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ORIGINAL ARTICLE

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Sensory irritations and pulmonary effects in human volunteers following short-term exposure to pinewood emissions

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Abstract Pinewood (*Pinus* ssp.) is widely used for furniture and building purposes. However, despite its widespread use, information on possible human sensory irritations and pulmonary effects caused by exposure to volatile organic compounds (VOC) emitted from pinewood is sparse. For this purpose, (1) sensory irritation of eyes, nose and throat, (2) lung function parameters (FVC, FEV_1), (3) exhaled nitrogen oxide (NO) concentration, (4) eye blink frequency, and (5) sensory evaluation (using the SD method) were investigated before, after, and partly during exposure of human volunteers to emissions from pinewood panels. Fifteen healthy nonsmokers were exposed for 2 h under controlled conditions to VOCs emitted from pinewood panels in a 48 m^3 test chamber. VOC concentrations were about 5 mg/m^3 (loading rate, 1 m^2/m^3), 8 mg/m^3 (loading rate, 2 m^2/m^3), and 13 mg/m^3 (loading rate, 3 m^2/m^3), respectively. Terpene and aldehyde exposure concentrations ranged from about 3.50 ± 0.51 mg/m^3 and 0.07 ± 0.008 mg/m^3, 5.00 ± 0.95 mg/m^3, and 0.20 ± 0.02 mg/m^3 or 9.51 ± 1.10 mg/m^3 and 0.21 ± 0.04 mg/m^3 for loading rates of 1, 2, and 3 m^2/m^3, respectively. The emissions consisted predominantly of α-pinene and Δ^3-carene. No concentration-dependent effects before or after exposure to the emissions were measured with respect to sensory irritation, pulmonary function, exhaled NO, and eye blink frequency. Only the odor of the emissions was perceived by the study subjects, rated as being closer to "pleasant" than to "unpleasant." In conclusion, the results of our study suggest that short-term exposure to high VOC concentrations, even up to 13 mg/m^3, released from pinewood does not elicit sensory irritation or pulmonary effects in healthy humans under controlled conditions.

Key words Pinewood emissions · VOC · Terpenes · Sensory irritation · Pulmonary

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Introduction

Energy-saving improvements of buildings have led to the construction of insulated houses and dwellings with high standards of air tightness. This process has led to a marked reduction in the air exchange that normally takes place through gaps around doors and windows. Consequently, the question whether volatile organic compounds (VOCs) emitted from building materials and furniture can cause adverse effects to human health, especially sensory irritation,[1] i.e., irritation of the eyes, nose, and the upper airways, has become increasingly important. Additionally, it is well known that wood from the pine tree (*Pinus sylvestris*) or the European spruce (*Picea abies*) emits numerous odorous VOCs responsible for the typical smell of wood. Hence, odor perception (hedonics) may play a crucial role during exposure to wood-related VOC.

The monoterpenes α-pinene and Δ^3-carene have been identified as the compounds predominantly emitted by pinewood.[2–4] The monoterpenes β-pinene, limonene, and terpinolene have also been identified as being emitted from pinewood. The composition of pinewood-related VOC varies among species, location, and growth season. As described in the literature, because wood is being used more and more extensively in interior finishing of buildings in Europe, terpenes often occur in the indoor environment. The concentration of terpenes such as α-pinene, β-pinene, and Δ^3-carene in indoor air has increased greatly in the past two decades.[5–8] However, typical median concentrations in indoor air are low, ranging between 7 µg/m^3 and 44 µg/m^3 for α-pinene and from 4 µg/m^3 to 17 µg/m^3 for Δ^3-carene.[9,10] It is only in new or newly renovated dwellings that maximum concentrations of α-pinene can reach several hundred

micrograms per cubic meter.[3,9,11–13] Terpene concentrations greater than 1,000 μg/m^3 are rarely encountered and generally indicate construction faults.

Despite the widespread use of pinewood, information on possible sensory irritative effects from exposure to these complex VOC emissions is sparse. The perception of wood-related VOCs is based on olfactory and trigeminal pathways. Especially, the trigeminal pathway might cause acute health effects triggered by physiological reflexes (e.g., neurogenic inflammation) or defense mechanisms (e.g., coughing, sneezing, lacrimation).[1,10,14–19]

Monoterpenes can easily penetrate the different barriers of the body. Their uptake can occur through the lungs, gastrointestinal tract and intact skin.[20] For instance, Hedenstierna et al.[21] found weak irritant effects of α-pinene in mouth and throat in exposure studies with healthy volunteers using a 100-mm visual analogue scale (VAS), although concentrations were high. Odor and nasal pungency thresholds have been investigated in humans for selected terpenes. Odor threshold ranged from 0.64 mg/m^3 for geraniol to 9.61 mg/m^3 for Δ^3-carene. Nasal pungency thresholds were about three orders of magnitude above odor thresholds, ranging from 1,504 mg/m^3 for cineole to 9,247 mg/m^3 for Δ^3-carene.[22] Furthermore, monoterpenes or their oxidation products may cause both nonallergic and allergic contact dermatitis.

However, most available studies focus only on high concentrations of single compounds; none of the controlled exposure studies has considered VOC mixtures emitted from pinewood. Hence, the objective of the present study was to examine healthy human volunteers under standardized experimental conditions for the presence of sensory irritations, lung function impairment, and subjective health complaints when exposed to VOC emissions from pinewood at worst case concentrations in indoor air.

To evaluate possible health effects, sensory irritations, lung function measurements, changes in NO exhalation, and eye blinking frequency were investigated. Additionally, to determine subjective symptoms and well-being, validated visual analogue scales (VAS) were used to describe eight uncomfortable sensations. The semantic differential (SD) method was used for evaluating responses to odor quality.

Materials and methods

Study design

Fifteen healthy volunteers participated in the study. Subjects were exposed to five sessions each of clean air (clean air I at the beginning and clean air II at the end) and emissions from pinewood panels under loading rates of 1, 2, and 3 m^2/m^3 in a 48 m^3 exposure chamber. Each subject was exposed to the five exposure conditions over a period of 2 h each while exercising on a cycle ergometer at 50 W (six sets of 20 min including 5-min rests). Air exchange rate was 1 h^{-1}. Exposure to pinewood emissions was conducted blind, i.e., the subjects were not aware of the kind of test material or the exposure conditions. The exposure sessions were separated by at least 2 weeks. At the beginning of each exposure session the subjects were given sufficient information about the objective and procedure of the experiment. They were instructed not to discuss their symptoms or assumed exposure levels with anyone. The exposure design generally followed the parameters used in various previous human exposure studies accomplished at the Karolinska Institute in Stockholm, Sweden.[23–26] The study was approved by the Ethics Committee of the Medical Faculty of the University of Freiburg.

