The effect on human eye blink frequency of exposure to limonene oxidation products and methacrolein

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Abstract

Oxidation products of terpenes (e.g. limonene) contain unidentified irritants, which may be responsible for a fraction of the reported eye and airway complaints in indoor environments. Here we report exposure to parts per billion (ppb) levels of limonene oxidation products (LOPs) and the terpene oxidation product methacrolein using blink frequency (BF) as a measure of trigeminal stimulation of the human eye. Ten male subjects averaging 43 (standard deviation 10.5) years were exposed for 20 min to LOPs, methacrolein, and clean air, respectively. A baseline BF was measured prior to and following each exposure (8 min and 4 min, respectively). The subjects were exposed locally in the non-dominant eye and single blind at 20% relative humidity (RH), while viewing an educational film. Blinking was video recorded and evaluated for full sessions of 36 min. Mean BF increased significantly during exposure to LOPs and methacrolein compared to the baseline of clean air, and the findings coincided with weak eye irritation symptoms. Lowest observed effect levels were 286 ppb methacrolein and a 10 min-old LOPs mixture of initially 92 ppb limonene and 101 ppb ozone (O3), which increased the BF comparably by 18% (p = 0.001) and 17% (p = 0.003), respectively. The increase in BF was smaller, although not significantly different, during exposure to LOPs at 50% RH to 20% RH in mixtures prepared from ca. 350 ppb limonene and 300 ppb O3. LOPs may cause trigeminal stimulation and possibly eye irritation at O3 and limonene concentrations, which are close to high-end values measured in indoor settings. The effects may be exacerbated by low RH.

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Keywords: Blink frequency; Eye irritation; Methacrolein; Terpene oxidation products

1. Introduction

Dryness, lacrimation and strained, burning, gritty or itchy eyes are common symptoms of “eye irritation” in epidemiological studies of the indoor environment (Brightman and Moss, 2000). The causes are not well documented, although a number of suspected indoor risk factors have been explored (Wolkoff et al., 2003). The apparent difficulty of explaining eye irritation including airway irritation by measured indoor concentrations of volatile organic compounds (VOCs), aldehydes, and particles in offices, respec-

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tively (Wolkoff et al., 2003; Schneider et al., 2003) has prompted the hypothesis that hitherto not measured re-active chemical species could be partly responsible for the reported irritation (Wolkoff and Nielsen, 2001). At present, it appears that products formed in the oxidation of certain biogenic VOCs, terpenes, contribute to eye and airway irritation as supported by mouse biosay and human exposure studies (Wolkoff and Nielsen, 2001). In addition, the effect of these terpene oxidation products appears to decrease with increasing relative humidity (RH) (Wilkins et al., 2003).

Terpene oxidation products are formed in the reaction of oxidants (e.g. ozone (O₃), the hydroxyl radical (OH), and the nitrate radical (NO₃)) with terpenes in the presence of nitrogen oxides. Terpenes are emitted from vegetation and certain wood-based building materials in new housing (Hodgson et al., 2000), and added to household products (e.g. the use of pine and citrus oils in cleaning agents). Limonene, α-pinene and isoprene are examples of often measured terpenes indoors (Wolkoff et al., 2000). High concentrations of limonene can be obtained at low air exchange rates in the absence of O₃. Typical mean indoor limonene concentrations are less than 10 parts per billion (ppb), but values as high as 70 ppb have been reported (Wolkoff et al., 2000; Sexton et al., 2004). The human sensory irritation threshold for R-(+)-limonene has been estimated to be more than 80 parts per million (ppm) based on 2 h chamber experiments (Falk-Filipsson et al., 1993). However, using the method in the present study, no adverse eye-irritation or trigeminal response was observed during 20 min exposure to 116 ppb limonene at 20% RH (Klenø and Wolkoff, 2004). Indoor O₃ concentrations are typically 20–70% of outdoor levels (Weschler, 2000) varying from tenths to hundreds of ppb in polluted cities especially during summer. While the outdoor source of O₃ is the predominant one, electrostatic equipment, like photocopiers, contributes to the overall indoor O₃ concentration (Brown, 1999; Leovic et al., 1996). No adverse eye-irritation was reported during 20 min human exposure to 40 ppb O₃ at 20% RH (Klenø and Wolkoff, 2004). The identity of the terpene oxidation products depends on the structure of the reacting terpene and the oxidant. In O₃ oxidation (ozonolysis) of terpenes, abundant products are formaldehyde, methacrolein, OH and methyl vinyl ketone for isoprene (a hemiterpene), and OH and formaldehyde for limonene (Atkinson and Arey, 2003). In addition to the stable irritating oxidation products such as formaldehyde and methacrolein, terpene ozonolysis is a major source of indoor radicals, which are suspected to cause eye- and airway irritation (Klenø and Wolkoff, 2004; Wolkoff and Nielsen, 2001). The identity of the radicals, which are formed in terpene oxidation includes OH, Criegee Intermediates, and other organic radicals (Atkinson and Arey, 2003).

