EYE IRRITATION FROM EXPOSURE TO PPB LEVELS OF LIMONENE OXIDATION PRODUCTS

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ABSTRACT
Terpene/ozone reaction products in realistic indoor concentrations may cause eye/airway irritation. This hypothesis has been tested by exposing the left eye in 8 male subjects single blind for 20 min to limonene oxidation products (LOPs) and the residual reactants, including clean air, in random order, while viewing an educational film. The reaction mixture of ozone and limonene was 10.5 min old and 65% completed with residual concentrations of 31 and 75 ppb, respectively. The subjects reported eye irritation and their blinking activities were recorded during exposure. The mean blink frequency (BF) increased 21-66% during exposure to LOPs relative to clean air (p<0.0001). The BF was unaffected by exposure to ozone, while a negligible reduction in BF was observed for limonene. Limonene and LOPs resulted in weak subjective eye irritation, but the perceptions differed. Only for LOPs, the increase of BF and irritation coincided. The findings substantiate the above hypothesis.

INDEX TERMS
Blink frequency, eye irritation, human exposure, limonene, ozone.

INTRODUCTION
Eye irritation (e.g., dry, tired or strained eyes) is often the most common symptom reported in the indoor environment. The symptom “dry eyes” and related ocular-surface diseases is one of the major eye complaints treated by eye physicians (Lemp, 1999). The cause(s) of eye irritation in the indoor environment is not known, although several suspected causal agents have been proposed. There are indications that gaseous pollution may be associated with objective changes of the eye tear film (TF). For example, in operating rooms compared with wards (e.g., Fenga et al., 2001). Outdoor air pollution, though particles cannot be excluded, has also been associated with eye complaints or defects of the TF (e.g., Smedbold et al., 2001), in particular reactive compounds like peroxy acetyl nitrates found in smog (Altshuller, 1978). One hypothesis is that eye (and airway) irritation should be caused by indoor air pollution (Norn, 1992). Recently, the focus has changed from chemically non-reactive VOCs to chemically reactive VOCs as a plausible chemical explanation for eye/airway irritation indoors, because strong eye/airway irritants are produced when they react with ozone (Wolkoff and Nielsen, 2001). To find further support for this hypothesis, exposure of the human eye has been examined by measuring the blink frequency (BF, blinks per min) as a function of realistic exposures to limonene oxidation products (LOPs), ozone, limonene, and clean air.

Eye physiology, background
The purpose of normal blinking is three-fold, to restore the TF, to defend the eye from environmental exposure, e.g., mechanically removing deposited particles and cellular debris, in addition to supply the ocular epithelium surface with vitamins and growth factors (Tsubota, 1998).

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Perceived eye irritation correlates with BF (Norn, 1983). An increase of BF has been found in human exposure studies with different VOCs, e.g., 1400 ppm butyl acetate resulted in a significant increase from 9 to 12 min⁻¹, though the tear film break-up time (BUT) was unchanged (Iregren et al., 1993). Exposure to a VOC mixture (12 – 24 mg/m³) resulted in a dose/time dependent increase in BF associated with eye irritation (Prah et al., 1993). The BF increased from about 21 to 35 min⁻¹ by 35 min of continuous formaldehyde exposure from 0.03 to 3.2 ppm, most clearly in the interval 1.2 to 3.2 ppm; similarly, eye irritation was reported to increase from 1.3 to just above 2 on a scale from 1 (not at all) to 4 (strong) (Weber-Tschopp et al., 1977). Unfortunately, these studies have been carried out at industrial levels rather than indoor levels where the effects cannot be predicted.

There also appears to be an inverse correlation between BF and BUT, because in persons with high BUT, the BF is low (e.g., Al-Abdulmunem, 2001; Prause and Norn, 1987). These findings support the hypothesis that BF is triggered by trigeminal stimulation. All in all, BF should be a good objective measure of eye irritation (cf., Walker et al., 2001), provided subjects are exposed under neutral conditions and unattended.

**METHOD**

**Human subjects and exposure of the eye**

Eight male subjects (seven Caucasians and one Afro-American) between 30 and 63 years (mean = 48) participated in the study. Inclusion criteria were; non-smoker, absence from any pathological eye history and otherwise healthy. One wearer of contact lenses was requested not to use them two weeks prior to exposure. The study was approved by the Local Research Ethics Committee, and informed consent for participation was obtained for all subjects.

The subjects were exposed in a well air-conditioned examination room, after a minimum of 30 minutes of staying indoors. Each exposure session was made up of an acclimation step with pure air to get used to the exposure conditions, see Table 1; initial baseline: 8 min of pure air (A); exposure: 20 min of either LOPs (BA), ozone (BB) or limonene (BC); final baseline: 8 min of pure air (C). The test program involved 3 exposure sessions for each subject, i.e. the impact of ozone, limonene and LOPs were single determinations carried out in random order. There was a minimum of 3 days between the individual exposure sessions.