Characterization of test material

The selected pinewood panels were produced in Germany and of commercial origin. They had been uniformly manufactured from pine (*Pinus sylvestris* L.) and were 18 mm thick, 800 mm long and 300 mm wide. To prevent exposure to or loss of VOC during transport and storage, the panels were wrapped in plastic foil. Upon arriving at the study location (WKI: Fraunhofer-Institute for Wood Research – Wilhelm-Klauditz-Institut, Braunschweig, Germany), the pinewood panels were unwrapped and placed in the exposure chamber in electropolished stainless steel holders. At that time, the moisture content of the panels was approximately 6% (m/m). The 48 m^3 exposure chamber was loaded with 200 panels giving loading rates of 1 m^2/m^3, with 400 panels of 2 m^2/m^3, and with 600 panels of 3 m^2/m^3. After completing the first day of testing, the chamber was cleaned, flushed with fresh air, and reloaded with new pinewood panels for the next groups.

Study subjects

Fifteen volunteers (six female and nine male) participated in all the study experiments. All the volunteers were nonsmokers; mean age was 23.4 ± 3.6 years (range, 20–30 years). None of the volunteers wore contact lenses during exposure to VOCs. Before the exposure experiments, clinical blood chemistry tests were undertaken. Two inflammatory markers, interleukin-6 (IL-6) and C-reactive protein (CrP), were analyzed in blood collected in vaccutainer tubes (with EDTA; Becton Dickinson, Heidelberg, Germany). The analyses were carried out by LADR (Medizinisches Versorgungszentrum, Braunschweig, Germany). Three subjects with IgE concentrations >100 kU/l showed no indication of clinical symptoms, i.e., inflammation or allergic diseases, as was also true for three outliers with relatively high CrP concentrations, 10–24 mg/l. Odor sensitivity was tested qualitatively with "Sniffin Sticks"[27–29] at study onset; the test result was a sum score of the correctly identified odors. Identification of 10 of 12 odorants was evaluated as normosmic. The rate of odor identification was found to be 58–100%; 80% of the volunteers were normosmic (i.e., odor identification rate ≥83%). The subjects were informed about the study design, possible hazards, and their right to immediately and unconditionally interrupt exposure. Each participant signed a written informed consent form on voluntary participation.

Exposure experiments

The exposures were carried out in a 48 m^3 exposure chamber ($3 \times 4 \times 4$ m, L × W × H) with an air exchange of 1 h^{-1}. The chamber was manufactured by Weiss Umwelttechnik (Giessen, Germany) of electropolished stainless steel. The chamber fulfilled international emission-testing standards such as ISO 16000-9[30] or EN 717-1.[31] Temperature, relative humidity, organic gases (as total organic carbon content), carbon dioxide level, and outlet flow rates of chamber air were continuously monitored. The airflow was conditioned to a temperature of 21° ± 0.5°C and relative humidity of initially 50% ± 3% with deionized water. To achieve low VOC background values, supply air was purged through an active charcoal and a fine particle filter, resulting in total volatile organic compounds (TVOC) values <20 µg/m^3 and individual VOC levels <1 µg/m^3.

Chemical analysis of the exposure chamber atmosphere

Samples of the chamber air were collected via stainless steel sampling lines from outside. VOC sampling of exposure chamber air took place according to ISO 16000-6,[32] collecting the exhaust air from the test chamber by active sampling (150 ml/min, 40 min; FLEC pump, Chematek, Roskilde, Denmark) with stainless steel desorption tubes (Perkin-Elmer, Santa Clara, CA, USA) filled with Tenax TA (Chrompack, Engstlingen, Germany). The organic compounds inclusive of acetic acid were desorbed thermally by a thermo desorber (320°C, 10 min; ATD 400, Perkin Elmer, Überlingen, Germany) and subsequently analyzed by capillary column gas chromatography with a Agilent 7890 GC system (Agilent, Santa Clara, CA, USA) combined with a Agilent mass selective detector 5975C (Agilent). The compounds were separated on a DB 5 MS column (60 m × 0.25 mm, 0.25 µm; Agilent) using helium as a carrier gas. Column temperature program was initial temperature 32°C and final temperature 320°C. Compounds were identified using the commercial NIST Spectral library (NIST; Wiley Registry 7th Edition, 2005); quantification used internal standards and mixtures of pure reference compounds as external standards. All VOC identified were quantified using their own response factor; their limits of quantification were <1 µg/m^3. Low molecular aldehydes and ketones, among them formaldehyde, were determined using the DNPH method (2,4-dinitrophenylhydrazine) as defined in ISO 16000-3.[33] For sampling the exhaust, air from the chamber is drawn for 1–2 h through cartridges (Supelco, Bellefonte, USA) coated with DNPH at a flow rate of 0.5–1.0 l/min. The sample cartridge is then eluted with acetonitrile; this eluate is directly used for high pressure liquid chromatography (HPLC) analysis (C_{18} column and water/acetonitrile solvent combinations with binary or ternary gradients). For detection by UV spectroscopy, the absorption maxima of different hydrazones range from 340 to 427 nm. The limit of quantification is <1 µg/m^3.

Examinations of the test subjects

Subjects underwent the following examinations: physical examination before and immediately after exposure including general health status, allergy, and/or skin diseases, acute infections, and acute diseases of the upper airways, lungs, heart, skin, and eyes. Pulse frequency, blood pressure, pulmonary function, and exhaled nitric oxide (NO) were also measured.

Lung function measurements

Spirometry was performed according to the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines[34] using a portable Spirometer (SpiroPro; Viasys Health Care, Hoechberg, Germany). The measurements included vital capacity (VC), forced vital capacity (FVC), and forced expiratory volume in 1 s (FEV_1). The highest value of three measurements was used. The difference between FVC or FEV_1 measurements before and after exposure to the different conditions in comparison to clean air I was used as an indication for the presence of respiratory alteration.

Measurement of exhaled nitric oxide (NO)

NO was measured according to the European Respiratory Society (ERS) with a handheld device (NioxMino; Aerocrine, Solna, Sweden).[34,35] The difference between the measurements of NO before and after exposure to different conditions in comparison to clean air I was used as an indication for the presence of an inflammatory effect.

Eye blinking frequency

Eye blinking was monitored throughout the entire 2-h exposure by electromyography (EMG).[36] The EMG device based on the Varioport system (Becker Meditec, Karlsruhe, Germany). Two miniature surface Ag/AgCl electrodes (internal diameter, 6 mm) were attached to the orbicularis oculi muscle of the left eye and one electrode (Ambu Blue Sensor electrode; Ambu, Ballerup, Denmark) on the cheek bone. The electrodes were connected to Becker Meditec amplifiers; the raw EMG signal was analyzed automatically by a four-channel contour-following integrator. With the help of video recordings, the complete EMG data were screened for artifacts. To standardize visual demands during the assessment, the volunteers were asked not to speak, read, or listen to music. All counting was performed by the same experimenter, blinded with respect to exposure conditions. For analyses of blinking frequency, specific sections were selected, representing time series between 15–20 min, 55–60 min, and 115–120 min after the beginning of exposure.

