom, 59 m^2, elementary school, Lahti	Electrostatic filter collection time 31 × 24 h, March	32 mg
ISL*	Detached one-family house, with motorised ventilation, Lahti	Collected by electrostatic filter during 30 days	81 mg
VOF-1*	Detached one-family house, motorised ventilation, Oulu	Collected by electrostatic filter during 30 days	160 mg
PKV-9*	Upstairs office room in a one-family house, with motorised ventilation, Viiala	Collected by electrostatic filter during 30 days	30 mg
Dust collected by vacuuming			
Pack*	Terraced two-family house, Lohja, with shared central vacuum cleaner	Contents of the fine-dust collection filter, accumulated ~3.5 months	65 200 mg
Par	Apartment of an elderly couple	From vacuum cleaner dust bag, accumulated >30 days	4000 mg
PKV-8*	One-family house, Viiala	Exhaust air filter (HEPA, 100 mm × 132 mm × 20 mm) of a household vacuum cleaner	55 mg
Reference materials			
Hei	Farm barn dust**, Hautjärvi	Settled dust, accumulated in a barn for hay storage over >3 years	220 mg
Blank	Reagent control	300 ml of ethanol used to leach the used glassware and equipment	<2 mg
Electro static filter	New tradeware	Substance leached with 300 ml of ethanol from an unused electro static filter	17.4 mg

concentration (μg of the dry substance per ml of the sperm suspension, three replicates), where the fraction of motile sperms was <50% of that in the identically prepared vehicle exposure, was recorded. EC_{50}[4] was calculated as an average of the three replicates, provided that no more than one of the three deviated from the other two by max. one dilution step (this condition was practically always met when the sperm quality was good); otherwise, the assay was repeated. With this protocol an SD of <40% was obtained for all assays.

Sperm motility in this context means rapid tail beating (the sperm flagellum is then visible as a bunch) and/or progressive movement of the sperm cell, from location A to location B, or in a circle. The subjective estimation of rapid and progressive motility was calibrated with a Hamilton–Thorne semen analyser as described in Andersson et al. (1997). The stock solutions of valinomycin (100 μg ml^{-1}) and of 3,5-dichlorophenol (10 mg/ml), used for the positive control assays, were prepared in ethanol. Valinomycin gave toxicity titer in the sperm assay of 1024 ml/μg and 3,5-dichlorophenol 128 ml/mg and toxicity endpoints (EC_{50}, 3–4 days exposure) of 0.002 and 1 μg, respectively, per ml of the expended boar sperm. The reference toxicants were prepared as described elsewhere (ISO standard 11348, 1998, Andersson et al., 2007).

2.4. Pretesting of labware for sperm toxicity

The disposables and all equipment used that comes in contact with the sperms, extraction solvents or the tested samples, were pretested for toxicity. To do this, extended semen was placed in, or into contact with the tested utensil and incubated for 1 day and 3–4 days at 20–22 °C. Sperm motility was then compared to that of similarly incubated sperms in a clean glass vial.

2.5. Origins of the bacterial strains and culturing

Valinomycin producing *Streptomyces griseus* (strains 10/ppi, DSMZ 41751) was isolated from a moisture troubled building (a commercial bakery where the workers suffered from building related ill health). *Bacillus cereus* 7/pk4 (HAMBI 2711) (Apetroaie et al., 2005) was from indoor wall of a hospital facility, where the occupants complained of building related illness (Andersson et al., 2005). *Bacillus amyloliquefaciens* 19b (HAMBI 2660) was isolated from a moisture damaged residence as described by Mikkola et al. (2004). *Aspergillus* sp. strains pp2 and KaIII and *Paenibacillus polymyxa* RS10 (HAMBI 3103) were isolated from dusts collected in this study. The 16S rRNA gene sequencing, microscopy (gram-staining, spore staining) and biochemical assays were obtained as service from the DSMZ (German Collection for Microorganisms and Cell Cultures, Braunschweig, Germany) as indicated in the references above. *Aspergillus* sp. strains were preliminarily identified based on colony morphology and microscopic characterisation.

For toxin isolation, TSA or MEA plates were inoculated with dust or with the strains, and grown for 10–30 days at 20–22 °C. For the chamber experiments, the *S. griseus* strain 10/ppi was inoculated on cellophane films (Ø 75 mm) placed on TSA agar, grown for 10 days at 20–22 °C.

2.6. The aerosolisation chamber

The flow-through chamber (stainless steel, Ø 240 mm, height 280 mm, volume 12.5 L) for studying aerosolisation is shown in Fig. 1. The air inlet valve is near the chamber floor and the outlet valve on the stainless steel lid. The temperature and relative humidity sensor was inserted through the lid to record the conditions inside the chamber without a need to open the chamber. The valves and the sensor were sealed with 5 mm butyl rubber O-rings. The lid was sealed with a rubber seal and firmly fastened with flanged joints, screws and nuts, to prevent any leakage. The air flow was measured with a Gilian flow meter (Gilian® Glibrator, Sensidyne Clearwater, FL, USA; high flow range 2–30 L min^{-1}). The air passing through the chamber was humidified and filtered (Microfiber 25 mm Ø, sterile, Whatman GF/A, 1.6 μm, Maidstone, UK). A similar filter was fitted to the chamber outlet valve to collect the microbial cells (spores) exiting the chamber. The chamber with its accessories was placed in a fume hood. To keep the humidity inside the chamber as constant as possible, a jar containing 250 ml of saturated aqueous K_2SO_4 was placed inside the chamber. A metal screen was placed 50 mm above the chamber floor, above the air inlet valve, as a pedestal for the rack holding the cellophane films with cultures. The chamber and all items that were used in the set-up, were rinsed with methanol, the rinsates evaporated to a small volume and analysed for toxicity by the sperm assay and for valinomycin by the LC/MS assay. This was done both before and after each chamber experiment.

