Acute Sensory Responses of Nonsmokers at Very Low Environmental Tobacco Smoke Concentrations in Controlled Laboratory Settings

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The objective of this study was to provide a basis for effectively protecting nonsmokers from acute sensory impacts and for preventing deterioration of indoor air quality caused by environmental tobacco smoke (ETS) emissions. With an olfactory experiment we determined odor detection thresholds (OT) of sidestream ETS (sETS), and with a full-body exposure experiment we investigated sensory symptoms at very low sETS exposure concentrations. OT concentrations for sETS are three and more orders of magnitude lower than ETS concentrations measured in field settings and correspond to a fresh air dilution volume of > 19,000 m^3 per cigarette, over 100 times more than had previously been suggested for acceptable indoor air conditions. Eye and nasal irritations were observed at one order of magnitude lower sETS concentrations than previously reported, corresponding to a fresh air dilution volume of > 3,000 m^3 per cigarette. These findings have great practical implications for defining indoor air quality standards in indoor compartments where ETS emissions occur. Our study strongly supports the implementation and control of smoking policies such as segregating smoking areas from areas where smoking is not permitted or instituting smoking bans in public buildings. Key words: environmental tobacco smoke, indoor air quality, odor threshold, sensory symptoms, ventilation. ***Environ Health Perspect*** **109:1045–1052 (2001). [Online 28 September 2001]**
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Over the past years, several studies evaluating acute health impacts and sensory responses from exposure to environmental tobacco smoke (ETS) have been performed. Chamber studies, evaluating lung functions of asthmatics and other sensitive subjects, have used sidestream ETS (sETS) concentrations between 2 and > 15 ppm carbon monoxide (*1–3*), and studies focusing on sensory symptoms have used ETS at lower concentrations (*4–7*). For eye irritations, a tolerable limit of 1.5–2 ppm CO has been reported (*5–8*). Significant increases of perceptive eye and nasal irritations as well as annoyance were observed at respirable suspended particulate matter (RSP) concentrations of 58 µg/m^3, corresponding to a time-weighted average concentration of 0.22 ppm CO, and led to a significant decrease in air quality acceptability (*7*). The authors estimated that an 80% air quality acceptability rate corresponded to an RSP concentration of 103.3 µg/m^3. Based on an average ETS-RSP yield per cigarette of 13.7 mg (*9*), this concentration corresponds to one cigarette diluted in an average western European living room. Cain et al. (*4*) reported similar observations.

Regarding the typical exposure concentrations encountered in field studies, RSP concentrations are reported at 120 µg/m^3 when someone is smoking (*10*). More recent personal exposure studies in the United States and in Europe showed median RSP concentrations that were markedly lower (*11–14*). However, these data are based on sample intervals averaged over 8-hr periods. Short-term RSP concentrations have been reported to be much higher (*10,15*). Furthermore, an alarming increase in the active smoking rate has been observed in some countries. In Switzerland, an increase of greater than 40% has been reported in the 14–24 years age group (*16*).

The awareness that perceptual and comfort aspects are important factors in a healthy building is growing, and indoor air quality guidelines are taking this more and more into consideration (*17*). ETS, as a contributor to sick building syndrome (*18*), potentially causes widespread sensory impacts and discomfort in many places where smokers and nonsmokers coexist. This concept is supported by the observation that people with a history of atopy or respiratory illness are more sensitive to the acute, irritating effects of ETS than people without such a medical history (*19*). However, odor thresholds and thresholds of perceptive irritations with respect to ETS have not been determined conclusively. The World Health Organization recommends that unwanted odorous compounds should not be present in concentrations exceeding the ED_{50} (effective dose that makes 50% of the exposed population respond) detection threshold. Sensory irritants should not be present in excess of the ED_{10} (effective dose that makes 10% of the exposed population respond) detection threshold (*20*). That many public buildings, schools, and restaurants still do not implement smoking policies in several parts of the world today indicates that ETS is potentially present and constitutes a social problem now and in the future.

The goal of this study was to determine odor detection thresholds of sETS in a laboratory setting. Acute sensory symptoms, breathing patterns, annoyance, and the indoor air quality acceptability were determined at very low sETS concentrations in an exposure chamber. On the basis of sETS emission rates, we established fresh air volumes necessary to dilute one cigarette to threshold concentrations. In addition, we used startle reflexes that are assessed by electromyogram recordings of the M. orbicularis oculi and elicited by an acoustic stimulus as an objective indicator of annoyance.

In this study, we aimed to determine air quality standards required to protect nonsmokers from adverse health effects caused by impacts of ETS on the human sensory system as well as to provide measures for establishing acceptable indoor air quality. We show that ETS odor thresholds are about 100 times lower, and nasal and eye irritations about 10 times lower, than reported in previous studies (*4,7*). On a practical level, separately ventilated areas for smokers and nonsmokers or a complete smoking ban are required to protect nonsmokers effectively from the sensory impacts and the annoyance potential of ETS.

Methods

Experimental design. In this study, we performed three experimental sessions. During one session, we conducted an olfactory experiment determining sETS odor detection thresholds. Data obtained laid the foundation of a laboratory exposure study investigating

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sensory symptoms in nonsmokers at very low sETS concentrations. Figure 1 illustrates the basic design scheme of the experimental setup for both studies. Moreover, we performed a cigarette emission study in the empty exposure chamber to describe the results obtained in terms of cigarette equivalents. We could thus compare sETS generated for both the exposure and olfactory study to sETS not biased by the experimental setup.

We generated sETS in a glove box 0.6 m^3 in volume by a Borgwaldt smoke generator (Borgwaldt, Hamburg, Germany). On the basis of sales statistics of the Swiss Community of the Cigarette Industry, we chose six cigarette brands and evenly distributed them on the smoke generator (*21*). Throughout the duration of a session, two randomly chosen cigarettes burned until they passively extinguished after 5–6 min. When burning ceased, another two cigarettes were lit. The mainstream fraction of the tobacco smoke aerosol was exhausted out of the glove box into a ventilation hood.