Visual analogue scales (VAS)

Symptom ratings were performed using 0–100 mm visual analogue scales (VAS) (Ernstgaard et al.[24–26]) graded from "not at all" (0 mm) through "hardly at all" (6 mm),

"somewhat" (26 mm), "rather" (48 mm), "quite" (72 mm), and "very" (90 mm) to "almost unbearable" (100 mm). The eight symptoms to be rated were (1) "discomfort in the eyes: burning, irritated, or running eyes"; (2) "discomfort in the nose: burning, irritated, or runny nose"; (3) "discomfort in the throat or airways"; (4) "breathing difficulty"; (5) "smell of emissions"; (6) "headache"; (7) "dizziness"; and (8) "fatigue". Ratings were performed immediately after entering the exposure chamber (time point, 0 min), during exposure (20, 40, 60, 80, and 100 min from the onset of exposure, with 5 min of recovery time between each and at the end of the exposure (time point, 120 min).

Sensory evaluation by semantic differential (SD) method

The semantic differential (SD) method was used for evaluating responses of 15 volunteers to odor quality in the chamber.[37] The SD consisted of 29 pairs of polar adjectives used to describe different sensory experiences (e.g., dark vs. light, happy vs. sad, soft vs. hard) and the evaluation scale ranged from –3 to +3.[37] The complete data set for determining odor perception and the representative profile value was evaluated according to the method for hedonic assessment of smell of emissions in plants and other technical installations.[37]

Statistical calculations

The underlying parameters were analyzed by nonparametric methods.[38] The Friedman test was applied to investigate differences between the different exposure conditions. In the case of a significant result ($P < 0.05$), a pairwise Wilcoxon signed-rank test was used to test the "concentration effects" more precisely. Because the underlying study investigated "safety aspects," no adjustment of the significance level was done. Furthermore, the Page's trend test[39] was used to detect a trend, whereas the different exposure conditions were arranged in an increasing manner, i.e., clean air I, pinewood emission with loading rate 1 m^2/m^3 < pinewood emission with loading rate 2 m^2/m^3 < pinewood emission with loading rate 3 m^2/m^3. Finally, the Wilcoxon signed-rank test was used to test for reversibility. Therefore, the two control conditions (clean air I and clean air II) were tested for differences. Gender differences of the median of the VAS ratings, lung function parameters, NO differences, and blink frequencies were tested by Mann–Whitney U test. The significance level was set at $P < 0.05$ in all statistical analyses.

Results and discussion

Study design

During the past decades, studies have been performed to evaluate the significance of health effects caused by various VOCs typically emitted from wood and wood-based materials. The studies focus on terpenes such as α-pinene, Δ^3-carene, and some of their mixtures, but also on aldehydes such as hexanal.[15–17,25,40] Most of these studies used exposure concentration measurements, lung function tests, and VAS as survey instruments for the identification of perception of odorous and irritating effects to the eyes and upper respiratory tract. However, all these studies dealt with single compounds or artificial mixtures and do not reflect the real situation of VOC mixtures emitted from pinewood. Based on the results available from previous studies, and to simulate a worst case scenario, we used high pinewood panel loading rates of 1–3 m^2/m^3 test chamber volume.

The study presented here used only validated and/or standardized methods: measurement of lung function parameters,[34] exhaled nitric oxide,[41–46] measurement of eye blinking frequency,[18,36,47–51] and VAS as a widely used survey instrument to record subjective symptoms.[15,16,23,24,52–55]

Chemical analysis of the exposure chamber atmosphere

The composition and average concentrations of the VOC emissions released from pinewood during the various exposure conditions are summarized in Table 1. The pinewood panels showed a typical pattern of VOC emission and release into the chamber air. The VOCs predominantly emitted were terpenes and aldehydes. At loading rates of 1, 2, and 3 m^2/m^3, total VOC (TVOC) emissions increased from 4.76 ± 0.47 mg/m^3 over 7.72 ± 1.46 mg/m^3 to 12.72 ± 1.27 mg/m^3 (Table 1). The pinewood loading rates investigated yielded approximate average exposure concentrations of 3.5 mg/m^3, 5.0 mg/m^3, and 9.5 mg/m^3 for terpenes, which consisted predominantly of α-pinene (up to 70%) and Δ^3-carene (up to 28%). Additionally, beside these main components, a number of other terpenes, such as β-pinene, limonene, and terpinolene, were emitted. In contrast, aldehydes such as pentanal, hexanal, or nonanal were emitted in considerably lower concentrations and accounted for only approximately 1.5% of the TVOC. Formaldehyde and acetic acid were also present in the chamber air under the various exposure conditions, reaching concentrations of about 0.05 mg/m^3 for formaldehyde and 0.9 mg/m^3 for acetic acid. For comparison, in some studies, especially those examining new or recently renovated housing, wood-specific VOCs were found in concentrations of more than 1 mg/m^3. Typical α-pinene concentrations in indoor air range between 10 and 40 $\mu g/m^3$.[10] Hence, the pinewood-specific VOC concentrations in our experiments were about 300- to 1,300 fold higher than these concentrations for α-pinene.

Examination of the test subjects

Subjects showed no clinical symptoms at the end of all five exposure experiments, especially, no apparent eye or throat redness and no neurovegetative symptoms (dizziness, nausea, orientation problems).

Table 1. Emissions of selected volatile organic compounds (VOCS) and very volatile organic compounds (VVOCs) from clean air and pinewood panels during 30 exposure sessions with $n = 15$ volunteers

Compound	VOC chamber concentrations [mg/m³]				
	Clean air I	Pinewood loading rate: 1 m^2/m^3	Pinewood loading rate: 2 m^2/m^3	Pinewood loading rate: 3 m^2/m^3	Clean air II
Terpenes					
α-Pinene	ND[d]	2.40 ± 0.35	3.04 ± 0.44	5.87 ± 0.77	ND
Δ^3-Carene	ND	0.92 ± 0.13	1.41 ± 0.32	2.24 ± 0.22	ND
Other terpenes	ND	0.17 ± 0.03	0.56 ± 0.18	1.40 ± 0.10	ND
Sum terpenes[a]	ND	3.49 ± 0.51	5.00 ± 0.95	9.51 ± 0.11	ND
Aldehydes					
Pentanal	ND	ND	0.05 ± 0.002	0.05 ± 0.01	ND
Hexanal	ND	0.07 ± 0.01	0.10 ± 0.007	0.13 ± 0.03	ND
Nonanal	0.006 ± 0.001	ND	0.021 ± 0.001	0.02 ± 0.003	0.003 ± 0.002
2-Heptenal	ND	ND	ND	ND	ND
2-Octenal	ND	ND	ND	ND	ND
Sum aldehydes[a]	0.006 ± 0.001	0.07 ± 0.01	0.20 ± 0.02	0.21 ± 0.04	0.003 ± 0.002
Formaldehyde	0.008 ± 0.002	0.026 ± 0.003	0.048 ± 0.008	0.043 ± 0.006	0.006 ± 0.002
Other aldehydes (DNPH)[b]	0.011 ± 0.003	0.137 ± 0.008	0.75 ± 0.076	0.435 ± 0.030	0.017 ± 0.007
Acetic acid	ND	0.37 ± 0.19	0.40 ± 0.07	0.92 ± 0.11	0.006 ± 0.005
Total VOC[c]	0.06 ± 0.04	4.76 ± 0.47	7.72 ± 1.46	12.72 ± 1.27	0.075 ± 0.047