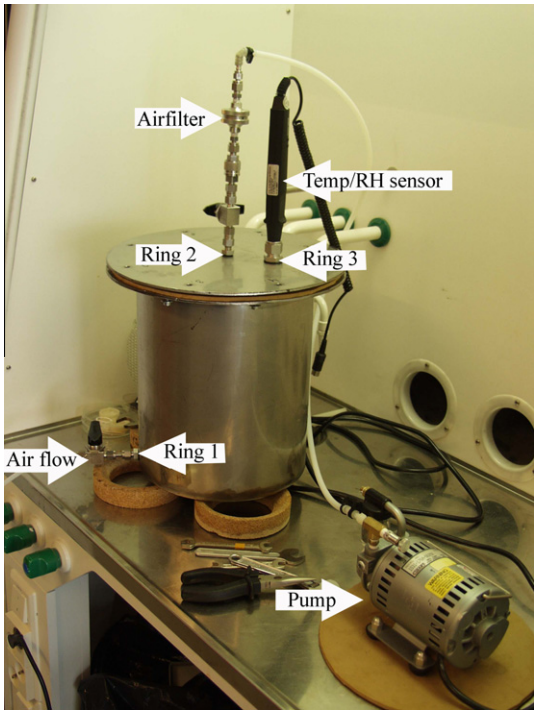

Fig. 1. The flow-through chamber. A cylindrical chamber, welded from stainless steel (AISI 304, contact angle with water 85°, Ø 240 mm, height 280 mm, volume 12.5 l) fitted with lid of the same steel, was used to evaluate the behaviour of valinomycin emitted by *Streptomyces griseus*. The chamber had air inlet (at bottom left) and outlet (at the top of chamber). The inlet and outlet had valves for sealing the chamber. The particles were removed from the incoming and outgoing air by glass fiber filters. During the experiment, the air flow through the chamber, temperature and relative humidity (RH) inside the chamber were measured. The air inlet, air outlet and the sensors were sealed with 5 mm butyl rubber O-rings. The lid was sealed with a rubber seal and fastened to the chamber with flanged joints, screws and nuts, to prevent any. The complete chamber system was placed in a fume hood.

[4] *Abbreviations*: EC_{50}, the lowest concentration of the toxicant that immobilised >50% of the boar sperm cells compared to vehicle control; HPLC, high performance liquid chromatography; log K_{ow}, logarithm of the octanol–water partition coefficient of a compound, LC–MS, liquid chromatography–mass spectrometry; TSA, tryptic soy agar; PCA, plate count agar; MEA, malt extract agar. DSMZ, German Culture Collection for Microorganisms and Cell Cultures; HAMBI, Culture Collection of Microorganisms at Helsinki University, http://www.helsinki.fi/mmkem/hambi.

To study the aerosolisation of microbial toxin cultures of *S. griseus* on cellophane films were placed in the flow-through chamber. These cultures of were obtained by overlaying preweighed, sterile cellophane films (Ø 75 mm) on TSA plates (n = 16) and inoculating the films with *S. griseus* 10/ppi. These, and similar cellophane overlaid plates (n = 8) with no inoculum were incubated in plastic bags at 20–22 °C for 10 days. The cellophanes were lifted from the culture plates, air dried and weighed to estimate the amount of grown biomass. Eight cellophanes with *S. griseus* were then placed on a rack on the metal screen inside the chamber. The other eight similarly grown cellophanes and eight cellophanes without biomass were separately immersed into methanol, and shaken overnight, toxicity and valinomycin concentrations in the methanol extracts were measured by the boar sperm motility inhibition assay and by liquid chromatography–mass spectrometry (LC–MS), respectively.

Fig. 2. The Conway chamber used for assaying sperm toxicants emitted by vinyl tiles of buildings.

2.7. The Conway chamber for measuring sperm toxicity of volatile and/or aerosolisable compounds

The Conway chamber (Fig. 3) consisted of a small desiccator (Ø 100 mm), precleaned with ethanol (70 vol %), and fitted with a liquid dispenser (cleaned with ethanol) in the valve. The material studied for volatile or aerosolisable substances was placed underneath or aside of the perforated porcelain support plate inside the chamber. An empty Petri dish (glass, Ø 60 mm) was placed on the plate. The valve was closed and after an equilibration time of 5–6 days, sperm suspension (10 ml) was dispensed into the glass Petri dish inside the chamber with the dispenser (without opening the chamber lid). Two days later, the sperm suspension was taken out from the chamber and evaluated for motility of the sperms.

2.8. Analytical procedures

The liquid chromatography–mass spectrometry (LC–MS) analyses for cereulide and valinomycin were performed as described by Jääskeläinen et al. (2003a), for amylosin as described by Mikkola et al. (2007) and by Apetroaie-Constantin et al. (2009). Stephacidin