We have recently applied increases of the human eye blink frequency (BF) as a measure of trigeminal stimulation of the eye (Klenø and Wolkoff, 2004). We observed that the BF increased significantly during 20 min exposure to limonene oxidation products (LOPs) prepared from ca. 200 ppb limonene and 130 ppb O₃, which had reacted for 10 min at 20% RH. These findings supported the hypothesis that terpene oxidation products can partly explain the prevalence of reported eye complaints in the indoor environment, although the applied reactant concentrations were atypically high. Two major questions arise from this study, namely (1) whether LOPs prepared from lower reactant concentrations can produce eye irritation in human subjects, and (2) how does stable terpene oxidation products (e.g. methacrolein) affect changes of the BF.

LOPs are known to cause eye and airway irritation by trigeminal stimulation in humans and mice, respectively (Wolkoff et al., 2000; Klenø and Wolkoff, 2004). The causative irritating products have yet not been identified, which implies that a biological response cannot be related to the concentration of a single species in the reaction mixture. Trigeminal stimulation in terms of airway irritation and thereby eye irritation has been related to the amount of chemically reacted O₃, that is the reaction extent (Wilkins et al., 2003). LOPs can be generated by mixing O₃ and limonene in a reaction flow tube, which imposes a specific reaction time to the exposure mixture. As the reaction proceeds, the decay of reactants can be estimated by the Eqs. (1) and (2):

\[ \frac{d(\text{limonene})}{dt} = k_2[\text{limonene}][\text{O}_3] + k_3[\text{limonene}][\text{OH}] \tag{1} \]

and

\[ \frac{d(\text{O}_3)}{dt} = k_1[\text{limonene}][\text{O}_3] + k_4[\text{O}_3] \tag{2} \]
where the residual reactant concentrations are shown in brackets, and \( k_1 \) and \( k_2 \) are second order rate constants for the reaction of limonene with \( O_3 \) and OH, respectively. \( k_3 \) is the first order rate constant for \( O_3 \) deposition onto the flow tube surface. A limonene oxidation mixture absent from direct sunlight (i.e. photochemistry is negligible) can be described by RH, reaction time, the reaction extent (the fraction of chemically converted reactants) and the initial reactant concentrations. RH affects the oxidation product distribution, e.g. the formation of hydroperoxides is favored by higher water content (Sauer et al., 1999). Some oxidation products build up in time as the reaction proceeds and the reactants are consumed. For example, the molar yield of formaldehyde is 19% of the reacted limonene concentration (Ruppert et al., 1999). Other products like the OH radical depends on the residual \( O_3 \) concentration (Weschler and Shields, 1996), since they react almost instantaneously and the driving force for OH production is ozonolysis of terpenes. Other terpene oxidation products show an even more complicated dependency of time, RH and concentration of reactants and products. In this work, the progress of the reaction will be expressed in terms of chemically reacted \( O_3 \) (as opposed to physically consumed \( O_3 \), i.e. deposition inside the reaction tubes as wall loss). The more chemically reacted \( O_3 \) for a particular LOPs mixture, the more limonene consumption and higher yield of stable irritants like formaldehyde. Since the concentration of the OH radical depends on the \( O_3 \) concentration, its concentration is similar in mixtures with the same residual \( O_3 \) concentration. It is therefore possible to prepare LOPs mixtures with different concentrations of stable irritants, but similar concentration of the OH radical. Dose-response information from human exposure to LOPs can thus provide information on the irritating product species: whether they build up with the reaction extent or depend on the concentration of \( O_3 \).

