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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 (O_3), 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 O_3. LOPs may cause trigeminal stimulation and possibly eye irritation at O_3 and limonene concentrations, which are close to high-end values measured in indoor settings. The effects may be exacerbated by low RH.

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 reactive 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 bioassay 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 (O_3), the hydroxyl radical (OH), and the nitrate radical (NO_3)) 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 O_3. 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 O_3 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 O_3 is the predominant one, electrostatic equipment, like photocopiers, contributes to the overall indoor O_3 concentration (Brown, 1999; Leovic et al., 1996). No adverse eye-irritation was reported during 20 min human exposure to 40 ppb O_3 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 O_3 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 O_3, 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 arose from that 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 O_3, that is the reaction extent (Wilkins et al., 2003). LOPs can be generated by mixing O_3 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):

$$-\frac{\mathrm{d(limonene)}}{\mathrm{d}t} = k_1[\mathrm{limonene}][\mathrm{O_3}] + k_2[\mathrm{limonene}][\mathrm{OH}] \quad (1)$$

and

$$-\frac{\mathrm{d(O_3)}}{\mathrm{d}t} = k_1[\mathrm{limonene}][\mathrm{O_3}] + k_3[\mathrm{O_3}], \quad (2)$$