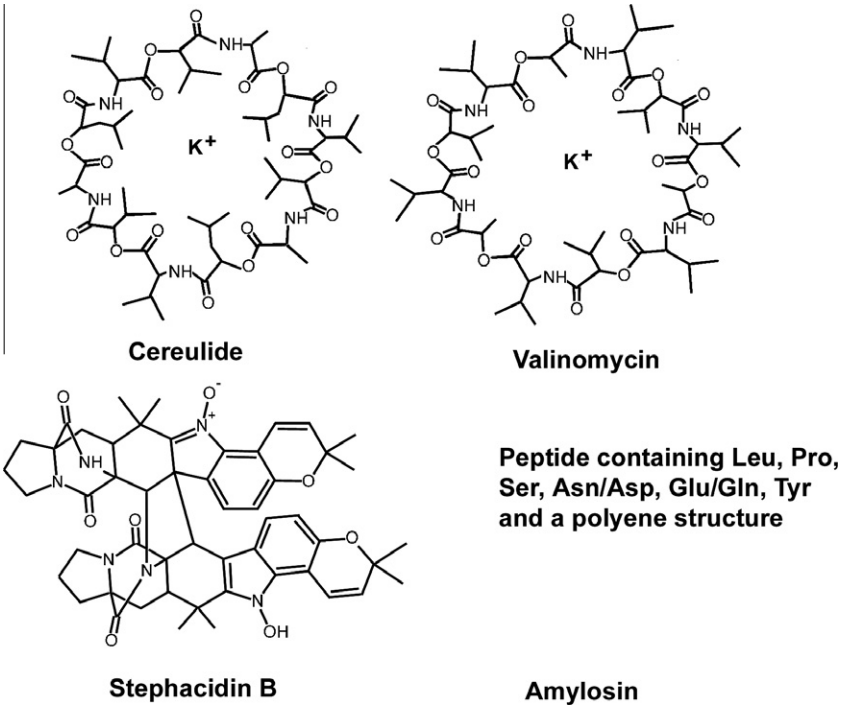

Fig. 3. Structures of toxic metabolites produced by microorganisms found in dusts and aerosols in this study. Valinomycin (MW 1110) and cereulide (MW 1152) are cyclic dodecadepsipeptides consisting of three repeating units (D-Val-L-*O*-Ala-L-Val-D-*O*-Val)$_3$ and (D-Ala-D-*O*-Leu-L-Val-L-*O*-Val)$_3$, respectively. *O*-Val is 2-hydroxyisovaleric acid residue, *O*-Leu is 2-hydroxyisocaproic acid residue and *O*-Ala is lactic acid residue. Amylosin (MW 1196) contains the amino acid residues Leu, Pro, Asp/Asn, Glu/Gln, Tyr and a chromophore group possessing polyene structure (Mikkola et al., 2007).

B was isolated from ethanol extracts of *Aspergillus* strains pp2 and KaIII (grown for 15 days on TSA agar at 22 °C) with HPLC–MS and HPLC–UV. The toxic HPLC fractions were detected by the sperm assay (see Section 2.2) and the toxic fractions identified with electrospray ionization ion trap mass spectrometry (HPLC–ESI-IT-MS) using an MSD-Trap-XCT_plus ion trap mass spectrometer equipped with Agilent ESI source and Agilent 1100 series LC (Agilent Technologies, Wilmington, Del., USA). The column used was SunFire C18, 2.1 × 50 mm, 2.5 μm (Waters, Milford, MA, USA). The eluent was a gradient of A, 0.1% formic acid and B, methanol from 60% A to 80% B in 15 min at a flow rate 0.200 ml min^{-1}. The HPLC–ESI-IT-MS and MS/MS analyses were performed using positive mode in mass range of 50–2000 *m/z*. For UV detection, absorbance at wavelengths of 215, 240, 254, and 280 nm was used. Stephacidin B (891.5 g/mol) was purified and quantified from the ethanol extracts of *Aspergillus* strains pp2 and KaIII using the above HPLC equipment and conditions except the column used was Atlantis C18 T3 4.6 × 150 mm, 3 μm (Waters, Milford, MA, USA) and flow rate 1 ml min^{-1}. Quantification was done with UV absorbance at 240 nm using commercially available stephacidin A (431 g/mol) as the reference compound. Stephacidin A resembles stephacidin B (commercially not available) in its structure (Qian-Cutrone et al., 2002).

For the analysis of the elemental composition the dusts were wet digested (hydrochloric acid/nitric acid) in microwave oven according to the EPA standard procedure 3051 (Environmental Protection Agency, 2007) and analysed by ICP-mass spectrometry according to the standard method ISO 17294-2 (International Standardisation Organisation, 2003).

3. Results

3.1. Dusts and aerosols collected from buildings with health complaints were found toxic in the sperm assay

The first objective of this work was to evaluate the mammalian cell toxicity of dust and aerosols in inhaled indoor air of spaces where building related ill health was suspected. The dusts and aerosols for toxicity assays were sampled by wiping from horizontal surfaces above the floor level, from dust bags of vacuum cleaners, by swabbing the interiors of personal computers or by electrostatic filtering of indoor air (30 × 24 h, 400 m^3 h^{-1}) from the respiration zone (Table 1). Of the sampled sites (n = 25) those with reported health complaints by the occupants are indicated with an asterisk * (n = 18) in Tables 1 and 2. The harvested aerosols and dusts were extracted by soaking in ethanol or methanol and the solvent soluble fraction was used for the toxicity assay.

Boar spermatozoan motility assay was used as an indicator of potential mammalian cell toxicity. The EC_{50} values, presented in Table 2, indicate the concentration of the dustborne or airborne substance (solvent soluble fraction) that inhibited the motility of more than 50% of the exposed sperms, compared to the vehicle

Table 2
Toxicity detected in indoor dust and aerosols by the boar spermatozoan motility inhibition assay. All samples were extracted with methanol or ethanol, evaporated and the residue dissolved in ethanol. Toxicity of was assayed by exposing boar sperm cells to ⩽1 vol % of the extract. The outcome is expressed as EC_{50}, μg of substance dry wt/ml of extended boar sperm that inhibited motility of >50% of the sperms compared to vehicle control, calculated as an average of three replicates, SD <40% for all samples. The sites were described in Table 1; those known or suspected to be connected to indoor health complaints are marked*.