Methacrolein was chosen as a model compound of a (fairly) stable terpene oxidation product and a strong airway irritant (Larsen and Nielsen, 2000). Methacrolein was chosen as a model compound of a (fairly) stable terpene oxidation product and a strong airway irritant (Larsen and Nielsen, 2000). Oxygen (99.999% pure) was from Hydrogas, Norway. \( O_3 \) was generated photochemically by irradiating molecular oxygen with a thermostated mercury lamp controlled by a high performance power supply (Weikoff et al., 2000). Limonene (>99.9% pure) was from Fluka and methacrolein (95% pure) was from Aldrich. A gas generator (Model 491M, KIN-TEK, TX) generated limonene and methacrolein vapors by evaporation of the liquids into a filtered air stream of medical grade. Clean humidified air was generated in a separate air supply from charcoal filtered air of medical grade. In the supply unit, the air stream was split in a dry channel and one passing through a sparger with clean deionized water. The RH was adjusted by combining the two channel flows.

2. Experimental

2.1. Chemicals

Oxygen (99.999% pure) was from Hydrogas, Norway. \( O_3 \) was generated photochemically by irradiating molecular oxygen with a thermostated mercury lamp controlled by a high performance power supply (Weikoff et al., 2000). Limonene (>99.9% pure) was from Fluka and methacrolein (95% pure) was from Aldrich. A gas generator (Model 491M, KIN-TEK, TX) generated limonene and methacrolein vapors by evaporation of the liquids into a filtered air stream of medical grade. Clean humidified air was generated in a separate air supply from charcoal filtered air of medical grade. In the supply unit, the air stream was split in a dry channel and one passing through a sparger with clean deionized water. The RH was adjusted by combining the two channel flows.

2.2. Measurements

All gas flows of oxygen, vapors and air were adjusted daily by flow controllers and measured before and after sampling with a traceable electronic bubble flow meter (Gillian Instrument Corporation, NJ). Temperature and RH were measured by a calibrated hygrometer (Model Testo 601, Testoterm GmbH & Co., Germany). Initial concentrations of the reactants were measured at sample point I in Fig. 1. Residual concentrations of the reactants were measured at the end of the Teflon tube at sample point II, which connected the polyethylene (PE) flow tube with the eyepiece. Limonene and methacrolein by multiplying with 0.03 (Alarie, 1973; Schaper, 1993). Furthermore, since eye- and airway irritation are both mediated through the trigeminal nerve, eye irritation in humans can be estimated and compared to the findings in the present study. The estimated TLV which should not alter the human BF is 10,400 ppb × 0.03 ~ 300 ppb.

The purpose of this work was to evaluate BF changes and reported irritation when exposed to increasing concentrations of: (1) methacrolein, (2) LOPs from three mixtures prepared from increasing reactant concentrations, and finally (3) two LOPs mixtures with high versus low RH.
were measured daily by sampling on Tenax TA adsorbent tubes followed by thermal desorption-gas chromatographic-flame ionization detection. The sampling rate was 200 ± 3 ml min⁻¹ using pump II (Ametek, PA). Analytical details are described elsewhere (Wolkoff, 1998). 

O₃ was monitored continuously in the reaction mixture using a newly calibrated chemiluminescence monitor (Model 265 A, API Inc., San Diego, CA). The limit of detection was 2 ppb. O₃ was partly consumed in surface reactions on the PE reaction flow tube (wall loss). A first order rate constant was measured in order to address the wall loss of O₃. For this particular reason, the chemical loss of O₃ was modeled in the four LOPs mixtures. See Section '2.6 Modeling.
of the chemical reaction’ for details. The loss of O₃ to the walls of the PE reaction flow tube was measured at the inlet (sampling point I) and outlet (sampling point II) for the applied flow and O₃ concentration.

The total number of ultrafine particles was measured at sampling point II by a condensation particle counter in the size range 7–1000 nm and a detection limit of 2 particles cm⁻³ (Model 3022A, TSI Inc., MN). The data were averaged over 10 min.

