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Human reference values for acute airway effects of five common ozone-initiated terpene reaction products in indoor air

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HIGHLIGHTS

- Airway effects of ozone-initiated terpene reaction products were assessed in mice.
- Sensory irritation, airway limitation, and pulmonary effects were observed.
- 3-Isopropyl-6-oxo-heptanal may be a sensory irritant of concern.
- 4-Oxopentanal may be of concern due to air-flow limitation.

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ABSTRACT

Ozone-initiated monoterpene reaction products have been hypothesized to cause eye and airway complaints in office environments and some have been proposed to cause skin irritation and sensitization. The respiratory effects of 60 min exposures to five common oxidation products from abundant terpenoids (e.g. limonene), used as solvent and fragrance in common household products or present in skin lipids (e.g. squalene), were studied in a head out mouse bioassay. This allowed determination of acute upper airway (sensory) irritation, airflow limitation in the conducting airways, and pulmonary irritation in the alveolar region. Derived human reference values (RFs) for sensory irritation were 1.3, 0.16 and 0.3 ppm, respectively, for 4-acetyl-1-methylcyclohexene (4-AMCH), 3-isopropenyl-6-oxo-heptanal (IPOH), and 6-methyl-5-heptene-2-one (6-MHO). Derived RFs for airflow limitation were 0.8, 0.45, 0.03, and 0.5 ppm, respectively, for dihydrocarvone (DHC), 4-AMCH, 4-oxo-pentanal (4-OPA), and 6-MHO. Pulmonary irritation was unobserved as a critical effect. The RFs indicate that the oxidation products would not contribute substantially to sensory irritation in eyes and upper airways in office environments. Reported concentrations in offices of 6-MHO and 4-OPA would not result in airflow limitation. However, based upon the RFs for IPOH and 4-OPA, precautionary actions should be considered that disfavor their formation in excess.

1. Introduction

There is an increasing health concern about the use of consumer and household products, e.g. air fresheners and cleaning agents, in indoor environments, because of their emission of terpenoid fragrances (Nazaroff and Weschler, 2004; Singer et al., 2006b). Especially, indoor chemistry of limonene (an abundant and ubiquitous volatile organic compound (VOC) indoors and generally a major fragrance component in numerous products) readily undergoes gas-phase reactions to produce a host of complex ozone-initiated terpene reaction products (called terpene reaction products). They comprise gaseous (Atkinson and Arey, 2003; Calogirou et al., 1999b; Singer et al., 2006a) and secondary organic aerosols (Glasius et al., 2000; Koch et al., 2000), in form of fine

Abbreviations: 4-AMCH, 4-acetyl-1-methylcyclohexene; AF, assessment factor; DHC, dihydrocarvone; IPOH, 3-isopropenyl-6-oxo-heptanal; LOAEL, lowest-observed-adverse-effect-level; 6-MHO, 6-methyl-5-heptene-2-one; NOAEL, no-observed-adverse-effect-level; 4-OPA, 4-oxopentanal; RD, depression in respiratory frequency; RF, human reference value; TB, time of brake; TE, time of expiration; TI, time of inspiration; TP, time of pause; VOC, volatile organic compound; VD, airflow at 0.5 tidal volume during expiration; VT, tidal volume.

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and ultrafine particles (Nøjgaard et al., 2006; Rohr et al., 2003; Singer et al., 2006a; Vartiainen et al., 2006; Wainman et al., 2000; Weschler and Shields, 1999). Further, both short (hydroxyl) and longer-lived radicals are formed (Chen et al., 2011). Products from surface ozonolysis of terpenoid compounds in household products (e.g. Destaillats et al., 2006; Ham and Wells, 2011) and sesquiterpenes in plants and skin lipids, like squalene (Fruekilde et al., 1998; Wisthaler and Weschler, 2010), may also be of concern as they are formed, for example in aircraft cabins and from ventilation filters (Destaillats et al., 2011; Forester and Wells, 2009; Wisthaler et al., 2005). Squalene is abundant in human skin lipids (Nicolaides, 1974) and for example present in Danish house dust in a mean concentration of 32 (95 percentile; 243) μg per g dust (Weschler et al., 2011).