Sample			EC_{50}, μg ml^{-1} in the boar sperm motility inhibition assay		
	Solvent extractable substances		Exposure time		
Code	Solvent	Yield μg mg^{-1} of collected dust or aerosol	30 min	1 day	3 or 4 days
Settled dusts collected from surfaces >1 m above the floor level					
RT5*	Methanol	90		0.8	0.8
RTK*	Ethanol	41	125	10	10
Tav*	Ethanol	8		12	12 (3 days)
PKV-4*	Ethanol	76		12	
Dusts accumulated in the interior of a computer					
TATK*	Methanol	94	125	25	3
C727*	Ethanol	22	125	50	25
C710*	Ethanol	50		25	5
HRTK*	Ethanol	100	125	25	3
Nas26	Ethanol	50	250	100	25
Aerosols collected by electrofiltration					
Uni*	Ethanol	83	50	10	2
Pip*	Ethanol	135	63 – 30	12	3
Mei*	Ethanol	68	125	12	3
Nei*	Ethanol	192	30	12	3
HSko	Ethanol	240	94	40	20
HMTu	Ethanol	660	125	50	25
HKi	Ethanol	520	225	85	30
LK10*	Ethanol	630	50	20	7
LA21	Ethanol	380	140	110	50
LA22	Ethanol	330	75	60	30
ISL*	Ethanol	620		100	<3
VOF-1*,	Ethanol	110			12
PKV-9*	Ethanol	220		12	6
Dust collected by vacuuming					
Pack*	Ethanol	34		10	5
Par	Ethanol	14	100	80	40
PKV-8*	Ethanol	360 μg per exhaust filter		<6	
Reference materials					
Hei	Methanol	27 μg mg^{-1} of dust	250	100	50
Blank	Ethanol	Too low to weigh	No toxicity	Non-toxic	Non-toxic
Electro filter	Ethanol	280		50	25
Vacuum cleaner	Ethanol	20,000 μg per extracted unused filter		>50	>50

exposed sperms in a parallel twin assay. Table 2 shows remarkable toxicity (EC_{50} of 10 μg ml^{-1} and lower, after the 3–4 days exposure) of the indoor substances collected from spaces (n = 18) where there was documented reporting of building related ill health. The EC_{50} values obtained for extracts of dusts and aerosols similarly collected from buildings with no reported health complaints (n = 7) ranged from 20 to >50 μg ml^{-1}.

Some of the dusts in Tables 1 and 2 were extremely toxic: the dust bag ("Pack"), containing the secondary, fine dust of a central vacuum cleaner of a two-family house (with heavy ill health symptoms of the residents), contained 2.2 g of ethanol soluble substance with an EC_{50} of 5 μg ml^{-1}, reportedly accumulated during 3.5 months of infrequent (less than once per week) use. We analysed the inorganic elemental composition (30 elements, data not shown) of this dust and of another residential vacuum cleaner bag (Par) and of farm hay barn dust (Hei) (Table 1). The "Pack" dust revealed an unusually high concentrations of As (34 mg kg^{-1} of air dry dust, vs. 1–4 mg in the dusts "Par" and "Hei"), somewhat elevated concentrations of Cr (83 mg kg^{-1} vs. 42–50 mg in Par and Hei) and Cu (120 mg kg^{-1} vs. 33–62 mg in Par and Hei). The high content of As, Cr and Cu in the dust "Pack" may have emanated from the Cr–Cu–As containing wood preservative that had reportedly been used in that building, but these toxic elements were not found in the samples of construction and insulation materials of that building, inspected in parallel. The EC_{50} values of arsenate, arsenite, chromate and copper hydroxide were >100 μg ml^{-1} giving no explanation for the extreme toxicity in the dust.

Another high toxicity was found in dust settled on lampshades that had not been dusted for a >year, with an EC_{50} of 0.8 μg ml^{-1} already after 1 day of exposure. This was a university office (non-laboratory) where the occupants suffered from serious ill health symptoms, but recovered after moving to another building.

Table 2 shows the results of aerosols of seven kindergarten spaces, 3 primary school classrooms and three residences sampled with electrostatic filters operated for 28–32 days each (Table 1). The EC_{50} values (3–4 days exposure time) of aerosol extracts retrieved from the eight sites with health complaints ranged from 3 to 12 μg ml^{-1} and the five sites with no reported health complaints from 20 to 50 μg ml^{-1} (Table 2). This result indicates that electrostatic aerosol sampling followed by a sperm motility inhibition test may be useful for identifying buildings associated with health problems. The aerosols collected with electrostatic filters (n = 13) contained generally more, av. 320 μg mg^{-1} (range 68–660 μg mg^{-1}), ethanol/methanol soluble substance than the dusts collected by wiping, swabbing or vacuuming (n = 9 + 2), av. 53 μg mg^{-1} (range 8–100 μg mg^{-1}) indicating that the dust collected on the electrostatic filter included also lipid containing parts of the aerosol.

In conclusion, the results described above show that the boar spermatozoan motility test displayed high inhibitory activities for indoor dusts and aerosols of most of the buildings associated with health complaints whereas those from buildings with no documented complaints displayed EC_{50} values were more similar to those of farm hey dust.