Fresh air was introduced into either the olfactometer or the full-body exposure chamber by a fresh air unit, equipped with two radial ventilators providing a fresh air flow of 1.5 m^3/min. The air was filtered by a glass fiber filter (Camfil 1E-110; Camfil AB, Trosa, Sweden) and an active charcoal granulate (CN-50 6 × 12 1.7–3.4 mm; Siegfried AG, Zofingen, Switzerland).

Cigarette emission experiment. To establish the amount of sETS emitted by one cigarette, we multiplied average baseline-corrected ETS concentrations throughout the burning time of the cigarette (570 sec) by the amount of fresh air introduced into the empty exposure chamber during the same time period (25.7 L/sec). During the cigarette emission experiment, one cigarette of the most commonly smoked brand in Switzerland was lit and inserted through the ceiling into the empty exposure chamber 2 m^3 in volume via a PVC tube. The experiment was repeated six times. Because the cigarettes smoldered passively, they remained burning for 9.5 min. During this time no mainstream smoke was generated (i.e., no puffs were taken). Because of the rather high air exchange rates (45/hr), we assumed a homogenous distribution of sETS. The cigarettes remained burning until they passively extinguished.

Subjects. We chose 24 female nonsmokers to participate in the olfactory and the full-body exposure experiments. Written consent was obtained from the subjects before the experiments. The Ethics Commission of the Federal Institute of Technology (Zurich, Switzerland) approved the study.

The subjects were required to be healthy, not to have a record of allergy to pollen or dust, not to be anosmatic, and not to have smoked in the last 5 years. Moreover, the subjects were not permitted to use either eyeglasses or contact lenses and were asked to refrain from being exposed to ETS on the day of the study. The subjects were between 18 and 35 years of age and were paid for their participation. Of the 24 who participated in the full-body exposure study, 18 took part in the olfactory experiment. In a preliminary questionnaire, the participants were asked to indicate their degree of annoyance by ETS, automobile exhaust fumes, solvents, and perfumes.

Olfactory experiment. To obtain sETS odor thresholds, we performed two types of experiments based on the method of limits (*22*). In one, the subjects were asked to evaluate the air by placing their nose into the duct of the olfactometer only upon presentation of the stimuli (type A); in the other, the subjects' noses remained within the duct throughout the duration of the experiment (type B). In four to eight repetitions, stimuli were presented in ascending concentrations for both experiments. A potential odor threshold value within a trial was obtained when the subject perceived the ascending concentration of stimuli for the first time. A valid odor threshold value was given when a subject stated perceiving an odor during two consecutively ascending concentrations. We calculated odor thresholds by subtracting the sETS baseline concentration before the stimuli had been presented from the maximum concentration of the sETS indicator during stimuli presentation. The data were obtained from 18 female nonsmokers who were divided into six panels of three subjects per panel.

An olfactometer developed at the Institute for Hygiene and Applied Physiology (Zurich, Switzerland) was used (*23*). Air is drawn via a Teflon-coated ventilator from the surrounding environment and guided through a system of glass tubing to four Teflon-coated nose ducts. Fresh air is constantly washed through the system at a rate of 147 L/min, reaching an air speed of 0.85 m/sec at the ducts from where the sensory measurements are carried out. One of the four nose ducts was used for monitoring ETS indicators. We fed sETS manually into the fresh air stream by rotameters. The maximum dilution factor of the olfactometer is 39,400. This was doubled with a further dilution before entering the olfactometer by a factor of two.

Full-body exposure experiment. The experimental procedure performed for each participant within the exposure chamber is described qualitatively in Figure 2.

Each session consisted of eight conditions of interest. In four of the eight episodes, different amounts of sETS, distinguished by the air flow rates of 200 mL/min, 500 mL/min, 1,200 mL/min, or 3,600 mL/min, were fed from the glove box into the fresh air stream passing though the exposure chamber (sETS condition). The smallest flow rate was determined to generate sETS concentrations that were approximately equivalent to concentrations observed at the 95th percentile of the odor threshold. Before each of these sETS conditions, air without sETS (zero condition) was administered. We randomized the sequence of sETS conditions over 24 subjects. For each subject the administered ETS episode pattern was

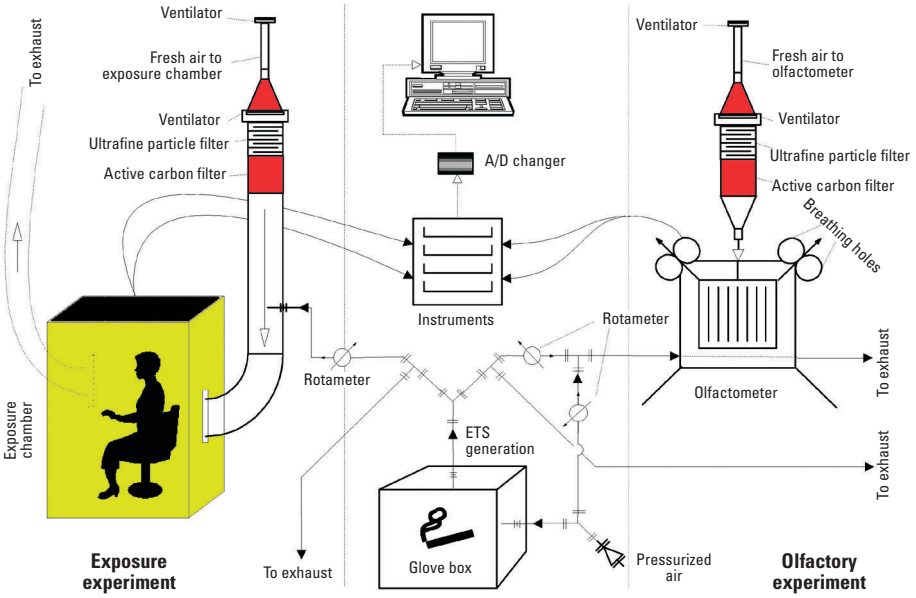

Figure 1. Scheme of the experimental setup for the odor threshold and the full-body exposure study. The equipment shown in the middle panel was used for both setups. Generated in the glove box and diluted by a fresh air delivery system, sETS was fed into either the olfactometer to determine odor detection thresholds or into the exposure chamber to assess sensory symptoms.

randomly selected out of a pool of 24 possible patterns. The session commenced with a zero condition that was succeeded by a randomly selected sETS condition. Zero condition and sETS condition then followed in alternating order. Each episode commenced with a 2-min time span of startle stimuli that was followed by a questionnaire and proceeded by an eye blink count. We continuously monitored breathing patterns throughout the session. To minimize distractions, a beige cotton curtain surrounded the exposure chamber. The experimenter did not have any eye contact with the subject.