Data are mean values ± standard deviation (MV ± SD)
[a] Sum of the average of each individual compound
[b] Acetaldehyde, propionaldehyde, butyraldehyde
[c] Includes all individual VOCs from C_6 to C_{16} (total VOC, TVOC)
[d] ND, not detectable

Lung function measurements

The results on differences in FVC and FEV_1 measurements before and after 2-h exposure under different exposure conditions are presented in Fig. 1. No differences or concentration dependencies were found between the different conditions ($P = 0.948$ and 0.980, respectively, Friedman test; $P = 0.3942$ and 0.4466, respectively, Page's trend test). No control differences were seen between clean air I and clean air II for FVC and FEV_1 ($P = 1.000$ and 0.607, respectively, Wilcoxon signed-rank test). No gender differences were found for pulmonary measurements.

Exhaled nitric oxide

Differences in exhaled NO (Fig. 2) were not significantly affected in the 15 volunteers before or after 2-h exposure to pinewood emissions under various exposure conditions ($P = 0.642$, Friedman test; $P = 0.2371$, Page's trend test). Furthermore, no control differences were seen between clean air I and clean air II ($P = 0.210$, Wilcoxon signed-rank test). No gender differences were found for exhaled NO. In the present study, the NO concentrations in exhaled air were in the same range as NO concentrations in adults not suffering from airway inflammation (10–20 ppb).[56] However, it should be noted that the validity of NO measurements is doubted, particularly in asthma management.[57]

Eye blinking frequency

Blinking frequencies ranged from 14 to 27 blinks per minute. Median blinking frequencies were detected in the range from 20.5 to 22.5 blinks/min (Fig. 3). Exposure conditions showed no effect on blinking frequencies at the time points 15–20 min, 55–60 min, or 115–120 min ($P = 0.145$, 0.210, and 0.164, respectively, Friedman test; $P = 0.221$, 0.235, and 0.442, respectively, Page's trend test). Furthermore, no control differences were seen between clean air I and clean air II ($P = 0.320$, 15–20 min; $P = 0.700$, 55–50 min; $P = 0.396$, 115–120 min; Wilcoxon signed-rank test). No gender differences were found for blinking frequencies. Increased blinking frequency has previously been reported in studies on industrial chemicals in workplace concentrations, e.g., 3-methylfuran and 1-octen-3-ol,[58,59] *n*-hexanal,[25] ε-caprolactam,[60] methacrolein,[51] and formaldehyde.[61] Increased blinking frequency has also been reported for environmental tobacco smoke[62] and limonene oxidation products.[47] Median blinking frequencies were detected in the range of about 20 blinks/min, and the results are therefore of the same order of a recently published study using the same technical equipment.[26]

Visual analogue scales (VAS)

The VAS ratings made during the five exposures at various time points showed large interindividual variation, sometimes with normal and extreme outliers (e.g., Fig. 4). Moreover, with the exception of the rating "smell of emissions," the median ratings were relatively low and only occasionally exceeded the verbal label "hardly at all" (≤6 mm). The VAS rating of "discomfort of the eyes," "discomfort in the nose," and "discomfort in the throat and airways" showed no consistent concentration- and time-dependent differences between the investigated exposure conditions (data not

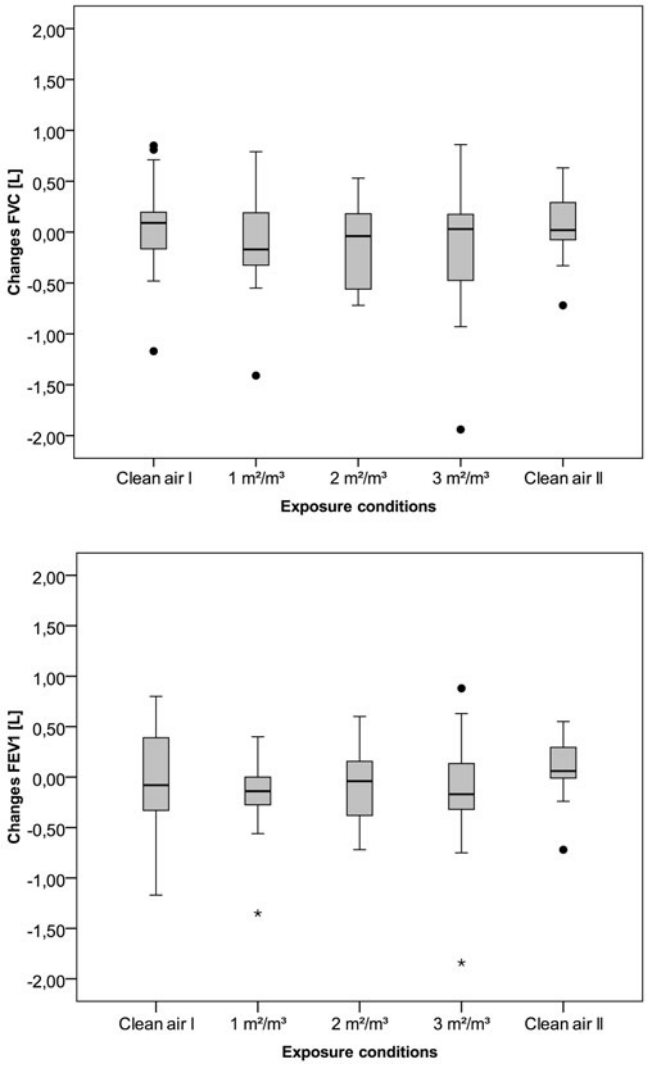

Fig. 1. Box plots of changes in forced vital capacity (*FVC*) (*upper plot*) and in forced expiratory volume in 1 s (FEV_1) (*lower plot*) in 15 volunteers measured before and after exposure to clean air or to pinewood emissions under various exposure conditions denote minimum observation, 25th percentile (lower quartile), 50th percentile (median), 75th percentile (upper quartile), and maximum observation. Normal outliers, *circles*; extreme outliers, *asterisks*