Epidemiological studies in public office buildings indicated associations between late afternoon outdoor ozone and upper respiratory and eye symptoms (Apte et al., 2008; Erdmann and Apte, 2004); these are among the top-three reported symptoms (Brightman et al., 2008). Furthermore, exposure of rodents to reaction products of limonene showed airway effects (Sunil et al., 2007; Clausen et al., 2001). Respiratory effects of the upper airways were dominated by sensory irritation, which is caused by stimulation of the trigeminal (5th cranial) nerve. Additionally, moderate long-lasting effects in the conducting airways were observed from ozonolysis of limonene (Rohr et al., 2002; Wolkoff et al., 2008). More recently exposure to denuded reaction products of limonene indicated that the generated ultrafine particles were without biological effect, rather the gaseous products appeared to be the offending agents (Wolkoff et al., 2008).

Contradictory findings from human exposure studies have been reported. Exposure of 130 women for 140 min to a mixture of 23 typical indoor VOCs, which included limonene and α-pinene, and ozone neither reported significant sensory irritation (Fiedler et al., 2005) nor was nasal inflammation observed (Laumbach et al., 2005). On the other hand, eye exposure of male subjects to reaction products of limonene significantly increased the eye blink frequency indicative of a trigeminal stimulation, but not necessarily of perceived sensory irritation (Klenø and Wolkoff, 2004).

It has also been hypothesized that terpene reaction products with multiple oxygen groups such as dicarbonyls may exhibit inflammatory and respiratory sensitizing properties. This was based on calculated sensitization potentials (Forester and Wells, 2009), pulmonary epithelial cell exposure studies (Anderson et al., 2010), and studies on combined dermal and pharyngeal aspiration (Anderson et al., 2012).

We have examined five common terpene reaction products on the basis of their general abundance with high ozone or hydroxyl radical yields from common terpenoids. Our objective was to determine the acute upper and lower respiratory tract effects of these compounds. We used inhalation exposure as this is the appropriate route for risk assessment of indoor air pollutants with the purpose to evaluate the terpene reaction products as causative of eye and respiratory symptoms in indoor environments. We are not aware of previous inhalation studies of these terpene reaction products. 4-AMCH (4-acetyl-1-methylcyclohexene), DHC (dihydrocarvone), IPOH (3-isopropenyl-6-oxo-heptanal), 6-MHO (6-methyl-5-heptene-2-one), and 4-OPA (4-oxopentanal) are common terpene reaction products from fragrances like limonene, e.g. Atkinson and Arey (2003) and Calogirou et al. (1999b); for precursors, see Table 1.

2. Materials and methods

2.1. Chemicals

Methanol (99%) and pentane (99%) were from Aldrich. See Table 1 for structures of the following terpene reaction products: 4-AMCH (93% and 3% 3-acetyl-6-methylcyclohexene) and DHC (97% purity; 77% n-(+)-dihydrocarvone, 20% iso (+)-dihydrocarvone) were from Aldrich, and 6-MHO (99%) from Aldrich–Sigma. IPOH (97%) and 4-OPA (97%) were synthesized according to (Wolinsky and Barker, 1960) and (Hutton et al., 2003), respectively, by (HM-Chemo Co., Shanghai Branch, CN) and (Shanghai Chempartner Co., CN). The terpene reaction products are stored at 4 °C. Electron impact and chemical ionization GC/MS analyses of methanol diluted samples were carried out for structural confirmation and identification of impurities; pentane was used as solvent for 4-OPA, due to instability in methanol. For GC/FID and GC/MS conditions, see (Wolkoff, 1998). The structure confirmation was either by high hit search (>0.9) in the Wiley Library (Ver. 7) or comparison with literature mass spectra; for 4-OPA (Fruekilde et al., 1998; Hutton et al., 2003; Molander and Cameron, 1993) and IPOH (Calogirou et al., 1999a). The purity was based on GC peak area integration in full scan mode and averaged ($n = 2$).

