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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