For the sensory questionnaires, each sensory symptom was scaled on a vertical axis within which the participants were told to mark a horizontal reference anywhere on the scale that reflected their perception of the given symptom (Table 1)

The exposure chamber was constructed out of Plexiglas (height, 1.6 m; length, 1.4 m; width, 0.9 m). It was possible to seat a subject comfortably in front of a small desk. The fresh air unit providing particle free air at a constant volatile organic compound (VOC) background concentration maintained a constant air flow (1.5 m^3/min). Air was fed into the chamber via a ventilation duct (0.25 m in diameter) situated knee height near the far corner of the chamber on the right hand side facing the participant. The exhaust air left the chamber by a duct (0.25 m in diameter) behind the subject's head. In this way the air was forced to pass by the subject's face. Although the air exchange rate of the ventilation system was 45/hr, air velocities in the vicinity of the face remained < 0.1 m/sec. Air sampling tubes were placed through holes in the center part of the ceiling near head height.

Instrumentation. A number of sETS constituents were continuously monitored throughout the duration of the experiments:

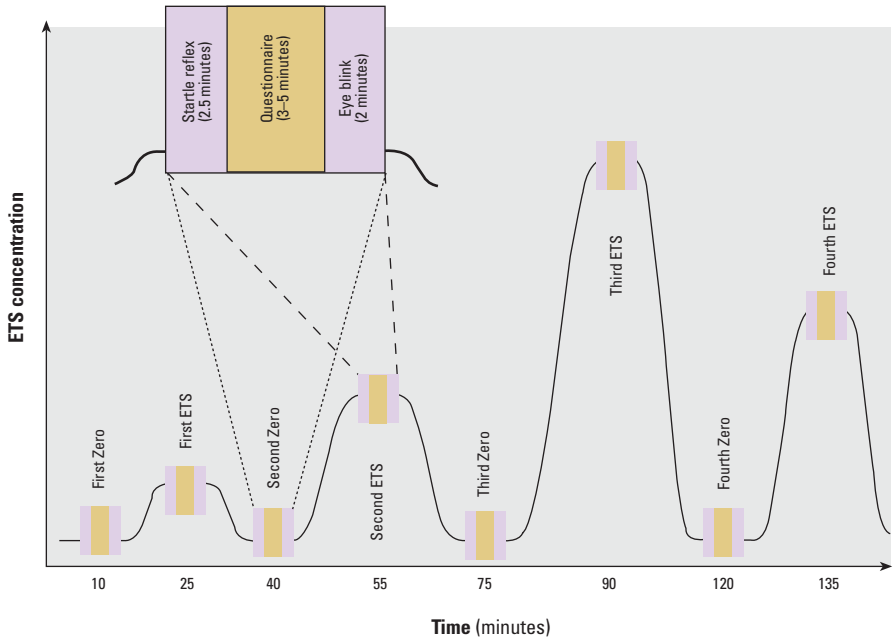

Figure 2. Experimental procedure during one session of the exposure experiment. The sequence of sETS concentrations was randomized over 24 subjects. Each episode commenced with a startle response measurement, followed by a questionnaire and an eye blink count. Breathing pattern measurements were performed during the entire session.

Table 1. A streamlined version of the sensory symptoms questionnaire.

Assessed judgment	Scale
Air temperature Relative humidity	3, too high; 0, just right; –3, too low
Odor strength Eye irritation Nasal irritation Throat irritation	6, overwhelming; 5, very strong; 4, strong; 3, moderate; 2, weak; 1, very weak; 0, not at all
Arousal Annoyance	6, overwhelming; 5, very strong; 4, strong; 3, moderate; 2, weak; 1, very weak; 0, not at all
Odor perception	1, extremely pleasant; 0.67, pleasant; 0.33, rather pleasant; 0, neutral; –0.33, rather unpleasant; –0.67, unpleasant; –1, extremely unpleasant
Odor perception, air quality	1, acceptable; –1, unacceptable; a value > 0 is acceptable; a value < 0 is unacceptable

particle-bound polycyclic aromatic hydrocarbons (pPAH), total volatile organic compounds (tVOC), and particle number concentrations. In the cigarette emission and full-body exposure experiment, CO was additionally monitored, and a number of discrete particle number and particle mass distributions were carried out. CO_2, parameters of thermal comfort, and VOCs were also assessed in the full-body exposure study.

We measured pPAH by means of a photoelectric aerosol sensor (PAS, type: LQ1-TV, Matter Engineering Inc., Wohlen, Switzerland) (*24,25*) For total volatile organic compounds a flame ionization detector was used (Model VE7; J.U.M. Engineering, Karlsfeld, Germany). We measured CO with an APMA-300E CO Monitor (Horiba Ltd., Japan). To assess the total particle number concentrations, we used a condensation nucleus counter (version 3025; TSI, St. Paul, MN, USA). For particle number versus size distributions, we used a scanning mobility particle sizer (version 2.3; TSI Inc.) in the size range between 0.015 and 0.673 µm mobility diameter at a resolution of 64 channels per decade on a logarithmic diameter axis. A 10-stage Quartz Crystal Microbalance Cascade Impactor System allowed the assessment of size-specific particle masses (Model PC-2; California Measurements Inc., Sierra Madre, CA, USA) at a mid-point aerodynamic cutoff ranging from 0.07 µm to 35 µm.