3.2. Toxic microbial cultures were obtained from the toxic indoor dusts

Next, we asked whether toxicity found in the collected aerosols and dusts would be of microbial origin, since the sampled buildings with health complaints had a history of moisture damage. We cultured those dusts and aerosol samples (Table 1) that were available at the time (n = 16) on two different agars, tryptic soy agar (pH 7.2), widely used for environmental bacteria, and malt extract agar (pH 5.4), traditionally used for cultivating molds and fungi. The plates were allowed to grow for 30–60 days at 20–22 °C, sealed with adhesive tape to prevent drying. The obtained mixed cultures were extracted with ethanol and analysed for toxicity similarly as for the dust samples in Table 2. The results, in Table 3, show that 11 out of the 16 dust samples yielded microbial cultures that were highly toxic (EC_{50} 0.03–6 μg ml^{-1} after one day of exposure) in the sperm assay. All these toxic cultures were obtained from the dusts implicated in building related ill health complaints.

Table 3
Boar spermatozoan motility inhibiting activity of extracts prepared from mixed microbial cultures obtained from dusts and aerosols described in Table 1. The samples from Table 1 were plated on tryptic soy (TSA) or malt extract (MEA) agar, sealed with adhesive tape to prevent drying, and allowed to grow for 30–45 days. The biomass was then harvested from the plates and extracted with ethanol and the toxicity of the extract assayed by the boar spermatozoan motility assay. When more than one plate was independently inoculated from the same dust, result of each plate is shown. "High toxicity" indicates dust and aerosol samples of which an ethanol extract was obtained with EC_{50} (4 days) of ⩽6 μg ml^{-1} (Table 2). The samples originating from sites with known or suspected building related health complaints are marked*. The table shows EC_{50} values (1 day exposure) of the extracts of mixed cultures grown from the indoor samples described in Table 1. The EC_{50} values were calculated as an average of three replicates (SD <40% for all samples).

Plating medium	TSA, pH 7.2	MEA, pH 5.4
Cultures obtained from indoor samples with high toxicity		
RTK*	27	62
Tav*	0.04, 0.8	
TATK*	6	
HRTK*	>100	
C710*	1	3
Uni*	3	1
Pip*	0.5	
Mei*	5	
ISL*	6	
VOF-1*	12	
PKV-8*	0.03, <6	
PKV-4 *	1, 12	
Pack*	3, 5	1
Cultures obtained from indoor samples with low toxicity or none		
Nas26	<100	100
Par	100	100
Hei	50	100

To get information on the microbial species responsible for the toxicity of the dust cultures, 129 colonies were picked from the plates, streaked to pure cultures and each of these tested for toxicity by the boar sperm assay. A total of 42 toxic substance producing isolates were found. Of these, 11 of these were identified to genus or species level.

The identified sperm motility inhibiting isolates were *B. cereus, Bacillus simplex, Bacillus subtilis, B. amyloliquefaciens, P. polymyxa, S. griseus* and *Aspergillus* sp. Four toxic substances were identified from the pure cultures: amylosin, cereulide, stephacidin B and valinomycin. Stephacidin B was produced by *Aspergillus* sp. almost as a monoculture from the fine dust bag of a residential vacuum cleaner (Pack); it was also found in a kindergarten (Uni) (Table 1). The boar sperm toxicities of these substances are shown in Table 4. Highly toxic *P. polymyxa* was obtained from dust swabbed from the interior of the computer of a taxi office (TATK, Table 1), but the responsible substance was not yet identified. The toxicities, measured by the boar spermatozoan motility inhibition assay, of the four substances identified from microbes in the airborn samples displayed EC_{50} values (3–4 days exposure) of 0.0002–0.2 μg ml^{-1} (Table 4). The high toxicities of the microbial toxins is evident when these EC_{50} values are compared to those of the positive reference toxicants, valinomycin, 3,5-dichlorophenol, zinc sulphate and potassium dichromate (Table 4).

Table 4
Toxicity in the boar spermatozoan motility inhibition assay of substances identified in one or more microbial isolates retrieved from the dust and aerosol mixed cultures in Table 3. The boar spermatozoan motility inhibiting activity was assayed as explained in this paper. The EC_{50} values were calculated as averages of three replicates (SD <40%). 3,5-Dichlorophenol, potassium dichromate and zinc sulphate were used as reference chemicals as described in the International standard 11348 (ISO, 1998). Valinomycin was used as a reference toxicant (Andersson et al., 2007). The highest tested concentration is indicated*.

Toxin and producer organism	EC_{50} in the sperm motility assay, μg dry wt/ml		
Exposure time	30 min	1 day	3–4 days
Substances produced by the dust isolates in this study			
Amylosin, *Bacillus subtilis*	0.15	0.03	0.03
Cereulide, *Bacillus cereus*	<0.01	0.0002	0.0002
Stephacidin B from *Aspergillus* sp. strains KalII and pp2	1–3	0.4	0.2
Valinomycin, *Streptomyces griseus*	<0.01	0.0005	0.0005
Reference substances			
3,5-Dichlorophenol	12	<3	1
Potassium dichromate	>400*	400	50 (3 days)
Zinc sulphate heptahydrate	>300*	>300*	50 (3 days)
Stephacidin A (commercial)	31	3.3	1

The outcome thus showed that substances with high toxicity (sperm motility inhibition assay) were produced by microorganisms grown from the airborn materials. The presence of even minute amounts of such substances may explain the toxicities observed for the indoor dusts and aerosols (Table 2). The results show that the boar spermatozoan motility inhibition assay was useful in tracking potentially toxic agents in the airborn materials collected from buildings.