2.3. Generation of LOPs and methacrolein

LOPs were generated by mixing flows of limonene vapor (600 ml min⁻¹), O₃ (50 ml min⁻¹), and a variable flow of air in a Teflon tube (l: 1.3 m, i.d.: 2 mm) connected to a wide bore PE reaction flow tube (l: 4.7 m, i.d.: 2.2 cm). The flow through the PE reaction tube (180 ml min⁻¹) was adjusted by pump I (Model s2500, Dupont, DE) connected by a T-union to the Teflon tube upstream the PE reaction flow tube. The flow tube was cleaned prior to each session with 500 ppb O₃ (4 l min⁻¹) for 120 min in order to remove adsorbed organics. Initial reactant concentrations were measured prior to reaction (Table 1). Following 10 min of reaction (i.e. the time it takes for the mixture to exit the PE reaction flow tube) the residual reactant concentrations were measured (Table 1). The reacted amount of O₃ was calculated to be 32–79% from measurements of the residual O₃ concentrations and modeling of the O₃ wall loss (Section 2.6).

Methacrolein was generated in the same system by replacing limonene with methacrolein in the gas generator, and O₃ turned off. All exposure concentrations were adjusted to 20±3% RH and temperature 21±2 °C, except for the LOPs IV mixture which was 50±3% RH. The mixing ratio of oxygen was 0.23 in the LOPs experiments and otherwise 0.21.

2.4. Human exposure

Ten healthy male subjects averaging 43 years (standard deviation 10.5 years) participated in the study. They were non-smokers, absent from any pathological eye history and used no systemic medication likely to promote dry eyes (Craig, 2002; Doughty et al., 1997). Women were excluded from the study, since they are known to be more sensitive than men with respect to reporting irritation symptoms (Wolkoff et al., 2003), which implies that the inclusion of both genders would require more subjects. The result of this study is therefore not representative of typical office workers, but merely serve to compare effects of methacrolein, LOPs and RH on changes of BF. Other exclusion criteria were deviations from a healthy general condition such as a common cold or fatigue. All subjects carried out office work in the same building. In the preceding 2 weeks...

<table>
<thead>
<tr>
<th>Reactant concentrations¹</th>
<th>Clean air</th>
<th>MET I</th>
<th>MET II</th>
<th>MET III</th>
<th>LOPs I</th>
<th>LOPs II</th>
<th>LOPs III</th>
<th>LOPs IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limonene (ppb)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>92 (1)</td>
<td>204 (2)</td>
<td>343 (11)</td>
<td>350 (1)</td>
</tr>
<tr>
<td>O₃ (ppb)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>101 (1)</td>
<td>180 (1)</td>
<td>304</td>
<td>301</td>
</tr>
<tr>
<td>Residual reactant and product concentrations²</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>41 (1)</td>
<td>74 (15)</td>
<td>111 (12)</td>
<td>110 (9)</td>
</tr>
<tr>
<td>Limonene (ppb)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>39 (1)</td>
<td>38 (2)</td>
<td>40 (2)</td>
<td>40 (5)</td>
</tr>
<tr>
<td>Methacrolein (ppb)</td>
<td>–</td>
<td>89 (1)</td>
<td>189 (3)</td>
<td>286 (2)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Particles (cm⁻³)</td>
<td>–</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Other parameters³</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>18/32</td>
<td>71/65</td>
<td>155/79</td>
<td>154/79</td>
</tr>
<tr>
<td>O₃ reacted (ppb%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>21±2</td>
<td>20±3</td>
<td>50±3</td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Relative humidity (%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

¹ LOPs are formed by mixing limonene and ozone in the specified concentrations (parts per billion (ppb)) following 10 min reaction in a PE flow tube. Mean concentrations are listed with one standard deviation in brackets.

² The mixtures are not fully reacted and contain reactants of the stated residual concentrations.

