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Acute effects of 1-octen-3-ol, a microbial volatile organic compound (MVOC)—An experimental study

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ABSTRACT

Acute effects were assessed from exposure to a common volatile compound of microbial origin, the aliphatic alcohol 1-octen 3-ol (octenol). Twenty-nine volunteers performed symptom reports, measurement of blink frequency by electromyography, measurement of the eye break-up time, vital staining of the eye, nasal lavage, acoustic rhinometry, transfer tests and dynamic spirometry. Subjects were during 2 h in random order exposed to either 10 mg/m^3 of octenol or clean air as control. During octenol exposure subjective ratings of smell and nasal irritation were increased together with higher nasal lavage biomarker levels of eosinophil cationic protein, myeloperoxidase and lysozyme. Also eye irritation and blinking frequency were increased together with throat irritation, mild dyspnoea after 1-h but not after 2-h, and a small decrease in vital capacity. Ratings of headache and nausea were also increased. Atopics did not have more reactions due to exposure, whereas females experienced more smell and mucosal irritation. Thus, there were both subjective and objective signs of mild mucosal irritation of eyes and airways together with symptoms of headache and nausea.

1. Introduction

Earlier review articles have concluded that there is consistent evidence for an association between damp housing conditions and respiratory symptoms among adults (Bornehag et al., 2001; Peat et al., 1998; Husman, 1996). These conclusions are mainly based on a large number of cross-sectional questionnaire studies, and there are less experimental or clinical studies on respiratory effects of building dampness or mould exposure (Ebbehoj et al., 2005; Hirvonen et al., 1999; Norbäck and Wieslander, 2002; Zock et al., 2002; Wieslander et al., 2007). There are different types of exposures related to indoor mould exposure, including mould allergens, mycotoxins, cell-wall compounds with immunomodulation properties, e.g. beta-1-3-glucanes, as well as microbial volatile organic compounds produced by fungi (MVOC) (Fischer and Dott, 2003). The biological mechanisms behind the observed relationship between dampness, moulds, and respiratory health remain unclear.

1-Octen-3-ol (CAS Registry Number: 3391-86-4, hereafter called octenol) is a common compound produced by mould (Fischer and Dott, 2003; Pasanen et al., 1997) and bacteria (Viehweg et al., 1989) as a result of the biodegradation of lipid containing organic materials by microbial lipoxygenases. Octenol has a boiling point of 180 °C at 101.3 kPa, a vapour pressure of 71 Pa at 25 °C and an octanol:water partition coefficient (log Kow) of 2.60. It has a characteristic fungal smell (Pyysalo, 1976) and has been suggested as an indicator of fungal growth in the indoor environment (Fischer et al., 1999). Enzymatic cleavage at one of the double bounds and oxidation of linoleic acid has been shown as one source of octenol from fungi (Matsui et al., 2003; Wurzenberger and Grosch, 1984). Linseed oil, which is rich in linoleic acid, is a common ingredient of solvents for paints, varnishes and linoleum carpets. Octenol is also used as flavouring agent and perfume ingredient (EPA, 2003). Other possible indoor sources are bodily odours from sweat (Cork and Park, 1996), breath (Ramoni et al., 2001) and composts (Fischer et al., 2000).

Indoor concentrations of octenol were up to 10 μg/m^3 in 38 buildings having problems with dampness or water damage (Ström et al., 1993), up to 1.5 μg/m^3 in 96 randomly selected classrooms (Smedje et al., 1996), and up to 0.2 μg/m^3 in a more recent school study (Kim et al., 2007). The concentration of octenol in indoor air in

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; BUT, break-up time; CO, carbon monoxide; ECP, eosinophil cationic protein; EMG, electromyography; FVC, forced vital capacity; MPO, myeloperoxidase; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; PEF, peak expiratory flow; TWA, time weighted average; VC, vital capacity.