For the chemical analysis of the VOC samples, a known volume of air was pumped through a stainless-steel tube filled with an adsorbent (Tenax TA; Tenax GmbH, Düsseldorf, Germany). The transfer of the sample to capillary gas chromatography (column: DB-5ms, 30 m; J&W Scientific, Agilent Technologies, Palo Alto, CA, USA) was done by thermodesorption (Perkin Elmer ATD 400; Perkin Elmer Instruments, Wellesley, MA, USA). The gas chromatrgraph (Fisons 6000; Fisons Instruments, Beverly, MA, USA) was equipped with an flame ionization detector for quantification and a mass spectrometer (Fisons MD800) for identification of the detected VOCs. The sorbent tubes were loaded with toluene-d8 as an internal standard. Concentrations are given as toluene equivalents. The Tenax tubes were exposed for 60 min at a sample rate of 100 mL/min. The sampling and analysis of these VOCs was performed by the Swiss Federal Department for Economics and Occupation (Zurich, Switzerland). For the aldehyde analysis, samples were drawn through a stainless-steel tube at a sampling rate of 1.3 L/min with 2,4-dinitrophenylhydrazine as an adsorbent. Aldehydes are desorbed, and via high pressure liquid chromatography the different species are determined. The aldehyde analysis was performed by the Institut

für Gefahrstoff-Forschung der Bergbau-Berufsgenossenschaft (Bochum, Germany). The data for both the VOCs and the aldehydes are not shown.

We measured the air temperature and relative humidity with an instrument from ROTRONIC AG (Bassersdorf, Switzerland). Wind speeds were assessed by a Dantec low velocity flow analyzer type 54N50 (Dantec Inc., Copenhagen, Denmark). Carbon dioxide measurements were performed with the EGQ-10 measuring instrument (Sauter AG, Basel, Switzerland).

We recorded respiratory parameters by Respitrace cardio respiratory diagnostic technology (SensorMedics Technology, Yorba Linda, CA, USA) based on inductive plethysmography. Data analysis was performed with RespiEvents software (version 4.2c; Nims, Miami Beach, FL, USA). Breathing bands that assessed breathing patterns were fitted over the subject's breasts and abdomen. We calibrated the bands before and after the experiment using a spirometer (Spiro-Junior; Erich Jaeger, Würzburg, Germany).

We used an SR-EMG System (San Diego Instruments Inc., San Diego, CA, USA) to assess the startle response signal. This device is a modularized electromyograhic system of two units, an amplifier modifier and a stimulus generator unit. For the startle response measurements, we placed two electrodes on the M. orbicularis oculi of the left eye of the subject. A broad-band white noise (100–1,000 Hz) at 65 dB_A as a background was presented to the subject during a 2-min period over a set of headphones. During this period a series of 10 acoustic impulses of 100 dB_A for a time span of 40 msec were generated.

Results

Cigarette Emission Experiment

The emission rates per cigarette for pPAH, $PM_{2.25}$, particle numbers, CO, and tVOC are shown in Table 2.

To estimate the degree that coagulation and adsorption processes may alter the physical characteristics of the sETS aerosol, we compared particle number and particle mass distribution measurements from directly emitted sETS to machine-generated sETS that had been transferred from the glove box to the exposure chamber. The particle number distribution of one cigarette burning in the exposure chamber shifted from a geometric mean diameter of 0.085 µm (geometric standard deviation = 0.002 µm) to an average geometric mean diameter of 0.172 µm (geometric standard deviation = 0.002 µm) when initially generated in the glove box (average of 3 measurements). Parallel to the increase in mean diameter, the particle number concentration would have to decrease over time. Based on the particle emission rate of 9.3×10^{12} particles per cigarette (Table 2), the estimated particle concentration in the glove box (0.6 m^3) after two cigarettes had burned was 3.1×10^7 particles/cm^3. The following calculations were performed to estimate the actual particle number concentrations if coagulation processes in the glove box had not taken place (*26*):

$$N(t) = \frac{N_0}{1 + N_0 K t} \quad [1]$$

$$K = C_c 3.0 \times 10^{-10} \quad [2]$$

$$\frac{d(t)}{d_0} = \left(\frac{N_0}{N(t)}\right)^{1/3} \quad [3]$$

where $N(t)$ = particle number concentration at time t; N_0 = initial particle number concentration = 3.1×10^7; K = coagulation coefficient; C_c = slip correction factor ~ 1.2 for a particle with a geometric mean diameter of 0.085 µm; t = approximate burning time of a cigarette including time to transfer to the exposure chamber, ~ 420 sec; $d(t)$ = particle diameter at time t; and d_0 = initial particle diameter = 0.085 µm.

The solution to Equation 1 equals 5.5×10^6 particles/cm^3 (i.e., 5.7 greater particle numbers if coagulation had not taken place), and the geometric mean diameter increased by a factor of 1.78 (Equation 3). Compared to the initial particle number concentration, this is equivalent to a theoretical decrease by a factor of 5.7 after coagulation in the glove box and adsorption of the smaller particles onto the PVC tubes has taken place. The observed increase in geometric mean diameter by a factor of 2.02 is similar to the calculated increase of 1.78. In addition, the particle mass distribution revealed a shift to larger diameters within the accumulation mode (0.1–2 µm) after sETS had been generated in the glove box and transferred to the exposure chamber (data not shown). These results show that substantial coagulation and particle removal have taken place in the time span between aerosol generation within the glove box and its analysis in the exposure chamber.

Table 2. Average sETS emission rates per cigarette.

Indicators	Mean concentration during burning time ± SD	Air volume during burning time of one cigarettes (m^3)	sETS generation per cigarette ± SD
pPAH	1,661 ± 117 ng/m^3	14.65	24.3 ± 1.7 µg
$PM_{2.25}$[a]	387 ± 78 µg/m^3	14.65	5.7 ± 1.1 mg
Particle numbers[b]	(6.3 ± 0.5) × 10^5/cm^3	14.65	(9.3 ± 0.7) × 10^{12}
CO	4.88 ± 0.47 ppm	14.65	89 ± 9 mg
tVOC[c]	3,722 ± 414 ppb	14.65	113 ± 13 mg

The experiment was repeated six times.
[a]One profile was generated. [b]Particle numbers were averaged out of three repetitions. [c]tVOC masses were calculated on the basis of propane equivalents.

Olfactory Experiment

The obtained odor thresholds of sETS expressed in terms of measured particle numbers, pPAH, and tVOC concentrations are depicted in Figure 3.