³ The term “O₃ reacted” refers to the amount of ozone consumed in chemical reactions, excluding deposition onto the tubing surface (wall loss) according to Eq. (4).
months before the experiments they were not exposed to methacrolein, O₃ or limonene, other than to the ubiquitous background concentrations of these compounds, and other species encountered in typical offices. Prior written consent was obtained from the subjects, and the study was approved by the local Danish Research Ethics Committee (01-009/03). The subjects were locally exposed single blind in their non-dominant eye using a specially designed transparent glass eyepiece, which was designed to fit the facial curve ensuring an effective air supply at atmospheric pressure. The linear velocity was calculated to be less than 4 cm s⁻¹ at a flow of 180 ml min⁻¹, and the subjects did not report any discomfort with respect to the air flow. The air flow itself may still produce irritation causing an increase of BF in time, irrespective of the exposure. Such an unspecific BF increase with time was observed for clean air exposure sessions equaling 1.1% min⁻¹ on average (Table 2). A time effect was therefore incorporated in the model (see Section 2.5). The gaze direction was slightly upward. The presence of the eyepiece may have caused some minor visual disturbances, although the dominant eye was unaffected, but the subjects did not pay attention to the eyepiece after a few minutes. The subjects were neither informed about the nature of stimuli nor could they smell it, because the low air flow exited the eye-piece above the ear following dilution into ambient air. Blinking and comments on perceived eye irritation (intensity, location, and description) were recorded using a digital video camera (Sony DCR-PC110E PAL; 25 frames per second). The subjects reported the intensity of the perceived irritation prior to the exposure on a linear scale equally divided into none, weak, moderate and strong irritation. It was stated whether irritation was located in the eye (left/right), on the skin, or at the eyelids. The subjects rated the intensity by marking a scale, and they were allowed to state intermediate intensities, e.g. “between weak and moderate”. Perceived irritation during exposures was verbally assigned to this scale in short comments, e.g. “weak irritation, left eye ball”. An educational video film was shown to avoid drowsiness during the entire session of 36 min.

Each of the eight sessions was made up of four successive stages: an acclimatization stage, which included 3 min of clean air; an initial baseline recording with 8 min of clean air (stage A); 20 min of either methacrolein, LOPs or clean air (stage B); and finally 8 min of clean air (stage C). The latter stage was divided into 4 min of recovery and 4 min of a final baseline recording. The two exposure shifts in stages B and C were carried out by turning a tap on an exposure controller. When clean air was on, the LOPs mixture or methacrolein exited a vent and vice versa. A partition

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Blink frequency change during exposure to methacrolein (MET) and limonene oxidation products (LOPs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test results/compounds</td>
<td>Clean air</td>
</tr>
<tr>
<td>Subjects whom perceived eye irritationᵃ</td>
<td>3/10</td>
</tr>
<tr>
<td>Relative change in blink</td>
<td>-9</td>
</tr>
<tr>
<td>Perceived intensityᵇ</td>
<td>&lt;weak</td>
</tr>
<tr>
<td>Relative change in blink</td>
<td>-9</td>
</tr>
<tr>
<td>Confidence interval (%)</td>
<td>-19; 3</td>
</tr>
<tr>
<td>p-value</td>
<td>0.14</td>
</tr>
<tr>
<td>Base line trend (% min⁻¹)ᵈ</td>
<td>1.1</td>
</tr>
<tr>
<td>LOEL (ppb)ᵉ</td>
<td>266</td>
</tr>
<tr>
<td>TLVᶠ</td>
<td>300</td>
</tr>
</tbody>
</table>

ᵃ The number of subject, who perceived eye irritation in the particular session.
ᵇ The average reported intensity of perceived irritation in the particular session. The intensity scale is linear and ranges from “none”; “<weak”; “weak”; “<moderate”; “moderate”; “<strong” and “strong” irritation.
ᶜ The results are stated as the blink frequency change relative to baseline (see Section 2.5 for details), the 95% confidence interval and the p-value, which is the probability of the blink frequency being equal to baseline.
ᵈ The modeled increase in BF from the initial (stage A) to the final baseline (stage C) in the particular session.
ᵉ The Lowest Observed Effect Level (LOEL) showing an increase in BF statistically different from that of clean air.
ᶠ The proposed occupational Threshold Limit Value (TLV) (Larsen and Nielsen, 2000).
A previously developed set-up for exposure of the human eye to irritants is used in this study (Klenø and Wolkoff, 2004). However, the original procedure was slightly modified. First, the subjects were unaware about the exposure shifts in stages B and C. It was recognized, that a visual shift between exposures could influence the subjects, although unaware of the type of exposure. Second, the present study involved exposure to higher concentrations of irritants in stage B, and a possible carry-over effect from stage B to the final baseline recording of session C could increase the BF, and thereby underestimate the effect of the irritants. The BF changes were therefore modeled in two different runs: one with a 4-min-recovery period imposed between stage B and C, and one without. When the recovery period was included, the BF changes were systematically higher in every methacrolein and LOPs exposures (the clean air session was unaffected), which indicated a carry-over effect and justified inclusion of the recovery period. Time trends in BF from stage A to C were modeled for the clean air session, and the methacrolein and LOPs sessions (Table 2). The trend in the clean air session was not significantly smaller than in the other sessions, and 4-min recovery was assumed to be sufficient.