3.3. Laboratory disposables may leach toxic substances leading to false positives if not pretested

To avoid false positives in cell toxicological assays it is important to keep the background response low. The results shown above were obtained with materials shown non-toxic by pretesting. The pretesting had shown that sperm toxic substances leached into ethanol and methanol from several brands laboratory disposables, test tubes, centrifuge tubes and gloves. When two fingers of latex and vinyl gloves were soaked in 80 ml of methanol, the solvent subsequently evaporated and the residue redissolved in 2 ml, sperm toxicity was found, typically with an EC_{50} (3 days exposure) <3 μg ml^{-1} of sperm suspension for the vinyl as well as for the latex gloves. Dipping two fingers of latex and vinyl gloves in 10 ml of the commercially obtained extended boar semen for 2 min, also caused >50% motility loss compared to vehicle control, when inspected 3 days later.

No background problem for the boar sperm assay was seen from any of the solvents (ethanol, methanol) or other reagents used. When 300 ml of the solvent (ethanol or methanol) was evaporated to dryness and the final residue dissolved in 100 μl and used *in toto* to expose the spermatozoa (Table 2), no toxic effect was seen in the boar sperm assay.

We did not make efforts to identify the chemicals leaching from the disposables. The sperm motility inhibiting activities of triclosan (Irgasan DP300) and six different phthalates were tested, as these substances are well known plastics additives. EC_{50} value for triclosan was 1 μg ml^{-1} and for the phthalates (listed in 2.1) 5 μg ml^{-1}, after 3 or 4 days exposure. These widely used substances may be present in indoor aerosols and dusts, but their contribution is unlikely to explain the high toxicity of many of the dust and aerosol samples nor the difference in toxicities between the airborne materials from buildings with and without health complaints, respectively (Table 2).

The outcome thus was that substances toxic to commercial boar spermatozoa may leach from many but not all laboratory disposables into the extraction solvents and into the commercial boar semen. Therefore, pretesting of all materials that come in contact with the sperms or the solvents used to prepare the extracts for the sperm assays must be pretested and only those materials that are found non-toxic, may be used.

3.4. How do microbially emitted toxic metabolites aerosolise?

Considering the presence of toxin producing viable microorganisms in aerosols and settled dusts (Tables 3 and 4), the question arises if and how toxins are emitted into indoor air from microbially colonised indoor sites. We investigated whether the boar spermatozoan motility assay could be useful in answering this question. A valinomycin producing indoor air isolate, *S. griseus* 10/ppi, was grown on cellophane films placed on agar plates and the cellophanes subsequently placed in a stainless steel flow-through chamber (Fig. 1), with temperature and humidity similar to those inside Finnish workplaces and residences in the cold season when doors and windows are mainly kept closed, a diurnal ventilation 0.23 $m^3\ h^{-1}\ m^{-3}$.

After 336 h of operation, the chamber was opened, all parts extracted with methanol. The boar spermatozoan toxicity was determined and the concentrations of valinomycin measured (by LC–MS) in the extracts. Toxicity was found at two sites inside the chamber, on the glass fiber exit filter and on the inside (steel) walls of the chamber (Table 5). In addition, toxicity was found on the blank cellophanes on agar plates that were incubated together with the inoculated ones in same plastic bags before the start of the chamber experiments. These same three sites also were the only ones (out of 10 items measured) where valinomycin was also detected by the LC–MS. On the exit filter, designed to retain any airborne spores, 0.4 μg of valinomycin (100 ng cm^{-2}) was found and on the chamber walls a total of 52 μg was detected (14.6 ng cm^{-2}). A similar load of valinomycin (5.3 μg, 15.1 ng cm^{-2}) was found on the blank cellophanes (with no colonies).

The experiment described in Fig. 1 and Table 5 thus shows that the valinomycin producing *S. griseus* culture emitted this toxin in its toxic form. Valinomycin deposited onto the chamber surfaces in measurable quantities. It was also found that the *Streptomyces* culture continued to increase its valinomycin contents (from ca. 3300 μg on day 0–4300 μg on day 14, Table 5) with no other humidity source than the inflowing air and no other carbon source than the cellophane (= extruded cellulose).

Fig. 2 shows a non-contact approach (Conway chamber) to studying emissions of toxic vapours or aerosols from building materials in the presence or absence of microbial contamination.

Table 5
Chamber experiment for evaluating aerosolisation of valinomycin from *Streptomyces griseus* growing on cellulosic material. A chamber of stainless steel (Fig. 2) was constructed to study the airborne emissions of valinomycin from cultures of a valinomycin producing indoor isolate, *S. griseus* 10/ppi. This was seeded on 8 (lot A) + 8 (lot B) cellophane foils placed on TSA plates, whereas 8 cellophane foils (lot C) on TSA plates were left blank. All were placed in the same bags and grown for 10 days at 20–22 °C. The contents of valinomycin were measured in the various chamber parts and in the cellophane foils with (lot A) and without (lot C) *S. griseus* culture. The foils of lot B with *S. griseus*-growth ($n = 8$) were placed in the chamber (on the racks above the grid).

Measured item	Valinomycin found, μg per item	
	Before chamber exposure	After exposure
Methanol rinse of the chamber inside walls, 0.36 m^2	<0.1	52*
Rubber ring 1 (0.2 g)	<0.005	<0.005
Rubber ring 2 (0.2 g)	<0.005	<0.005
Rubber ring 3 (0.55 g)	<0.02	<0.02
O-ring sealing the lid on the chamber (10 g)	<0.02	<0.02
Cellophanes with biomass of *Streptomyces griseus* 10/ppi on, lot A ($n = 8$, 0.17 g each)	3260 ± 10% (in 400 mg of biomass)*	
Cellophanes with biomass of *Streptomyces griseus* 10/ppi, lot B ($n = 8$, 0.17 g each)		4250 ± 10% (in 470 mg of biomass)*
Cellophanes without biomass of *S. griseus*, lot C ($n = 8$, 0.17 g each)	5.32 ± 10% (no biomass)*	
Outlet valve membrane filter, effective surface area 4 cm^2	0.002 (weight of filter 25 mg)	0.40 (weight of filter 26 mg)*