**Table 1.** Test matrix for eye exposure.

<table>
<thead>
<tr>
<th>session</th>
<th>acclimatization</th>
<th>A (pure air)</th>
<th>BA (LOPs)</th>
<th>BB (ozone)</th>
<th>BC (limonene)</th>
<th>C (pure air)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 min</td>
<td>8 min</td>
<td>20 min</td>
<td>-</td>
<td>-</td>
<td>8 min</td>
</tr>
<tr>
<td>2</td>
<td>3 min</td>
<td>8 min</td>
<td>-</td>
<td>20 min</td>
<td>-</td>
<td>8 min</td>
</tr>
<tr>
<td>3</td>
<td>3 min</td>
<td>8 min</td>
<td>-</td>
<td>20 min</td>
<td>20 min</td>
<td>8 min</td>
</tr>
</tbody>
</table>

The subjects were exposed single blind in their non-dominant eye, only, by means of a glass eyepiece. It was designed to fit the facial curve, which ensured an effective air supply at a low air velocity. The unattended subjects watched an educational film with their dominant eye, only. However, shielding did not annoy the none-dominant eye. A VHS video camera recorded the subject’s blinking and comments simultaneously, in order to evaluate the BF later. Comments during the exposure were compared with those in the questionnaire.
The subjects filled out a questionnaire about eye conditions before, during and after exposure. They were asked if they felt eye irritation, its location and magnitude (none-weak-moderate-strong-unbearable) and the nature of the perception (e.g., itching, stinging, smarting).

**Chemical composition of the exposure agents**
Ozone was generated photochemically by irradiating oxygen (99.999%, Hydrogas, Norway) with a thermostated mercury lamp controlled by a high performance power supply (for details, see Wolkoff et al., 1999). Limonene was generated in an AID 350 (AID inc., Avondale, PA) gas generator by evaporation of limonene (>99.9 %, Fluka) into a filtered air stream. Ozone and limonene were mixed with humidified air and allowed to react in a wide bore Teflon flow tube (l: 4.7 m, φ: 2.2 cm). The LOPs were prepared from 200 ppb limonene and 130 ppb ozone. The consumption of ozone was 65%, excluding wall loss, after 10.5 min reaction time in the Teflon flow tube. The residual reactants in the exposure mixture are listed in Table 2. Turning off the ozone or limonene channel, allowed for the generation and dosage of one reactant alone. Pure humidified air was generated in a separate air supply from charcoal filtered air (medical grade). The exposure conditions are shown in Table 2.

<table>
<thead>
<tr>
<th>Table 2. Eye exposure conditions.</th>
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<tr>
<td>exposure room</td>
</tr>
<tr>
<td>RH (%)</td>
</tr>
<tr>
<td>T (°C)</td>
</tr>
<tr>
<td>air velocity (cm/s)</td>
</tr>
<tr>
<td>concentration (ppb)</td>
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</table>

1) Estimated values based on airflow and geometry.

A calibrated chemiluminescence monitor was used for the ozone measurements before and after exposure (model 265 A, API Inc., San Diego, CA). Limonene was sampled on Tenax TA and measured by Thermal Desorption - Gas Chromatographic - Flame Ionization Detection (for analytical parameters, see Wolkoff 1998).

**Determination of blink frequency**
The VHS recordings were critically viewed and each blink was stored along with the exposure time on a computer. A blink was defined as a downward movement of the upper eyelid discernible by the researcher, but twitches and partial blinks were excluded. The individual sequences were arranged in four-minute intervals from which BFs were calculated. The mean BF of sequence A and C constituted the baseline. The effects of LOPs, ozone and limonene relative to pure air were tested by a variance analysis (SAS ver. 8.1). The variation in BF increased with BF, and hence the data were not normal distributed. Taking the logarithm to BF transformed the data into a normal distribution.

**RESULTS**
Eye irritation reported in the questionnaires was generally none-weak or absent during sequences A and C (pure air), and the overall difference in BF differed by less than 14%.

<table>
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<th>Table 3. Variance analysis on the effects of LOPs, limonene and ozone on blink frequency.</th>
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<tr>
<td>LOPs</td>
</tr>
<tr>
<td>p</td>
</tr>
<tr>
<td>95% confidence interval of BF increase (%)</td>
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</table>

1) A positive number designates an increase in BF relative to that of pure air and vice versa.
Exposure to LOPs caused a significant (p< 0.0001) increase in BF of 21-66 % in contrast to the residual reactants (see Table 3). The increase was associated with weak-moderate irritation in the eye, the eyelid and/or the corner of the eye. Skin irritation around the eye was not reported. Irritation was perceived as stinging, warmth, itching, soreness or smarting sensation. One subject neither reported irritation nor showed an increase in BF. Exposure to ozone caused an insignificant increase in BF. Two subjects reported none-weak irritation, and one reported weak-moderate irritation. However, none of these statements were associated with an increase in BF.