The same researcher critically viewed all video recordings, and each blink (averaging ca. 500 blinks/session) excluding those in the acclimatization stage, was stored along with the time on a computer. Only blinks with a downward movement of the upper eyelid covering more than 50% of the cornea were counted. Twitches were ignored. A few incidents of accidentally rubbing one’s eyes and forced extended closure were counted as one blink observation. Each session was counted only once, since pilot studies showed that repetitive counting differed by less than 2%. The BF was calculated in sequences of non-overlapping 1 min averages. Blinking during exposure to clean air for 36 min exhibited an alternating pattern (Fig. 2). Due to the alternating BF with time, blink measurements shorter than ca. 4–5 min are considered unreliable in accordance with Doughty, (2001). The alternating pattern was conserved during exposure to methacrolein and LOPs. Blinking data was transformed by taking the logarithm of the BF, since the variation increased with the BF. A linear mixed model could then be used to model the BF changes. The eight exposure sessions were modeled separately. For each of the eight exposure sessions, data consisted of measurements at 32 time points (time $t = 4, 5, \ldots, 31$ min and $t = 36, 37, 38, 39$ min) on ten subjects. The logarithm of the BF at time $t$, for person $p$ was modeled using a linear mixed model:

$$
\log(\text{BF}_p) = \beta + \mu_p + t\gamma + I(t)\delta_t + \epsilon_{tp}
$$

where $\beta$ is a fixed session level, $\mu_p$ is a random person level, $t\gamma$ is a linear effect of time, and independent normally distributed error terms $\epsilon_{tp}$. The variation within persons, $\sigma^2_w$, and the variation between persons, $\sigma^2_B$, was modeled. The effect $\delta_t$ was included only at the time points where the exposure was present ($I(t) = 1$ for $t = 12–31$ min and zero otherwise), estimated values

![Exposure to a full session of clean air](image-url)

Fig. 2. Five examples of alternating eye blink frequency curves during exposure to clean air.
represented changes in the mean value of the log transformed BF and were transformed to relative changes. This linear mixed model is comparable to a repeated measurements ANOVA analysis, but more general, because it includes the time trend $\gamma$. SAS version 8.02 was used to model the BF.

### 2.6. Modeling of the chemical reaction

A simple one-compartment model was used to estimate the extent of the gas-phase oxidation reactions in terms of chemically reacted O$_3$. The involved reactions and rate constants are shown elsewhere (Klenø and Wolkoff, 2004). VISSIM version 3.0 (Visual Solutions, Incorporated, MA) was applied for this purpose using a Backward Euler (stiff) integration method with a 0.001 minimum step size. The O$_3$ concentrations in the reaction flow tube were measured at sample points I and II in Fig. 1 in the absence of other compounds, and used to calculate the first order rate constant for the wall loss according to Eq. (3):

$$k_3 = -\frac{\ln (O_3, \text{sample point II}) - 1}{t}$$

The wall loss was modeled for LOPs by implementation of the 1st order rate expression using the rate constant above. The chemically reacted O$_3$ (excluding wall loss) in percent was calculated according to Eq. (4):

$$O_3, \text{reacted} = \frac{(O_3, \text{sample point I} - O_3, \text{sample point II} - O_3, \text{wall loss})}{O_3, \text{sample point I} - O_3, \text{wall loss}} \times 100\%$$

### 3. Results

The changes in BF from exposure to clean air, methacrolein and LOPs are shown in Table 2 and Fig. 3. Clean air decreased the BF by 9% on average, while 36% of the subjects rated irritation as “less than weak”, however typically transient within the session. While a perception generally involved an increase of BF, the reverse situation was not always true, and 40% of the subjects did not report irritation in any session.