The comparison of both experiment types shows an increase in sensitivity of the odor threshold based on median sETS concentrations by a factor of 2–4 while the subjects' noses remained in the ducts. The variability of all measurements expressed by the ratio between the 95th and 5th percentile lies between 9 and 35 (type A) and between 6 and 21 (type B). The variability based on the ratios between maximum and minimum odor threshold concentration do not exceed 300 for type A, while for type B a maximum ratio of 175 was observed.

Subjects

We chose 24 healthy, female nonsmokers for the full-body exposure study assessing a variety of sensory symptoms, startle responses, and breathing patterns in a range of very low ETS concentrations. Before the study the participants were asked to state how bothered they

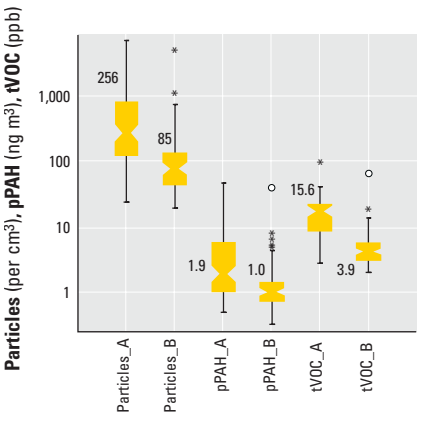

Figure 3. Odor detection thresholds of sETS expressed in terms of particles per cubic centimenter (number of values: 98 for type A, 60 for type B), pPAH, in nanograms per cubic meter (number of values: 98 for type A, 74 for type B), and tVOC, in parts per billion (number of values: 51 for type A, 75 for type B) concentrations. Two experiment types were performed: type A, nose in (5 sec) and out (30 sec) of duct; type B, nose stays in duct. Box plots were generated with Systat 8.0. Number in boxes are median concentrations.

generally felt toward ETS, automobile exhaust fumes, perfumes, and solvents. On a voting scale from 1 to 5 (1 = not at all bothered; 5 = very bothered), the subjects were, on average, more bothered by ETS (4.3) and automobile exhaust fumes (3.9) than by perfumes (2.2) and solvents (2.6). None of the subjects was very bothered by all of these agents.

Full-Body Exposure Experiment

The following sections describe the environmental conditions and the chemical species the subjects were exposed to. The results of the sensory symptom questionnaire, breathing patterns, eye blink rates and startle reflex measurements are presented.

Environmental conditions. Table 3 summarizes the average ETS concentrations of the four ETS conditions the subjects were exposed to. The ETS conditions were randomly distributed in an odd succession. Also shown are four alternating zero conditions.

The tVOC concentrations during the zero-air condition represent values that can cause possible discomfort and irritation according to the guidelines suggested by Mølhave (*27*). This remains unexplained, because the subsequent VOC concentrations measured by Tenax tubes with a sampling time of 60 min were not above the limit of detection (data not shown). The study population perceived the odor as neutral (neither pleasant nor unpleasant), and judged the quality of indoor air as acceptable.

The concentrations of VOCs and aldehydes to which the subjects were exposed increased with the degree of sETS infiltration into the exposure chamber. The data (not shown) suggest that for nicotine and 3-ethenyl-pyridine the surfaces of the glove box and the tubing acted as a sink.

Sensory responses. On the basis of the questionnaire results, we compared the average absolute sensory symptom values during the four ETS conditions to the sensory symptom values of the directly preceding zero-air condition (Table 4; only lowest sETS condition shown). The differences between the intensity of a sensory symptom at an ETS condition and symptom intensity of the preceding zero condition were statistically significant for all perceived sensory symptoms except perceived air temperature and relative humidity. The average concentrations at the lowest sETS were 468 particles per cm^3, 7.3 ng/m^3 pPAH, and 19 ppb tVOC. This corresponds to an estimated ETS-$PM_{2.25}$ (particulate matter ≤ 2.25 µm diameter) concentration of about 4.4 µg/m^3. At these concentrations the percentage of occupants judging the quality of air to be acceptable was 33%.

The results for the sensory symptoms show that even at very low ETS concentrations, subjects perceived a significant increase in sensory impact (eye, nasal, and throat irritations). Furthermore, they felt significantly more annoyed and reported the quality of air to be less acceptable than under zero conditions.

Humans are capable of discriminating relative changes only in perception (*28*). Figure 4 takes this circumstance into account. Plotted are relative increases of the intensity of a sensory symptom (intensity at an ETS condition minus intensity at the preceding zero condition) against relative increases of log-transformed sETS concentrations (ETS concentration at an ETS condition minus ETS concentration at its preceding zero condition). Furthermore, *p*-values of a linear regression model are depicted.

Based on a Pearson's linear regression model, the log-transformed ETS indicators such as particle numbers, pPAH, and tVOC concentrations show a linear trend with odor strength, eye irritation, arousal, annoyance, odor perception, acceptability of indoor air quality, wanting to open the window, wanting to leave the room, and complaining at work. Nasal irritations, on the other hand, show a linear trend with the particulate indicators only.

To determine which sensory channel (odor, nose, eyes, throat, arousal) contributes most to the observed decline of the indoor air quality, we performed a stepwise multiple linear regression model. Only the linear combination of the variables odor strength ($F = 12.1$, $p = 0.001$) and arousal ($F = 7.39$, $p = 0.008$) related to the degree of indoor air quality acceptability ($r^2 = 0.5$, $p < 0.001$). The contributions of eye, nasal, and throat irritations, however, did not show a significant relation ($p > 0.4$).

Startle reflex measurements. In the past, the startle reflex has been used as a tool to evaluate emotional qualities of a foreground stimulation (*29,30*). Because the startle reflex is not confounded by voluntary muscle activity, it is well suited to assessing motor behavior caused by a foreground stimulus. The startle reflex amplitude is affected by the extent to which the foreground stimulus can attract attention (*31*), especially when foreground stimuli and startle stimuli constitute different modalities (*32*). The startle response is facilitated when attention is directed to an acoustic startle stimulus, whereas the response is attenuated when attention is drawn away from the stimulus (*33*). These findings suggest that the redirecting of attention toward an annoying stimulus can be measured by the startle reflex.