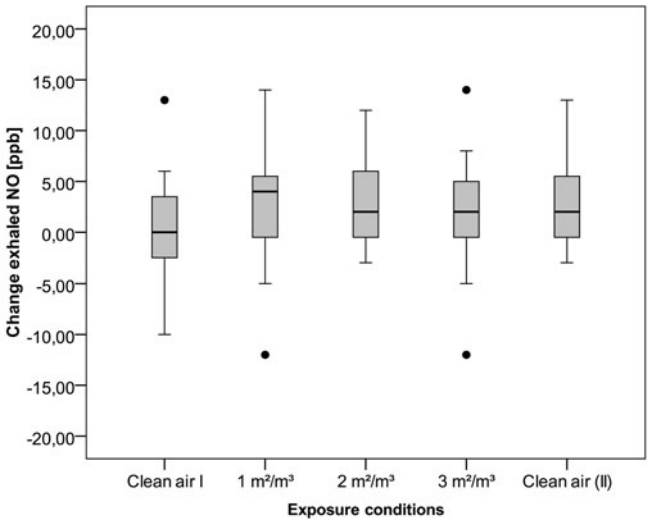

Fig. 2. Box plots of changes in *exhaled NO* concentrations in 15 volunteers exposed to clean air and pinewood emissions under different exposure conditions measured before and after exposure denote minimum observation, 25th percentile (lower quartile), 50th percentile (median), 75th percentile (upper quartile), and maximum observation. *Circles*, normal outliers

shown). Significant differences between the different exposure conditions were found during exposure to pinewood emissions for the rating "smell of emissions" (Fig. 4: $P < 0.001$, Friedman test; $P < 0.001$, Page's trend test). Regarding the time-course of this rating, subjects showed strong adaptation during the 2 h of exposure to pinewood emissions. However, VAS ratings did not reach the control level of clean air. No concentration- or time-dependent gender differences in symptom ratings were observed. The only measurable response from volunteers to exposure to pinewood emissions was the VAS rating for smell of emissions. In general, the ratings given in the individual experiments for smell were highest immediately upon entering the exposure chamber (0 min). The ratings subsequently declined during the 2 h of exposure as part of an odor adaptation but did not reach the control value of clean air. Interestingly, "smell of emission" was not concentration dependent, the main reason possibly being the flat concentration–odor intensity perception curve over a wide range. Reduction of the VOC concentration has no effect so long as the level is on the flat plateau.[63] Which substances are responsible for the odor effect caused by the complex VOC mixtures emitted from pinewood can only be speculated. Odor threshold levels for terpenes, i.e., for α-pinene, Δ^3-carene, or limonene, are quite high compared to the terpene concentrations identified in the test chamber atmosphere. Thus, in the present study, terpenes released from pinewood (maximum, 9.5 mg/m^3) may be responsible for the odor perceived by the study volunteers. The general trend is that reported odor thresholds are orders of magnitude too high.[19,64,65]

Sensory evaluation by SD

The sensory responses of the volunteers to pinewood emissions was estimated by the SD method. The curve in Fig. 5 shows the representative profile values of the 15 volunteers exposed to pinewood emissions at a loading rate of 3 m^2/m^3. The subjects judged the odor quality closer to "pleasant" than to "unpleasant." The mean representative profile value for "unpleasant" and "pleasant" were –1.46 and +1.36, respectively. For the other exposure conditions the mean scores were –0.14, +0.45, +0.49, +0.36, and +0.41 for clean air I, loading rates of 1 m^2/m^3, 2 m^2/m^3, and 3 m^2/m^3 for clean air II, respectively (data not shown). In conclusion, using the SD method, the study subjects rated the quality of the pinewood emissions in the chamber air as "pleasant" for all pinewood exposure conditions; i.e., they accepted the indoor air quality perceived and did not express dissatisfaction. In

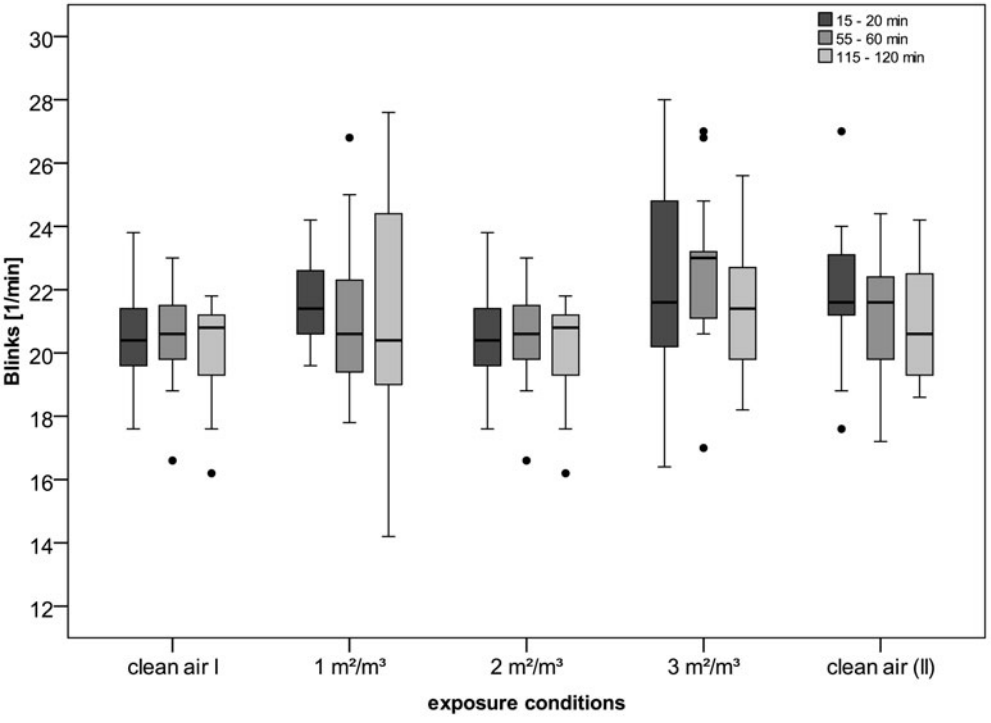

Fig. 3. Box plots of blinking frequency in 15 volunteers during exposure to clean air and to pinewood emissions at various exposure conditions. Results are presented in time series between 15–20 min, 55–60 min, and 115–120 min. Box plots denote minimum observation, 25th percentile (lower quartile), 50th percentile (median), 75th percentile (upper quartile), and maximum observation. *Circles*, normal outliers