Forty to fifty percent of the subjects experienced itching, stinging or gritty eyes rated as “less than weak” to “weak” during exposure to methacrolein (Table 2). The methacrolein exposure concentrations including a regression line are shown in Fig. 3. No dose-response relationship is apparent at concentrations below the proposed TLV. The lowest observed effect level (LOEL) was 286 ppb methacrolein, which increased the BF by 18% ($p=0.001$).

Forty to fifty percent of the subjects reported eye irritation rated as “weak” to “less than moderate” during exposure to LOPs I-III, and between “less than weak” to “weak” during exposure to LOPs IV. Eye irritation was experienced as a feeling of gravel in the eyes or a stinging sensation. All LOPs exposures increased the BF significantly more than clean air (Table 2). No dose-response relationship was apparent for LOPs as shown in Fig. 3. LOPs III, which was prepared from the highest reactant concentrations and had reacted the most, increased the BF significantly more than LOPs I and II (34% versus 17% and 12%). It is of note, that the mean BF increases of LOPs I and II did not overlap the confidence interval of LOPs III. This shows that the effects were separated by more than two standard deviations. Similarly, an increase of the RH from 20% to 50% showed a reduction of BF (34% versus 22%).
but not significantly. The LOEL of LOPs was 18 ppb of chemically reacted O$_3$ in a mixture of initially 92 ppb limonene and 101 ppb O$_3$.

4. Discussion and conclusion

The purpose of this study was to measure weak eye irritation, during exposure to oxidation products. An exposure set-up developed for eye irritation assessments using increases of BF as a measure of trigeminal stimulation was applied. Eye irritation effects have previously been measured using this set-up for LOPs, isoprene oxidation products, and nitrate radicals (Klønø and Wolkoff, 2004). We now report changes in BF from exposure to low concentrations of methacrolein at 20% RH corresponding to ca. 1/3, 2/3 and 3/3 of a recently proposed TLV of 300 ppb, which had been estimated from a mouse bioassay (Larsen and Nielsen, 2000). Changes in BF by low concentrations of methacrolein were subject to large variation, and a dose-response relationship was not apparent at concentrations below the proposed TLV (Table 2 and Fig. 3). Concentrations above TLV are irrelevant for indoor environments and therefore not examined. Forty to fifty percent of the subjects perceived irritation during the methacrolein exposures, and the subjective ratings were comparable in all three exposure settings (Table 2). LOEL was 286 ppb methacrolein, which increased the BF by 18% equal to an average increase from 14 to 16.5 min$^{-1}$. Indeed, the LOEL was expected to be somewhat higher than the proposed TLV, which should cause no eye irritation. However, the proposed TLV is subject to uncertainty, since it is estimated from an empirical relationship between RD$_{50}$ and TLV values (Alarie, 1973; Schaper, 1993).

LOPs were prepared in three concentrations at 20% RH, and the highest concentration was prepared at 50% RH as well. Exposure to LOPs I and II increased the BF by 17% and 12%, which was significantly more than the clean air exposure (Table 2). LOEL was 286 ppb methacrolein, which increased the BF by 18% equal to an average increase from 14 to 16.5 min$^{-1}$. Indeed, the LOEL was expected to be somewhat higher than the proposed TLV, which should cause no eye irritation. However, the proposed TLV is subject to uncertainty, since it is estimated from an empirical relationship between RD$_{50}$ and TLV values (Alarie, 1973; Schaper, 1993).

LOPs were prepared in three concentrations at 20% RH, and the highest concentration was prepared at 50% RH as well. Exposure to LOPs I and II increased the BF by 17% and 12%, which was significantly more than the clean air exposure (Table 2 and Fig. 3). Doubling the reaction extent from 71 ppb (LOPs II) to 155 ppb reacted O$_3$ (LOPs III) increased the BF by 34%. A dose-response relationship was not apparent (Fig. 3), and the regression analysis was poorly correlated ($R^2 = 0.7$). LOPs II did not increase the BF significantly more than LOPs I and II, the subjective ratings failed to show such a relationship. Fifty percent of the subjects reported irritation from LOPs III versus 40% in the other LOPs sessions, but the rating was somewhat lower (Table 2).