Figure 5 depicts differences between startle electromyographic amplitudes determined during the four ETS episodes and their directly preceding zero-air condition. All EMG signals have been normalized by the startle amplitude of the first zero episode. Log-pPAH concentrations correlated nearly significantly to EMG amplitudes (negative *r*) when rank orders ($p = 0.058$) were not considered. Although we observed a negative trend as sETS concentrations increased, a significant difference existed only between

Table 3. Average concentrations of the continuously monitored environmental parameters during four exposure episodes depicted as sETS flow rates.

Environmental parameters	1st Zero	200 mL/min	2nd Zero	500 mL/min	3rd Zero	1,200 mL/min	4th Zero	3,600 mL/min
Particle numbers (cm^3)	0.02 (0.03)[a]	468 (110)	0.03 (0.03)	1,456 (266)	0.04 (0.04)	3,860 (795)	0.05 (0.04)	17,343 (1.891)
PPAH (ng/m^3)	1.6 (0.9)	9.3 (2.4)	1.8 (1.0)	22.8 (4.2)	2.1 (0.9)	58.5 (9.9)	2.2 (0.9)	218.8 (32.0)
CO (ppm)	0.16 (0.08)	0.21 (0.10)	0.16 (0.08)	0.27 (0.10)	0.15 (0.07)	0.39 (0.10)	0.15 (85)	1.07 (370)
tVOC (ppb)	1,244 (58)	1,256 (56)	1,238 (56)	1,282 (52)	1,232 (59)	1,340 (54)	1,231 (64)	1,702 (137)
CO_2 (ppm)	636 (79)	628 (44)	630 (62)	632 (83)	636 (93)	648 (61)	622 (74)	639 (46)
Temperature (°C)	23.8 (3.4)	24.0 (3.0)	23.8 (2.9)	23.8 (2.8)	23.9 (2.7)	23.9 (2.8)	23.9 (2.8)	23.7 (2.9)
Relative humidity (%)	27.9 (3.8)	27.2 (3.3)	27.4 (3.6)	27.3 (3.4)	27.1 (3.4)	27.3 (3.5)	27.0 (3.2)	27.1 (3.5)
ETS-$PM_{2.25}$[b] (µg/m^3)	0.7 (0.0)	5.1 (1.4)		34.0 (5.4)		115.5 (31.6)		430.7 (96.4)

The 1st, 2nd, 3rd, and 4th zero correspond to conditions without sETS exposure (compare to Figure 2).
[a]SDs in parentheses. [b]$PM_{2.25}$ measurements were performed on a separate occasion with the quartz crystal cascade impactor while the exposure chamber was vacant.

the highest concentrated sETS episode and its preceding zero condition (pairwise *t*-test, $p < 0.05$).

IAQ acceptability and ventilation requirements. Because detection of an sETS odor can be the key factor for indoor air quality acceptability, the question arises of how much fresh air is needed to dilute the sETS emissions of one cigarette to concentrations where no odor would be perceived. We divided sETS emissions per cigarette depicted in Table 2 by median odor threshold concentrations (Figure 3; while noses remained in the ducts). Thus, we obtained dilution volumes per sETS indicator. We then calculated the average dilution volumes based on volumes obtained from particle number, pPAH, and tVOC concentrations. To correct for coagulation and adsorption, we multiplied particle numbers by a factor of 5.7 (see "Cigarette Emission Experiment"). We assumed that the mass of the sETS emissions per cigarette is homogeneously distributed within a compartment and that no sinks are present. This produced an average fresh air volume of > 19,000 m^3 per cigarette in order to dilute to sETS concentrations where no odor would be perceived.

By the same method we observed eye and nasal irritations at dilution volumes corresponding to 3,000 m^3 per cigarette (lowest sETS concentration episode). At these sETS concentrations, 67% of the occupants judged the air unacceptable.

Breathing patterns and eye blink rates. Breathing pattern parameters (inhalation volume and inhalation flow rate) used as markers for olfactory or trigeminal activation (*34,35*) did not show any significant decrease during ETS exposure. There was a positive yet insignificant correlation between eye blink counts and log-transformed ETS particle concentrations.

Discussion

Cigarette Emission Experiment

Compared to other investigations, particle mass emissions observed in this study are about half as high as stated in the literature (*8,10*). This result is caused partly by the circumstance that our study measured not RSP (aerodynamic diameter of 3.5 µm) but $PM_{2.25}$. Furthermore, the cigarettes were not actively smoked but smoldered passively. The absence of exhaled mainstream smoke can reduce particulate matter of ETS by 15–43% (*36*). As for CO, concentrations are about 50% higher than reported by Martin and colleagues (*9*), whereas $tVOC_{FID}$ concentrations are approximately four times higher than reported by the same authors, possibly caused by the longer burning time of the cigarette that extinguished passively in our experiment. The greater relative contribution of tVOC measured in propane equivalents may result from organic compounds emitted from the smoldering filter material.

Olfactory Experiment

We hypothesize that the observed increase in sensitivity of the odor threshold while the subjects' noses remained in the olfactometer ducts compared to when the subjects' noses were placed into the ducts only upon presentation of the stimuli originates from an increase in mental concentration. Compared to an odor threshold variability of several orders of magnitude reported for some single chemicals (*37*), the variability of the observed sETS odor thresholds not exceeding a maximum value of 300 are low.

Odor thresholds of sETS obtained from the olfactory experiments showed that a median odor sensation was perceived at very low concentrations equivalent to an ETS-$PM_{2.25}$ concentration of approximately 0.6–1.4 µg/m^3. Because the olfactory stimuli were presented in ascending order, odor threshold values obtained in this experimental setting are considered to be the lowest attainable. The absolute values of these thresholds in terms of particle numbers, tVOC, and pPAH concentrations point out that, for field settings, an odor sensation would lie in the noise of the background concentrations. Typical long-term average concentrations reported in indoor settings where smoking takes place (*10,11*) are two orders of magnitude higher than concentrations at these threshold values. Compared to short-term concentrations, however, the determined odor threshold concentrations is up to three or more orders of magnitude lower than reported in field settings (*10,15,38*). The reason for the low threshold values found here is most likely the fact that our reference fresh air was cleared by an ultrafine particle filter and by an active carbon filter (see Figure 1).