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schools has been related to the prevalence of symptoms of asthma in school personnel (Smedje et al., 1996), pupils (Kim et al., 2007) and clinical signs of eosinophil activation (Norbäck et al., 1993). However, no human controlled studies of exposure to octenol were found in the literature.

The aim of the study was to assess acute effects of octenol via a battery of physiological and biochemical tests for airborne pollutants (Walinder et al., 2005). A study on mice showed a RD50-value (respiratory frequency reduced 50%) of 182 mg/m^3 indicating sensory irritation (Korpi et al., 1999). Numerical values of occupational exposure limits based on irritation have been shown to be on average around 3% of the respective RD50 values (Schaper, 1993). This corresponds to about 5 mg/m^3 for octenol. With no other data available, and in view of the relatively short exposure duration of 2 h, we chose a slightly higher exposure level of 10 mg/m^3 in our study.

2. Methods

2.1. Subjects

Twenty-nine healthy subjects (14 females) aged 20–54 (mean 33 and S.D. 9) years participated in the study. The study was performed after written consent from the participants, and approval by the Regional Ethical Committee at the Karolinska Institutet, Stockholm, Sweden. The subjects were medically examined prior to the first exposure. Atopy was tested by laboratory verified Ig-E antibodies to common Swedish allergens: cat, dog, horse, birch pollen, timothy, mugwort, *Cladosporium herbarum*, *Dermatophagoides pteronyssinus*, and *Dermatophagoides farinae* (Phadiatop test, Pharmacia Diagnostics, Uppsala, Sweden). Twelve (41%) of the volunteers had laboratory verified atopy.

2.2. Chamber exposure

The subjects were exposed to clean air or octenol (10 mg/m^3). The exposure time was 2 h and conducted during resting conditions with the subjects seated. Up to 5 subjects at a time were exposed in a 20 m^3 exposure chamber with a high air exchange rate (18–20 air changes per hour) to assure that exposure conditions were constant irrespectively of the number of persons in the chamber. There was a minimum period of two weeks between the exposures, which were performed in a balanced and random order between air and octenol during December to February. The temperature and the humidity in the chamber were set to 24 °C and 30%, respectively. Temperature and humidity were continuously recorded (Vaisala HMP 36) and logged (Squirrel Meter Logger 1200 Series, Grant, Sweden). Vapour of octenol was generated by injecting liquid solvent into inlet air by means of a high-pressure piston pump (Gilson 302, France). The inlet air was dispersed throughout the entire chamber ceiling. Air was sampled from the upper central part of the exposure chamber to monitor the concentration of octenol during exposures. The air samples were transferred through a Teflon®-coated tube to a gas chromatograph by means of a pump (DDA-P101-BN, Gast, Benton Harbor, USA). The gas chromatograph (Auto system, PerkinElmer) was equipped with a wide bore capillary column (CP-sil 8, 10 m, 0.53 mm i.d., 2 μm, Chrompack) and a flame ionization detector. Helium was used as a carrier gas and the temperatures of the oven and detector were 55 and 250 °C, respectively.

2.3. Symptom ratings

Subjects were asked six times (immediately before, after 3, 60 and 118 min of exposure and at 15 and 200 min after exposure) to answer a questionnaire with ten questions related to smell, irritative symptoms (of the eyes, nose and the throat), dyspnoea, headache, fatigue, dizziness, nausea and intoxication. Answers were given by marking along a 100 mm visual analogue scale graded from "not at all" (0 mm) to "almost unbearable" (100 mm). The questionnaire was originally elaborated for exposure to solvents (Iregren et al., 1993) but has been used in several other inhalation studies (Ernstgård et al., 2006; Walinder et al., 2005).

2.4. Blinking frequency

The blinking movements of the left eye were measured by electromyography (EMG) via three skin electrodes, two on *M. orbicularis oculi* and one reference electrode on the cheek bone. The EMG signal was amplified and transferred via telemetry to a personal computer. A software program in C++ was used for the identification of the characteristic signal pattern. Identification of blinks was performed by comparison against nine conditions related to the size, shape and appearance of the pattern (Walinder et al., 2005). Blinking frequency was continuously recorded from 2 min before exposure and throughout the entire exposure period. Blinks were counted in 2-min intervals and presented as blinks per minute.