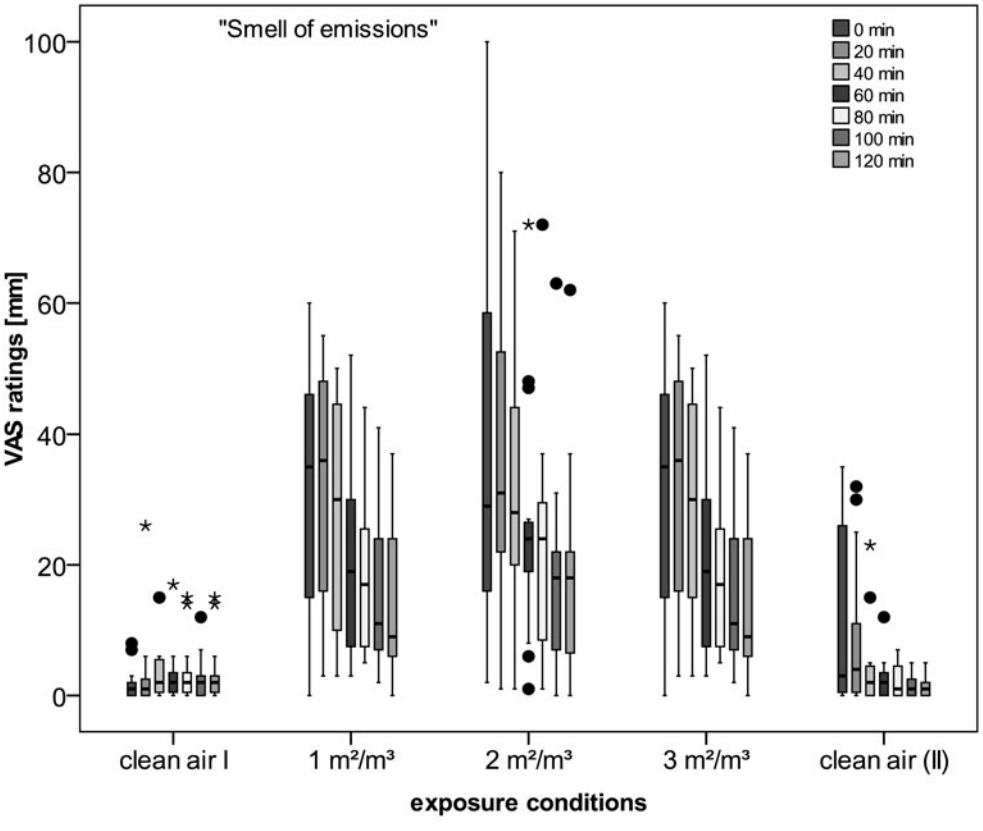

Fig. 4. Box plots of ratings "smell of emissions" of 15 volunteers exposed to clean air and to pinewood emissions under various exposure conditions and different time points during an exposure period of 2 h denote minimum observation, 25th percentile (lower quartile), 50th percentile (median), 75th percentile (upper quartile), and maximum observation. *Circles*, normal outliers; *asterisks*, extreme outliers. Symptoms ratings were performed in a questionnaire using a 0–100 mm visual analogue scale (*VAS*) graded as 0 mm, not at all; 6 mm, hardly at all; 26 mm, somewhat; 48 mm, rather; 72 mm, quite; 90 mm, very; 100 mm, almost unbearable

this study, odor seems to be the most important response caused by VOCs emitted from pinewood into the indoor environment. It is well known that almost all building materials derived from pine species possess an odor. Usually, the odor intensity decreases to low and acceptable levels within a few weeks or months after completion of the building.[66] However, odor perception varies and represents a wide range from pleasant to unpleasant.[19] The interaction

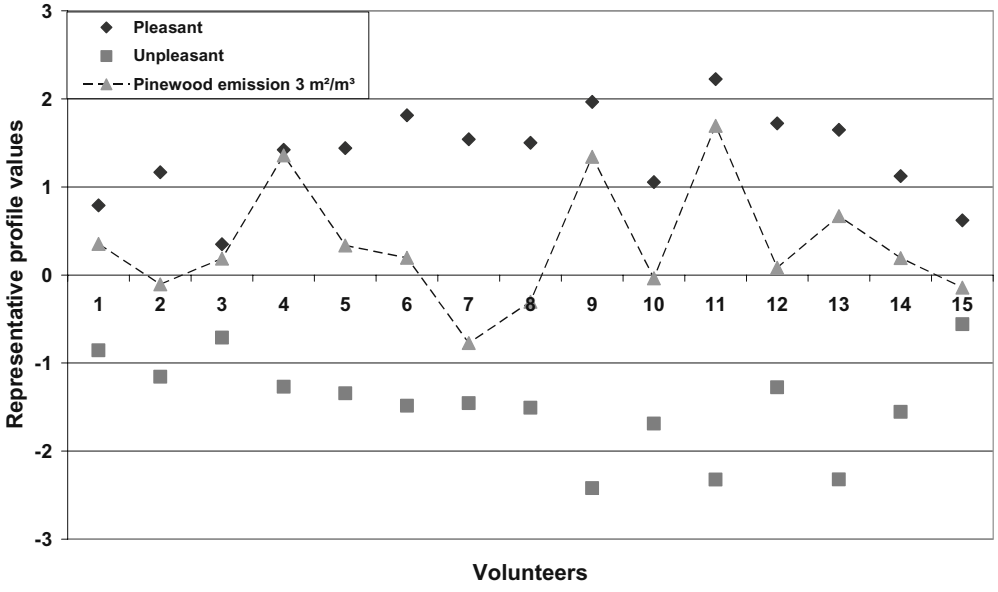

Fig. 5. Ratings of odor in chamber following exposure of 15 volunteers to emissions of pinewood (loading rate, 3 m^2/m^3) using a semantic differential (SD) method are shown as representative profile values of 15 volunteers according to GIRL[37]

between odor and a person's psychological state (e.g., emotion/mood) or manipulation[67] is complex, and cultural differences also exist.[68,69]

Conclusions

No evidence was found for eye, nose, throat, or upper airway irritation or for lung function impairment following exposure (2 h) to various levels of VOCs emitted from pinewood panels. Because no irritant effects could be identified, even at VOC concentrations to almost 12.7 mg/m^3, and irritant effects caused by irritant receptor-mediated mechanisms are early-onset mechanisms with threshold values and without cumulative characteristics, the study provides important and robust data with respect to the health evaluation of pinewood-mediated emissions in indoor air. It is not likely that health effects will arise long after a one-time short-period inhalation. Although the possibility of long-term health effects such as obstructive pulmonary disease emerging after repeated short-period inhalation over a long time cannot be ruled out with certainty, no basis to support this assumption has been reported. In summary, our experiments lead to the conclusion that only odor effects can be expected during exposure to even high VOC concentrations emitted from pinewood into the indoor environment. In view of the uncertainties regarding (i) long-term exposure, (ii) individual susceptibility to odorous or irritant substances,[70] or (iii) chemical reaction of pinewood-specific VOCs with other indoor air contaminants such as ozone,[12,19,71–73] exposure to VOCs in indoor air should, from a preventive point of view, also be minimized wherever possible.

Integrative concepts focusing on low-emission building products, energy-saving technologies, and heat, ventilation, and air-conditioning systems are necessary to reconcile environmental (energy-saving) and health issues in future.