2.2. Animals

Inbred BALB/cA male mice were purchased from Taconic, Denmark. At the initiation of the study, the mean weight and SD of the mice was 25.8 ± 1.3 g. Mice were housed in polypropylene cages (380 mm × 220 mm × 150 mm) with pinewood sawdust bedding (Lignocel S8, Brogaarden, Denmark). The photoperiod was from 6 a.m. to 6 p.m., and the temperature and relative humidity in the animal room were 22 ± 2 °C and 50 ± 5%, respectively. The cages were sanitized twice weekly. Food (Altromin no. 1324, Altromin, Lage, Germany) and tap water were available ad libitum.

Treatment of the animals adhered to procedures approved by The Animal Experiment Inspectorate, Denmark with Permission numbers 2006/561-1123 and 2011/561-1990.

2.3. Generation and monitoring of test atmospheres

The terpene reaction products were evaporated in Pitt No. 1 VOC generator (Wong and Alarie, 1982), diluted with medical dry air, and fed into a 24 L exposure chamber (Larsen and Nielsen, 2012). The airflow rates in the chamber were set between 18.8 and 23.2 L/min. The chamber exposure concentrations were monitored every fourth minute by 15 sequential 1.0 mL air samples on Tenax TA steel tubes (PerkinElmer), taken by syringe (size: 2.0 mL) suction, followed by thermal desorption within 12 h, and GC/FID analysis, as described previously (Wolkoff, 1998). Six-point calibration of the weighed compound in methanol (0.08–2.5 μg/mL) was applied for determination of air concentrations ($R^2 \geq 0.98$), except for 4-OPA that was dissolved in pentane.

Initially, a starting concentration was selected on the basis of the relation for non-reactive compounds according to Alarie et al. (1996). However, for reactive compounds, i.e. with an aldehyde group, a lower starting concentration was decided. Other exposure concentrations were decided upon the first observation of a bioresponse. The resulting exposure concentrations are shown in Table 2.

2.4. Bioassay

The respiratory effects were studied in a head out mouse bioassay (Alarie, 1998). The bioassay allows detection of respiratory effects on the upper airways (sensory irritation), effects on the conducting airways, and at the alveolar level by continuous computerized monitoring of the breathing pattern.

The inhalation effects are investigated by analyses of the breathing patterns in mice (Alarie, 1973; Nielsen et al., 1999). Briefly, the breathing pattern analysis recognizes and quantifies specific deviations from the normal breathing pattern (for terms and definitions, see Fig. 1 in Nielsen et al. (1999)). Thus, after end of inhalation, a short brake occurs before the exhalation is initiated, *termed time of brake* (TB, ms). An increase in TB leads to a decrease in the *respiratory frequency* (*f*, breaths/min). TB is a specific marker of sensory irritation and it increases with increasing exposure levels of sensory irritants due to a stimulation of the trigeminal nerves. Formaldehyde, ammonia, and methacrolein are examples of compounds being sensory irritants (Nielsen et al., 1999, 2007; Larsen and Nielsen, 2000).

Other parameters are *time of inspiration* (TI, ms), *time of expiration* (TE, ms), and *mid expiratory flow rate* (VD; mL/s), which are used for evaluation of airflow limitation. This is due to bronchial constriction, mucous accumulation, or inflammation of the conducting airways (for simplicity termed 'bronchoconstriction'). This extends TE and thus causes an associated decrease in *f*. To quantify the effect, the airflow rate at 0.5 VT (*tidal* volume, mL) during expiration is measured. VD decreases as the exposure concentration to a bronchoconstrictor increases. The decrease has been shown to be correlated with an increase in resistance to airflow (Vijayaraghavan et al., 1993) as measured by the classical method of Amdur and Mead (1958). If VT changes, it is attempted to normalize for differences by plotting the VD/VT ratio versus the exposure concentration.

Pulmonary irritation comprises two types of reflex patterns, which are both caused by stimulation of vagal nerve endings at the alveolar level (for simplicity termed 'pulmonary irritation'). First, one reflex reaction is characterized by rapid shallow breathing. The modification of the normal breathing pattern includes a decrease in VT, TI and TE. All three parameters decrease in a concentration-dependent manner. Due to the decrease in TI and TE, an increase in *f* will be observed, thus causing rapid shallow breathing. This type of reaction is typically seen shortly