Regarding the VOCs that can induce an odor sensation at concentrations near the determined odor threshold values, published odor thresholds for single chemicals suggest that not many compounds would be able to produce these thresholds (*39,42*). Among them, only pyridine could potentially create an odor sensation provided that minimum reported odor threshold values are taken as a criterion. This leads to the conclusion that other, perhaps unidentified compounds with an odor threshold in the nanogram or even picogram per cubic meter range could be responsible for the observed odor sensations. Furthermore, particles may be able to facilitate an odor sensation. Cain and colleagues (*8*) observed a slight decrease in odor intensity when ETS particles were electrostatically precipitated.

Full-Body Exposure Experiment

Environmental conditions. Based on the cigarette emission experiment, the highest episode concentration the subjects were exposed to is equivalent to one cigarette being smoked in a room about 100 m^3 in volume. Particle numbers concentrations averaged $1.7 \times 10^4/cm^3$; pPAH concentrations averaged 218 ng/m^3. Although these indicators are not typically assessed in ETS exposure studies, these values correspond to measurements obtained in field settings. A study performed by Morawska et al. (*39*) measured particle numbers of 5×10^4 at a rock concert. Junker et al. (*40*) reported pPAH concentrations of 336–990 ng/m^3in buildings for recreational activities. The lowest episode concentration is equivalent to one cigarette being smoked in a space of about 3,000 m^3, given a homogenous distribution of the emission. The average particle number and pPAH concentrations measured 468/cm^3 and 9.3 ng/m^3, respectively. As discussed above, the absence of exhaled mainstream ETS in this study underestimates the particulate exposure concentrations of the subjects compared to field settings (*36*). The gas-phase constituents of exhaled mainstream smoke, however, contributes only a small amount to ETS (*36*), so discrepancies in field settings are assumed to be small.

Table 4. Average perceived sensory responses of the sETS condition at a flow rate of 200 mL/min and the preceding zero condition.

Response	Symptom at zero air condition	Symptom at 200 mL/min
Temperature (–3, 3)	–0.56	–0.53
Relative humidity (–3, 3)	0.61	0.79
Odor strength (0, 6)	0.65	2.09#
Eye irritation (0, 6)	0.61	0.97*
Throat irritation (0, 6)	0.82	1.49**
Nasal irritation (0, 6)	0.55	0.94**
Arousal (0, 6)	0.41	1.79#
Annoyance (0, 6)	0.44	1.94#
Odor perception (–1, 1)	0.06	–0.22#
Air quality acceptability (–1, 1)	0.58	–0.03#
Percent acceptable	92	33#

The values in the parentheses correspond to the minimum and maximum values referred to on the vertical scale (Table 1). *$p < 0.05$, **$p < 0.01$, and #$p<0.001$, based on a pairwise *t*-test. Values of *p* for higher flow rates (not shown) are even lower.

Compared to field settings, the observed coagulation and particle removal processes overestimated the geometric mean diameters of the sETS aerosol. It has been reported that geometric mean diameters of ETS 10 min after having been generated by a human smoker increase 20–50% (*39*). In this study, the geometric mean diameter of the aerosol doubled, probably because the initial particle number concentration within the glove box is greater than would be measured in the field. Additionally, the interaction of small sETS particles with other surfaces would likely be larger than in a typical field setting.

Cain et al. (*8*) reported that the types of cigarettes generating sidestream smoke may create variations in the concentrations of ETS constituents. However, Nelson et al. (*43*) observed that ETS generated from a mix of the most widely used cigarette types is not significantly different from one country to another. The cigarette brands used in this study were chosen on the basis of sales statistics of the Swiss Community of the Cigarette Industry (*21*) and therefore represent ETS similar to that generated in other countries.

Sensory symptoms, startle reflex measurements, and eye blink rates. Because significant perceived sensory symptoms were observed at the lowest sETS exposure tested in this study, we conclude that thresholds of perceived sensory symptoms are even lower. Observed concentrations facilitating eye, nasal, and throat irritations correspond to an estimated ETS–$PM_{2.25}$ concentration of about 4.4 µg/m^3. This is equivalent to a dilution volume of about 3,000 m^3 per cigarette. Before this study, similar findings were reported at an ETS–RSP concentration of 58 µg/m^3 (*7*), although significant nasal irritations were not observed.

Only a few studies investigated the effect of odors on the startle reflex. Ehrlichman et al. (*44*) and Miltner et al. (*30*) investigated acoustic startle reflex modulation during short exposure to pleasant and unpleasant odors. Unpleasant odors enhanced startle amplitude, whereas pleasant odors had no effect. Later work (*45*) provided some evidence that a decreased startle reflex resulted from pleasant odors. These findings agree with the interpretation of Lang et al. (*29*) that the startle reflex amplitude is modulated by the emotional valence of the foreground stimulus. In contrast, we found a dose-dependent decrease in startle reflex amplitude with increasing concentrations of ETS. The differences between previous results and those of our study lie in the duration of the presented stimulus and in the analysis technique. Ehrlichman and Miltner presented the foreground odor stimulus for a very short period (one sniff) as Lang did with slides, rated high or low in valence. Startle amplitude was analyzed between the different trials only. We analyzed the difference in startle amplitude between, before, and during ETS stimulation, separately for each ETS concentration. Schicatano and Blumenthal (*33*) showed that distracting attention by attending to a visual search task reduced acoustic startle response amplitude. Therefore, we interpret our finding of a dose-dependent decrease of startle reflex amplitude as a directing of attention toward the increasing concentration of ETS.

Significant eye blink increases have been reported at concentrations > 1.3 ppm CO (*46*) and have been observed to increase in time (*5,7*). In this study, the concentration level as well as the duration of the episodes was not sufficient to create a significant increase in eye blink rates.