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References

1. Doty RL, Cometto-Muniz JE, Jalowayski AA, Dalton P, Kendal-Reed M, Hodgson M (2004) Assessment of upper respiratory tract and ocular irritative effects of volatile chemicals in humans. Crit Rev Toxicol 34:85–142
2. Risholm-Sundman M, Herder P, Lundgren M, Vestin E (1998) Emissions of acetic acid and other volatile organic compounds from different species of solid wood. Holz Roh-Werkstoff 56:125–129
3. Baumann M, Battermann A, Zhang GZ (1999) Terpene emissions from particleboard and medium-density fiberboard products. For Prod J 49:49–56
4. Baumann M, Lorenz L, Batterman S, Zhang GZ (2000) Aldehyde emissions from particleboard and medium fiberboard products. For Prod J 50:75–82
5. Scholz H, Santl H (1999) Occurrence and assessment of selected volatile organic compounds (VOC) in indoor air. Proc Indoor Air 1:481–486
6. Ullrich D, Weiland SK, Seifert B (1999) VOC in homes of children with asthma: a case control study of indoor air quality. Proc Indoor Air 4:131–132
7. Schleibinger H, Hott U, Marchl, D, Braun P, Plieninger P, Rüden H (2001) VOC-Konzentrationen in Innenräumen des Großraums Berlin im Zeitraum von 1988–1999. Gefahrstoffe Reinhaltung Luft 61:26–38
8. Schreiner H, Wetzel H, Kirbach I (2001) Innenraumluftbelastung deutscher Kindergärten mit flüchtigen organischen Verbindungen (VOC). Umweltmedizin Forschung Praxis 6:143–149

9. Lux W, Mohr S, Heinzow B, Ostendorp G (2001) Belastung der Raumluft privater Neubauten mit flüchtigen organischen Verbindungen. Bundesgesundheitsblatt 44:619–624
10. Mersch-Sundermann V (2007) Gesundheitliche Bewertung von α-Pinen in der Innenraumluft: Aktueller Erkenntnisstand. Umweltmedizin Forschung Praxis 12:129–151
11. Hodgson AT, Rudd AF, Beal D, Chandra S (2000) Volatile organic compounds concentrations and emission rates in new manufactured and site-built houses. Indoor Air 10:178–192
12. Wolkoff P, Clausen PA, Wilkens CK, Nielsen GD (2000) Formation of strong airway irritants in terpene/ozone mixtures. Indoor Air 10:82–91
13. Rehwagen M, Schlink U, Herbarth O (2003) Seasonal cycle of VOCs in apartments. Indoor Air 13:283–291
14. Bodin L, Juto JE, Mølhave L (2006) Upper airway inflammation in relation to dust spiked with aldehydes or glucan. Scand J Work Environ Health 32:374–382
15. Falk AA, Hagberg M, Löf A, Wigeaus Hjelm EW, Wang Z (1990) Uptake, distribution and elimination of α-pinene in man after exposure by inhalation. Scand J Work Environ Health 16:372–378
16. Falk A, Löf A, Hagberg M, Wigeaus Hjelm EW, Wang Z (1991) Human exposure to 3-carene by inhalation: toxicokinetics, effects on pulmonary function and occurrence of irritative and CNS symptoms. Toxicol Appl Pharmacol 110:198–205
17. Kasanen JP, Pasanen AL, Pasanen P, Liesivuori J, Kosma VM, Alarie Y (1999) Evaluation of sensory irritation of delta3-carene and turpentine, and acceptable levels of monoterpenes in occupational and indoor environment. J Toxicol Environ Health A 57:89–114
18. Wolkoff P, Skov P, Franck C, Pedersen LN (2003) Eye irritation and environmental factors in the office environment. Hypotheses, causes, and a physiological model. Scand J Work Environ Health 29:411–430
19. Wolkoff P, Wilkins CK, Clausen PA, Nielsen GD (2006) Organic compounds in office environments: sensory irritation, odor, measurements and the role of reactive chemistry. Indoor Air 16:7–19
20. Edman K, Löfstedt H, Berg P, Eriksson K, Axelsson S, Bryngelsson I, Fedeli C (2003) Exposure assessment to α- and β-pinene, Δ3-carene and wood dust in industrial production of wood pellets. Ann Occup Hyg 47:219–226
21. Hedenstierna, G, Alexandersson R, Wimander K, Rosen G (1983) Exposure to terpenes: effects on pulmonary function. Int Arch Occup Environ Health 51:191–198
22. Cometto-Muniz JE, Cain WS, Abraham MH, Kumarsingh R (1998) Sensory properties of selected terpenes. Thresholds for odor, nasal pungency, nasal localization, and eye irritation. Ann N Y Acad Sci 855:648–651
23. Ernstgaard L, Gullstrand E, Löf A, Johanson G (2002) Are women more sensitive than men to 2-propanol and *m*-xylene vapours? Occup Environ Med 59:759–767
24. Ernstgaard L, Iregren A, Sjögren B, Johanson G (2006) Acute effects of exposure to vapours of acetic acid in humans. Toxicol Lett 165:22–30
25. Ernstgaard L, Iregren A, Sjögren B, Svedberg U, Johanson G (2006) Acute effects of exposure to hexanal vapors in humans. J Occup Environ Health 48:573–80
26. Ernstgaard L, Iregren A, Juran, S, Sjögren B, van Thriel C, Johanson G (2009) Acute effects of exposure to vapors of standard and dearomatized white spirits in humans. 2. Irritation and inflammation. J Appl Toxicol 29:263–274
27. Kobal G, Hummel T, Sekinger B, Barz S, Roscher S, Wolf S (1996) 'Sniffin' Sticks': screening of olfactory performance. Rhinology 34:222–226
28. Hummel T, Kobal G, Gudziol H, Mackay-Sim A (2007) Normative data for the "Sniffin' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. Eur Arch Otorhinolaryngol 264:237–243
29. Katotomichelakis M, Balatsouras D, Tripsianis G, Tsaroucha A, Homsioglou E, Danielides V (2007) Normative Values of Olfactory Function Testing Using the 'Sniffin' Sticks'. Laryngoscope 117:114–120
30. ISO 16000-9 (2006) Indoor air – Part 9: Determination of the emission of volatile organic compounds from building products and furnishing. Emission Test Chamber Method
31. EN 717-1 (2005) Wood-based panels – determination of formaldehyde release: Part 1: Formaldehyde emission by the chamber method
32. ISO 16000-6 (2004) Indoor air – Part 6: Determination of volatile organic compounds in indoor and test chamber air by active sampling on Tenax TA sorbent, thermal desorption and gas chromatography using MS/FID
33. ISO 16000-3 (2001) Indoor air – Part 3: Determination of formaldehyde and other carbonyl compounds: active sampling method
34. American Thoracic Society/European Respiratory Society Recommendations (2005) Recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. Am J Respir Crit Care Med 171:912–930
35. Hemmingsson T, Linnarsson D, Gambert R (2004) Novel handheld device for exhaled nitric oxide analysis in research and clinical applications. J Clin Monit Comput 18:379–387
36. Kiesswetter E, van Thriel C, Schäper M, Blaszkewicz M, Seeber A (2005) Eye blinks as an indicator for sensory irritation during constant and peak exposures to 2-ethylhexanol. Environ Toxicol Pharmacol 19:531–541
37. GIRL (2004) Feststellung und Beurteilung von Geruchsimmissionen (Geruchsimmissions-Richtlinie – GIRL) in der Fassung vom 21. September 2004 mit Begründung und Auslegungshinweisen.
38. Hollander M, Wolfe DA (1973) Nonparametric statistical methods. Wiley, New York
39. Page EB (1963) Ordered hypotheses for multiple treatments: a significance test for linear ranks. J Am Stat Assoc 58:216–230
40. Falk Filipson A (1996) Short term inhalation exposure to turpentine; toxicokinetics and acute effects in men. Occup Environ Med 53:100–105
41. Kharitonov SA, Gonio F, Kelly C, Meah S, Barnes PJ (2003) Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. Eur Respir J 21:433–438
42. Vahlkvist S, Sinding M, Skamstrup K, Bisgaard H (2006) Daily home measurements of exhaled nitric oxide in asthmatic children during natural birch pollen exposure. J Allergy Clin Immunol 117:1272–1276
43. Maniscalco M, Sofia M, Pelaia G (2007) Nitric oxide in upper airways inflammatory diseases. Inflamm Res 56:58–69
44. Turner S (2007) The role of exhaled nitric oxide in the diagnosis, management and treatment of asthma. Mini Rev Med Chem 7:539–542
45. Price D, Berg J, Lindgren P (2009) An economic evaluation of NIOX MINO airway inflammation monitor in the United Kingdom. Allergy 64:413–414
46. Brindicci C, Ito K, Barnes PJ, Kharitonov SA (2007) Differential flow analysis of exhaled nitric oxide in patients with asthma of differing severity. Chest 131:1353–1362
47. Kleno J, Wolkoff P (2004) Changes in eye blink frequency as a measure of trigeminal stimulation by exposure to limonene oxidation products, isoprene oxidation products and nitrate radicals. Int Arch Occup Environ Health 77:235–243
48. van Thriel C, Seeber A, Kiesswetter E, Blaszkewicz M, Golka K, Wiesmuller GA (2003) Physiological and psychological approaches to chemosensory effects of solvents. Toxicol Lett 140–141: 261–271
49. van Thriel C, Kiesswetter E, Blaszkewicz M, Golka K, Seeber A (2003) Neurobehavioral effects during experimental exposure to 1-octanol and isopropanol. Scand J Work Environ Health 29: 143–151
50. van Thriel C, Kiesswetter E, Blaszkewicz M, Golka K, Seeber A (2003) Neurobehavioral effects during experimental exposure to 1-octanol and isopropanol. Scand J Work Environ Health 29: 143–151
51. Nojgaard JK, Christensen KB, Wolkoff P (2005) The effects on human eye blink frequency of exposure to limonene oxidation products and methacrolein. Toxicol Lett 156:241–251
52. Iregren A, Tesarz M, Wigaeus-Hjelm E (1993) Human experimental MIBK exposure: effects on heart rate, performance, and symptoms. Environ Res 63:101–108
53. Järnberg J, Johanson G, Löf A (1996) Toxicokinetics of inhaled trimethylbenzenes in man. Toxicol Appl Pharmacol 140:81–288
54. Sundblad BM, Larsson BM, Acevedo F, Ernstgaard L, Johanson G, Larsson K, Palmberg L (2004) Acute respiratory effects of expo-