**Figure 1.** Blink frequency-time curves for subject 1 (a-c) and subject 2 (d-f). Triangles are exposures to pure air and diamonds are LOPs (a, d), ozone (b, e) or limonene (c, f).

Exposure to limonene caused an insignificant reduction in BF. However, the trend (p<0.13) could be confirmed by more subjects. All but one subject reported none-moderate irritation from exposure to limonene, which was generally associated with a smarting sensation on the skin around the exposed eye. One subject reported weak soreness in the eye itself.

Examples of BF-time curves during the course of the sessions are shown in Figure 1. In Figure 1a, a short delay is followed by a gradually increase in BF from exposure to LOPs. The BF returns to the initial level of pure air. In Figure 1c, the BF increases steadily during the exposure. In Figure 1d, the BF increases by approximately 40% for 8 min, then decreases to below the baseline. After another 8 min, BF increases. Finally BF returns to the initial level of pure air. Figure 1f shows a typical decrease in BF from exposure to limonene.
DISCUSSION
The self-reported irritation from LOPs, ozone and limonene was weak-moderate, if any at all. In addition, none or only weak irritation persists in the final sequence with pure air (C). The mean difference in BF on baseline sequences A and C is less than 14%. Hence, the mean BF from sequences A and C is used as baseline to compare effects of LOPs, ozone and limonene.

The exposure to LOPs included residual limonene and ozone, see Table 2. However, skin irritation was not reported. The perception was isolated to the eye, the eyelids or the corner of eyes, and supported by a significant increase in BF of 21-66%. Ozone did not cause irritation or influenced the BF. Exposure to limonene caused weak skin irritation around the eye region, but not eye irritation, which is supported by the observation that the BF was unaffected or slightly lowered (p<0.13).

The increase in BF is biased by a natural variation in BF, which is probably more pronounced for people with low baseline BFs. Thinking, speaking and concentration are mental states, which are known to affect BF. The subjects watched an educational film during the exposure in order to maintain a neutral mental state and to avoid napping. People responded differently to a particular topic, but the topics were the same in each sequence in a particular session. Hence, the mental state should be alike in all sequences.

The subjects were exposed to only a small amount of LOPs, yet it introduced weak eye irritation. This classifies LOPs among potent eye irritants like peroxybenzoyl nitrate, which produces light eye irritation at 10 ppb (Heuss and Glasson, 1968). The increase in BF from exposure to LOPs is similar to that reported for formaldehyde in much higher concentrations (Weber-Tschopp et al., 1977). However, the exposure conditions were different. Trigeminal stimulation of the ocular surface is believed to be the cause of irritation in contrast to an immunological mechanism, since the observed BF response showed a fast reversibility, which also is the case in the corresponding bioassay with mice (Rohr et al., 2002). However, it cannot be excluded that destabilization of the precorneal tear film with resulting desiccation, ruptures, and dry spot formation, including direct exposure to nerve endings could be an alternative explanation (cf., Wolkoff et al., 2002).

The age of the mixture was 10.5 min, i.e. the LOPs formed are realistic in an indoor environment with air exchanges as high as 6 hr\(^{-1}\) for the chosen initial concentrations and loss terms. A value of 0.5 hr\(^{-1}\) is common in Scandinavia. Hence, lower concentrations can produce the same level of LOPs in real-life scenarios. Lowering the air exchange, however, influences the loss terms as well as the age of the mixture and hence the concentration of short-lived and stable LOPs. The exposure time of 20 min is ca. 1/24 of a typical working day. LOPs are therefore assumed to result in an impact at lower concentrations than given in this study. All subjects were exposed during the first half of the working day after no more than a few hours of office work. It is believed that exposure after a full working day or after completion tasks requiring intense visual attention or exposure to other risk factors, the biological effect would have been greater. It is anticipated the effect would be greater among women, possibly due to gender differences (Wolkoff et al., 2002).

CONCLUSION
The hypothesis that reactive indoor air chemistry is more likely to cause eye irritation than the chemically non-reactive VOCs has been substantiated in a human eye exposure study with 8 males. The significant 21-66% increase of the eye blink frequency by exposure to limonene
oxidation products is interpreted as a trigeminal stimulation and concurrent with perception of weak eye irritation. These effects are anticipated to be even greater if the subjects prior to exposure have had intense visual attention tasks, a full working day or the subjects were women.

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