2.5. Tear film break-up time

Precorneal tear film stability was assessed by measuring the tear film break-up time (BUT) by scanning the precorneal tear film with a biomicroscope (Topcon SL1E; Topcon, Tokyo, Japan). The time in seconds was recorded from the last blink until a rupture in the precorneal film was observed. Tear film stability was also estimated by recording the self-reported tear film break-up time. The subjects were asked to keep their eyes open, and the time was recorded until they felt an urge to blink, assuming that this feeling was the appearance of a dry spot on the cornea. Measurements of tear film break-up time were performed on three occasions in each eye: before entering the chamber; at the end of exposure; and 4 h after exposure.

2.6. Vital staining of the eye

Epithelial damage to the cornea and conjunctiva was assessed with a semi-quantitative method. Lissamine green (4 μL of a 1% physiological saline solution) was instilled into the lower conjunctival sac. After 1 min the cornea and conjunctiva were examined by a binocular microscope with a slit lamp (Topcon SL1E; Topcon, Tokyo, Japan) giving each eye a score of 0–9 (Norn, 1993). Vital staining was performed once, at 4 h after exposure.

2.7. Acoustic rhinometry

Rhinometric measurements were performed at three occasions during the exposure day: prior to entering the chamber, immediately after leaving it and 2 h after leaving the chamber. The nasal volume (from the nostril and 7 cm into the nasal cavity) and the minimal cross-section area were determined as an average of three measurements in each nostril. The measurements were performed under standardized forms, in a sitting position and after at least 30 min acclimatization to room temperature. The rhinometer has previously been described by Hilberg et al. (1989). It uses a single-click signal of audible frequencies which is analyzed with the Nasal Area-Distance Acquisition Program, version 1.0 (University of Aarhus, Aarhus, Denmark).

2.8. Nasal lavage

Lavage of the nasal mucosa was made with a 20-mL plastic syringe attached to a nose olive at three occasions, directly before entering the chamber, directly after leaving and 2 h after exposure. The subjects were standing, with their head flexed ca 30° forward. Room tempered (20–22 °C) 0.9% sterile saline solution was introduced into the nasal cavity. Each nostril was washed with 5 mL saline that was flushed back and forth five times via the syringe, at an interval of a few seconds. The fluid was then transferred into a 10-mL polypropylene centrifuge tube. The tube was kept on ice and centrifuged at 800 g for 5 min within 60 min. The supernatant was centrifuged again at 1400 × *g* for 5 min, and immediately frozen at −20 °C. References for the chemical analysis of lavage biomarkers, comprising eosinophil cationic protein (ECP), myeloperoxidase (MPO), lysozyme and albumin, have been given elsewhere (Walinder et al., 1998).

Table 1
Eye measurements in 29 subjects (mean ± S.D.) exposed to 10 mg/m^3 1-octen-3-ol or clean air for 2 h

Exposure	Measured break-up time (s)			Self-reported break-up time (s)			Blinking frequency	Lissamin staining
	Before	After[a]	4 h after[b]	Before	After[a]	4 h after[b]	During exposure[c]	After exposure[d]
Air	36 ± 19	−3 ± 17	−3 ± 17	32 ± 19	3 ± 17	3 ± 14	5.8 ± 0.7	0.2 ± 0.3
1-Octen 3-ol	34 ± 18	3 ± 14	−1 ± 13	35 ± 23	−2 ± 14	−4 ± 11	8.1 ± 0.9***	0.3 ± 0.4

[a] Change directly after exposure compared to before exposure.
[b] Change 4 h after exposure compared to before.
[c] Blinking frequency (blinks per minute) during exposure.
[d] Epithelial damage score 4 h after exposure.
*** $p < 0.001$ by repeated measurements ANOVA.