The subjective irritation ratings of LOPs I and II were higher than all methacrolein exposures even though the BF increases were of the same order. It is noteworthy, that the BF increased during exposure to both LOPs and methacrolein as the concentration/reaction extent increased, however not in a linear manner. An increase of the reaction extent neither influenced the subjective ratings of irritation, nor the number of subjects whom perceived irritation. An explanation could be that blinking increases prior to perceived irritation. In LOPs I, II, III and IV 32%, 65%, 79% and 79% of the initial O$_3$ was consumed in chemical reactions, respectively. The highest concentrations of stable irritants could thus be found in LOPs III and IV, and vice versa. The amount of reacted O$_3$ is a measure of the reaction extent and thereby the concentration of stable products, which build up during the reaction. The OH radical behaves differently: it is too reactive to build up in the reaction mixture and depends on the concentration of residual O$_3$ (Weschler and Shields, 1996). The reactant concentrations were carefully chosen in order to maintain similar OH concentrations in LOPs mixtures I–IV. Similar residual O$_3$ concentrations ensured OH concentrations of $1 \times 10^6$ molecules cm$^{-3}$ estimated by the model. For this reason OH is not considered responsible for the observed effect, because the significantly greater trigeminal response of LOPs III relative to LOPs I and II suggest that irritants in the mixture build up with the reaction extent.

LOPs III differed from LOPs I and II by the presence of particles, i.e. secondary organic aerosols (Table 1). Particle concentrations were expected to be formed in all LOPs mixtures, but the PE reaction flow tube apparently worked as a reverse denuder for low particle counts (Table 1). The higher BF increase of LOPs III can presumably be ascribed to gas-phase products, which are known eye irritants (Klønø and Wolkoff, 2004), but an additional effect of particles cannot be excluded. Secondary organic aerosols from ozonolysis of limonene contain several higher molecular weight oxygenated products (Glasius et al., 2000), which may exert a trigeminal response. A higher RH (LOPs IV) resulted in lower
rating of subjective irritation and a smaller trigeminal response. This was anticipated, because a low RH increases water evaporation from the precorneal tear film (PTF), which may exacerbate the susceptibility towards irritants (Wolkoff et al., 2005). Although the trigeminal responses at 20% and 50% RH were not significantly different, a similar negative biological effect of low RH on irritation from LOPs has been observed in a mouse bioassay (Wolkoff et al., 2003).

Subjects were exposed to a LOPs mixture in a previous study, which was comparable to LOPs II (Klenø and Wolkoff, 2004). The observed BF increase was higher (42% versus 12%) than in this study, and the difference is likely to be explained by the modification of the set-up, the inclusion of other human subjects, and day-to-day variation in the subjects.

The LOEL for LOPs corresponded to 18 ppb consumed O₃ in a mixture prepared from ca. 92 ppb limonene and 101 ppb O₃. This increased the BF by 17% during 20 min exposure comparable to the trigeminal effect of 286 ppb methacrolein. The applied limonene- and O₃ concentrations in LOPs I were higher than typical indoor values, but close to high-end values measured in various indoor settings (Wolkoff et al., 2000). Since the reaction time in the LOPs mixture was 10 min, the products can be formed at air exchange rates as high as 6 h⁻¹. The result indicates that incidents of high air exchange rate and high limonene- and O₃ concentrations may cause trigeminal stimulation and possibly eye irritation, indoors under atypical conditions. Average limonene concentrations in typical indoor environments are 10-20 times lower than in LOPs I (Wolkoff et al., 2000), this, however, may be counteracted by longer exposure time during a working day (typically 8 h versus 0.33 h in this study). The human eye may become further susceptible to airborne irritants under typical working conditions, which may alter the PTF (Wolkoff et al., 2003, 2005). Whether lower limonene concentrations lead to eye irritation in occupational environments should be pursued in future studies of prolonged exposure under simulated working conditions, including both genders.

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References