The chamber was sealed with a lid. Glass fiber membrane filters were placed in the inlet and the outlet air filter holders. The chamber was connected to a pump drawing air from the outlet valve of the chamber, at a flow rate of 0.8 $m^3\ m^{-3}\ h^{-1}$. The flow was kept constant during the day time and stopped at night and weekends, resulting to an average diurnal flow 0.23 $m^3\ m^{-3}\ h^{-1}$ during 14 days. The temperature and the relative humidity inside the chamber were recorded five times a day. The temperature was 18–22 °C, average 20.7 °C and the relative humidity (RH) was 80–90% for the first ½ h of each day, and 20–30% for the remaining 6–8 h per day. After 14 days, the total time of air flow was 92 h and the volume of air 96 m^3. All parts of the chamber were then dismantled and extracted with methanol. The extracts obtained were evaluated for toxicity (boar sperm motility inhibition assay) and analysed for valinomycin by LC/MS. The extracts that were toxic to sperm cells (EC_{50} <5 μg methanol soluble d.s./ml of sperm suspension) are marked with *.

Pieces cut of the building materials were enclosed inside the chamber and equilibrated in the chamber in stationary air for a sufficient time. The sperm suspension was then dispensed into the closed chamber via a funnel fitted on the chamber lid. Table 6 shows the results of an experiment with vinyl floor tiles. It shows that out of 11 samples of tiles from residencies with indoor air complaints (i) four samples emitted toxic substances and (ii) toxic substances leached into methanol from two other tiles (EC_{50} <5 μg ml^{-1}). The toxic tiles were not those with the highest burdens of culturable microorganisms, when cultured on conventional aerobic plate counting media. Out of 4 unused tradewares two brands (M1, M4) were sperm toxic by emissions into the air. These results indicate that stationary emission chambers can be used with spermatozoa as indicator cells to detect substances toxic to mammalian cells, emitted from indoor materials.

4. Discussion

In this paper, we describe an *in vitro* method for assessing the toxicity of indoor aerosols. The assay was performed within a reasonable effort, time and cost using boar spermatozoa as the indicator cells. The boar spermatozoa are equal in sensitivity in common *in vitro* procedures for cytotoxicity (neutral red uptake, MTT assay; Severin et al., 2005) but more sensitive towards toxins targeted to mitochondria (Apetroaie-Constantin et al., 2009; Hoornstra et al., 2003; Jääskeläinen et al., 2003b).

A method for measuring toxicity in aerosols, sampled directly from the indoor air is described in this paper. It can be used for determining the toxic burden in the air inhaled by the employees or residents when building related ill health is suspected. We did not find in the literature any effect-based toxicity studies to compare our results to.

As the reference toxicants for the assays we tested 3,5-dichlorophenol, potassium dichromate and zinc sulphate. These are the standard positive controls for inhibition of light emission of the luminescent bacterium *Vibrio fischeri* (ISO standard 11348, 1998), the most widely used assay for environmental toxicity (Richardson, 1993; Kaiser, 1998). The microbial toxins found in the present study from airborn microorganisms, were orders of magnitude more toxic than the ISO standard toxicants potassium dichromate and zinc sulphate (Table 4). We therefore used in this study valinomycin as a positive reference. Valinomycin was earlier found useful for assaying toxicity in indoor materials from sick buildings (Andersson et al., 1998b, 2005, 2007, 2009; Mikkola et al., 2007).

Electrostatic filters operated for ca. 30 days yielded 16–88 mg of aerosol from each of the kindergarten spaces and school classrooms (sized 26–62 m^2) and 30–160 mg from residences (Table 1). On the average, 32% of this yield dissolved in ethanol

Table 6
Non-contact method for detecting toxic substances in vinyl floor tiles from buildings. Vinyl tiles were cut to pieces and placed in Conway chamber (Fig. 3). The chamber was hermetically sealed, and after 5–6 days of equilibrating at 20–25 °C boar sperm suspension was dispensed into the chamber through the funnel fitted to the lid of the chamber. Two days later the chamber was opened and motility of the sperm cells was inspected. In parallel, the tile materials were extracted into methanol and analysed for toxicity similarly as for Table 2. The unused tradeware formed gel when extracted with methanol, making motility inhibition assessment impossible (ND, not done).

	Exposure of the sperms to the vinyl materials		Microbial contamination, 1000 cfu/g	
	Head space exposed	EC_{50} of methanol extracts, μg ml^{-1}	TSA agar	PCA agar with cycloheximide
Sampled from buildings of 1 up to 10 years of age				
2 samples	Not toxic	<5	0.2–1.6	0.3–1.2
2 samples	Not toxic	50	1.2–15	1.3–1400
1 sample	Not toxic	50	14,000	9100
2 samples	Not toxic	50	0.2–1.4	0.1–1.3
4 samples	Toxic	50	<0.1–9	0.4–2.4
Unused tradeware				
M1, M4	Toxic	(ND gel formation)		
M2, M3	Not toxic	(ND gel formation)		

and was assayed for toxicity. In many cases the aerosol was remarkably toxic, EC_{50} (4 days exposure) of 3–6 μg ml^{-1} in 10 of the studied 25 sites.