IAQ acceptability and ventilation requirements. Cain et al. (*8*) found that the degree of dissatisfaction evoked from ETS, strongly correlated to the perceived intensity of irritation or odor, depends on the channel (eye, nose, throat, odor) most severely affected. We found that the detection of the arousing sETS odor alone was sufficient to create dissatisfaction. However, dissatisfaction was not facilitated by the intensity of the perceived irritation, mainly because the sETS concentrations our subjects were

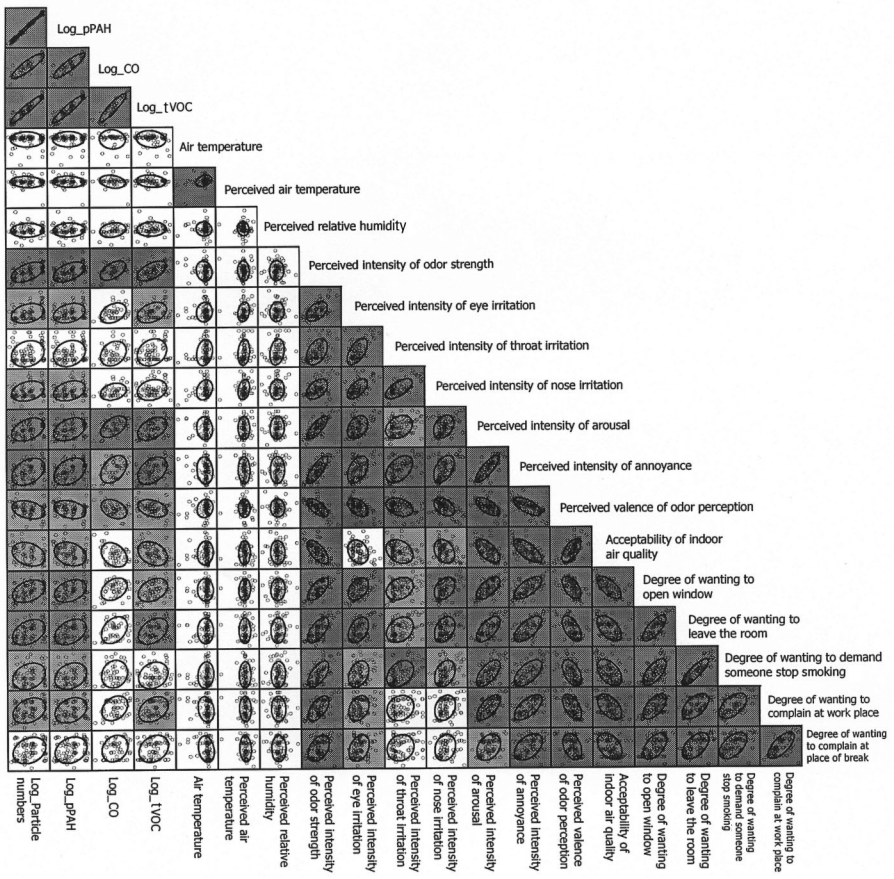

Figure 4. Scatter plots of background-corrected sensory responses (response at an ETS concentration episode minus response at the preceding zero concentration episode) and log-transformed ETS concentrations of 24 exposed subjects. The data depicted in the white boxes do not correlate significantly in a Pearson's linear regression model ($p > 0.01$). The data in the light gray boxes are highly significantly correlated to the linear trend ($p < 0.01$), and for the data in the dark gray boxes a very highly significant correlation exists ($p < 0.001$).

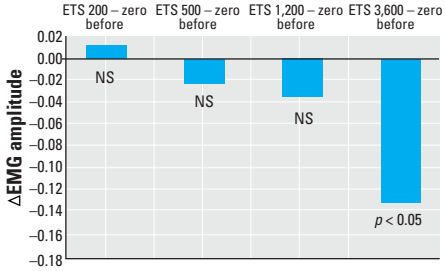

Figure 5. Differences between startle EMG amplitudes measured at an ETS condition (200 mL/min, 500 mL/min, 1,200 mL/min, and 3,600 mL/min) and the directly preceding zero-air condition for 22 subjects. The data of 22 of the 24 subjects were analyzed. Two data sets were rejected because they consisted of incomplete startle responses (this was possibly due to an inadequate placement of the electrodes onto the subject). The data have been normalized by EMG amplitudes measured during the first zero episode for each subject. NS, nonsignificant difference; significant difference determined by a pairwise *t*-test ($p < 0.05$).

exposed to were much lower than in the study of Cain and colleagues.

To create acceptable indoor air quality conditions, the sETS emissions of one cigarette would have to be diluted by an estimated fresh air volume of 19,000 m^3. This is at least two orders of magnitude higher than proposed by Cain et al. (*4*) for an estimated acceptability of 75–80% and an 80% acceptability by Walker and colleagues (*7*) in a full-body exposure study. These discrepancies are large. As stated above, the main reason is most likely the extremely clean reference air used in our study. Another factor may be that in our study a full-body exposure experiment was performed, whereas in the investigation of Cain et al. (*4*) subjects perceived the air at a sniffing station. Although these subjects did not smoke throughout the duration of the study, no information was given concerning their smoking status. Discrepancies with Walker et al.'s (*7*) study may emanate from the questions the subjects were asked about acceptability. Walker et al. employed a yes/no response to determine overall acceptance, whereas our study employed a voting scale ranging from clearly acceptable to just acceptable and from just unacceptable to clearly unacceptable. Studies by both Cain et al. (*4*) and Walker et al. (*7*) extrapolated the required fresh air volume (or the ETS concentrations) to where 80% of the subjects judged the quality of air to be acceptable. However, small changes in the slope of the log-scaled dose–response curves (ETS versus acceptability) will greatly influence the estimation of the of the 80% acceptability threshold. Obtained estimations must therefore be interpreted with great caution.

Controlled laboratory exposure studies conducted to date have not adequately considered low sETS concentrations that have adverse effects on perceived sensory symptoms. Furthermore, these studies have used ETS concentrations well above threshold concentrations of acceptable indoor air quality. To obtain realistic threshold concentrations for perceived sensory symptoms as well as acceptable indoor air quality, much lower exposure concentrations must be considered. In this study, we observed perceived sensory effects and a deterioration of indoor air quality at much lower sETS concentrations than previously reported. As Repace and Lowry (*48*) concluded, investigating cancer risk associated with ETS exposure, the degree to which ventilation rates would have to be increased to preserve indoor air quality in smoking areas would be impractical and economically unfeasible. We conclude that to protect nonsmokers effectively from adverse sensory symptoms and to provide acceptable indoor air quality, segregation of smoking and nonsmoking areas or smoking bans within public buildings should be enforced.

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