sure to ammonia on healthy persons. Scand J Work Environ Health 30:313–321
55. Wieslander G, Norbäck D, Venge P (2007) Changes of symptoms, tear film stability and eosinophilic cationic protein in nasal lavage fluid after re-exposure to a damp office building with history in flooding. Indoor Air 17:19–27
56. Kharitonov SA, Yates D, Barnes PJ (1995) Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. Eur Respir J 8:295–297
57. Stick S, Franklin P (2009) NO more dogma. Nitric oxide marker in asthma. Am J Respir Crit Care Med 179:87–92
58. Walinder R, Ernstgaard L, Johanson G, Norbäck D, Venge W, Wieslander G (2005) Acute effects of a fungal volatile compound. Environ Health Perspect 113:775–778
59. Walinder R, Ernstgaard L, Norbäck D, Wieslander, G, Johanson G (2008) Acute effects of 1-octen-3-ol, a microbial volatile organic compound (MVOC): an experimental study. Toxicol Lett 181: 141–147
60. Ziegler AE, Zimmer H, Triebig G (2008) Exposure study on chemosensory effects of caprolactam in the low concentration range. Int Arch Occup Environ Health 81:743–753
61. Lang I, Bruckner T, Triebig G (2008) Formaldehyde and chemosensory irritation in humans. A controlled human exposure study. Regul Toxicol Pharmacol 50:23–36
62. Junker, MH, Danuser B, Monn C, Koller T (2001) Acute sensory responses of nonsmokers at very low environmental tobacco smoke concentrations in controlled laboratory settings. Environ Health Perspect 109:1045–1052
63. Shusterman D (1992) The health significance of environmental odor pollution. Arch Environ Health 47:76–87
64. Cain WS, Schmidt R, Wolkoff P (2007) Olfactory detection of ozone and D-limonene: reactants in indoor spaces. Indoor Air 17:337–347
65. Cain WS, Schmidt R (2009) Can we trust odor databases? Example of *t*- and *n*-butyl acetate. Atmos Environ 43:2591–2601
66. Knudsen HM, Nielsen PA, Clausen PA, Wilkins CA, Wolkoff P (2003) Sensory evaluation of emissions from selected building products exposed to ozone. Indoor Air 13:223–231
67. Wilkins K, Wolkoff P, Knudsen HN, Clausen PA (2007) The impact of information on perceived air quality – "organic" vs. "synthetic" building materials. Indoor Air 17:130–134
68. Ayabe-Kanamura S, Schicker I, Laska M, Hudson R, Distel H, Kobayakawa T, Saito S (1998) Differences in perception of everyday odors: a Japanese–German cross-cultural study. Chem Senses 23:31–38
69. Dalton P (2003) Upper airway irritation, odor perception and health risk due to airborne chemicals. Toxicol Lett 140–141:239–248
70. Shusterman D, Murphy MA, Balmes J (2003) Differences in nasal irritant sensitivity by age, gender, and allergic rhinitis status. Int Arch Occup Environ Health 76:577–583
71. Glasius M, Lahaniati M, Galagirou A, Di Bella D, Jensen NR, Hjorth J, Kotzias D, Larsen BR (2000) Carboxylic acids in secondary aerosols from oxidation of cyclic monoterpenes by ozone. Environ Sci Technol 34:1001–1010
72. Docherty KS, Wu W, Lim YB, Ziemann PJ (2005) Contributions of organic peroxides to secondary aerosol formed from reactions of monoterpenes and O_3. Environ Sci Technol 39:4049–4059
73. Chen X, Hopke PK (2009) Secondary organic aerosol from α-pinene ozonolysis in dynamic chamber system. Indoor Air 19: 335–345