Toxin producing microorganisms were isolated from cultured subsamples of the airborne substance from buildings connected with ill health complaints. These cultures produced amylosin, cereulide, valinomycin and stephacidin B (structures shown in Fig. 3), in addition to a number of unidentified toxins. Amylosin is a linear peptide (1196 g/mol) with a polyene tail, forming K^+ and Na^+ permeant channels in biomembranes (Mikkola et al., 2004, 2007). Amylosin is produced by certain strains of members of the *B. subtilis* group of species prevalent in "sick" buildings (Andersson et al., 2005) and implicated with food poisonings (Apetroaie-Constantin et al., 2009). Amylosin was shown cytotoxic at low exposure concentrations, towards all tested types of mammalian cells, by collapsing the cellular Na^+/K^+ ion homeostasis of the mammalian cell (Mikkola et al., 2007).

Cereulide is the emetic toxin of *B. cereus* (Agata et al., 1995; Andersson et al., 1998a). It is a cyclic dodecadepsipeptide (1152 g/mol) and toxic by being a ionophore specific for K^+ (Teplova et al., 2006). Cereulide is produced by members of a specific clade within *B. cereus* (Agata et al., 1996; Ehling-Schulz et al., 2005) that are rare in the natural environment (Altayar and Sutherland, 2005), but common in "sick" buildings (Andersson et al., 2005) and foods implicated with food poisoning (Jääskeläinen et al., 2003b; Nishikawa et al., 1996; Pirhonen et al., 2005). Cereulide is highly toxic to man and ape, but not to rodents, by catalysing an efflux of K^+ and by causing mitochondrial dysfunction (Hoornstra et al., 2003), *in vivo* resulting into serious hypoglycemia (Mahler et al., 1997; Shiota et al., 2010).

Valinomycin, produced by several species of *Streptomyces*, is a well known K^+ ionophore, resembling cereulide in structure (1110 g/ml). Like cereulide, it is toxic to mammalian cells by affecting the cellular K^+ homeostasis and causing mitochondrial dysfunction (Hoornstra et al., 2003; Teplova et al., 2006). Valinomycin producers were found frequent in "sick" buildings (Andersson et al., 1998b, 2005).

Stephacidin B is first time described from indoor environment in this paper. Previously, stephacidin B (891.5 g/mol) was described as the product of a single strain of *Aspergillus ochraceus* WC76466 (ATCC 74432) (Cutrone et al., 2001; Qian-Cutrone et al., 2002). Stephacidin B contains a bicyclo[2,2,2]diazaoctane ring system common to alkaloids (Artman et al., 2007; Baran et al., 2005). It was reported to bind to nucleophosmin, the proposed oncogenic protein overexpressed in many human tumors, as well as several other proteins containing active site of reactive cysteine residues (Wulff et al., 2007). In the sperm cells, its target could be the sperm mitochondrial cysteine rich protein (SMCP) that is localized in the mitochondrial capsule and enhances sperm motility (Hawthorne et al., 2005). We found large numbers of the *Aspergilli* producing this substance in airborne materials of two locations out of the 25 investigated in this study. Stephacidin B is more hydrophobic ($\log K_{ow}$ 7.05) than stephacidin A ($\log K_{ow}$ 2.57) and it was reported to be 10 times more cytotoxic to various human tumor cell lines than stephacidin A (Cutrone et al., 2001), supporting the high toxicity found for stephacidin B in this study.

The indoor aerosol and dust collected by electrostatic filters contained a higher fraction of lipophilic (ethanol soluble) substance than dusts collected by other methods (wiping, swabbing, vacuuming). This indicates air carriage of organic substances also by vehicles other than settleable dust. It is known that the air-side of the air–water interface of water vapour is extremely hydrophobic, comparable to nonpolar materials such as Teflon. This predicts that the air-side of nanodroplets of water in air will hydrophobically attract apolar compounds (Horinek et al., 2009; Van Oss et al., 2005). The behaviour of nanosized water droplets in air of unsaturated humidity may explain how the highly hydrophobic nonvolatile molecule, valinomycin (1110 g/mol, $\log K_{ow}$ = 5.99, Teplova et al., 2006) became aerosolised and deposited on the walls of the steel chamber, as observed in this study. Steel surface is hydrophobic (θ 85°, Raulio et al., 2008) and thus attracts the hydrophobic-substances from the humid aerosols inside the simulation chamber. The transit of hydrophobic molecules or microbial cell fragments by moist air may also have caused the migration of *S. griseus* produced valinomycin from culture plates to noninoculated plates, as observed in this study. Such events may lie behind the symptoms described by physicians of "sick building" syndrome patients who, after the moisture damage in the building was remediated, continue to complain ill health symptoms (Kanazawa et al., 2010; Storey et al., 2004). The source of exposure may then be items or office materials "coated" with toxic substances but not necessarily by culturable microorganisms. There are literature reports on the transit of hydrophobic contaminants on air–water interfaces can be so high that water was successfully used to mobilise hydrophobic pollutants from soil and water vapour can be used to remove dioxins ($\log K_{ow} \geqslant 6$) from flue gases (Fazaelipoor, 2009; Freepatentsonline.com 2010).

Tissue toxicity of substances leaching from surgical plastics was noted already in the 1980s (Cormio et al., 1993, Talja et al., 1986; Virtanen and Andersson, 1986). Our experiences on the toxicity responses of boar spermatozoa towards plastic disposables confirm that the toxicity problem continues to exist. The causative agent of the toxicity is not clear.

In this study the toxicity assessment focused on the ethanol (or methanol) soluble fraction of the indoor aerosols and dusts. This choice was made because ethanol (i) dissolves the lipophilic substances that are most likely to spontaneously permeate the skin and mucous membranes; (ii) gives low vehicle background of toxicity even when large volumes were concentrated by evaporation; (iii) is well tolerated by boar spermatozoa as well as by numerous other cells employed for *in vitro* assays (Honkalampi-Hämäläinen et al., 2010).

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