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Monocyclic and bicyclic monoterpenes in air of German daycare centers and human biomonitoring in visiting children, the LUPE 3 study

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ABSTRACT

To investigate the assumed association between indoor air pollution with monoterpenes (MTps) and the internal MTp exposure of occupants, a comparative study was performed in daycare centers in two federal states of Germany. Three well-known monoterpenoid air pollutants, viz. α-pinene (αPN), Δ^3-carene (CRN), and *R*-limonene (LMN), were measured in indoor air in 45 daycare centers. Additionally, urine samples of 222 children visiting these facilities were collected in the evening after a full-day stay. Altogether 11 MTp metabolites were analyzed in the urine samples using a novel highly sensitive and selective gas chromatographic–tandem-mass spectrometric procedure. The medians (95th percentiles) of the MTp levels in indoor air were 9.1 μg m^{-3} (94 μg m^{-3}) for LMN, 2.6 μg m^{-3} (13 μg m^{-3}) for αPN, and <1.0 μg m^{-3} (3.2 μg m^{-3}) for CRN. None of the day care centers exceeded the German health precaution or hazard guide value. In spite of the low MTp air exposure, the urine analyses revealed an exposure to the three monoterpenes in almost all children. The median levels of MTp metabolites in urine were 0.11 mg L^{-1} for LMN-8,9-OH, 0.10 mg L^{-1} for LMN-1,2-OH, 49 μg L^{-1} for PA, 2.9 μg L^{-1} for POH, 5.2 μg L^{-1} for tCAR, and 4.1 μg L^{-1} for cCAR (LMN metabolites), 7.2 μg L^{-1} for MYR, 19 μg L^{-1} for tVER, and 19 μg L^{-1} for cVER (αPN metabolites), as well as 8.2 μg L^{-1} for CRN-10-COOH (CRN metabolite). Statistically significant and strong correlations among the urinary metabolites of each MTp were found. Moreover, statistical associations between LMN metabolites and the LMN indoor air levels were revealed. However, the weakness of the associations indicates a considerable impact of other MTp sources, e.g. diet and consumer products, on the internal exposure.

1. Introduction

Monoterpenes (MTps) are isoprenoid, biogenic, volatile organic compounds (VOCs) that may be detected in most indoor environments (Kephalopoulos et al., 2007; Koistinen et al., 2008). Especially α-pinene (αPN), Δ^3-carene (CRN), and *R*-limonene (LMN) are emitted in considerable amounts into indoor air e.g. from wooden construction materials, furniture, cleaning agents, air refreshers, paints or varnishes (Ho et al., 2011; Król et al., 2014; Offermann and Hodgson, 2011). In addition, various foods and beverages such as fruits, vegetables, spices, and wines contain extensive and characteristic volatile monoterpene fractions that may either be emitted into indoor air or be directly ingested. Particularly LMN is one of the most prominent monoterpenes in foods as well as in cosmetic and household products with citrus-like fragrance (Kim et al., 2013). The oral intake in the United States and Europe is estimated at about 465–700 μg kg^{-1} bw d^{-1} for LMN, 5–41 μg kg^{-1} bw d^{-1} for αPN, and 0.2 μg kg^{-1} bw d^{-1} for CRN (Adams et al., 2011; EFSA, 2011a,2011b). Moreover, since MTps are emitted into indoor air, there is a ubiquitous inhalation background exposure. Depending on the respective sources of emission, there may be large variations in MTp indoor air levels. Usually the mean background levels are in the lower to medium μg m^{-3} range (1–500 μg m^{-3}) (Brown et al., 1994; De Bortoli et al., 1986; Hippelein, 2004; Król et al., 2014; Montgomery and Kalman, 1989; Ostendorp and Heinzow, 2010; Sagunski and Heinzow, 2003; German Working Group on Indoor Air Guidelines, 2010). However, long-term observations reveal possible peak concentrations up to the mg m^{-3} range (Król et al., 2014). For LMN, the uptake caused by background exposure was estimated from indoor air measurements to be approximately 10 μg kg^{-1} bw d^{-1} (WHO, 1998). Though, for the more specific wood-related VOCs αPN and CRN, such an evaluation is missing.

MTps are appraised as relevant indoor air pollutants which require further research with regard to dose–response and long-term effects. Since significant exposure data are still lacking, a concluding risk assessment for airborne MTps is not carried out so far (Koistinen et al., 2008).

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Due to various toxicological effects after sub-acute and chronic exposure of humans and animals, such as irritation of the respiratory tract and histological lesions of liver tissue, the German Working Group on Indoor Air Guidelines of the Federal Environment Agency and the States' Health Authorities issued indoor air guide values for mono- and bicyclic monoterpenes to protect public health. A health precaution guide value of 1 mg m^{-3} for LMN and 0.2 mg m^{-3} for bicyclic MTps, as well as a health hazard guide value of 10 mg m^{-3} for LMN and 2 mg m^{-3} for bicyclic MTps were derived (Sagunski and Heinzow, 2003; German Working Group on Indoor Air Guidelines, 2010).

For decades, human biomonitoring has been shown to be a versatile tool for the identification and assessment of the general population's exposure to chemicals (Göen et al., 2012). Compared to air-monitoring, which reflects external exposure, biomonitoring focuses on the analysis of metabolites in biological materials and, therefore, it may increase significance for risk assessment (Angerer et al., 2007). Following uptake, MTps are rapidly metabolized to oxidized species which are excreted via urine, mainly in conjugated forms. The main renal metabolites of LMN are the vicinal diols limonene-8,9-diol (LMN-8,9-OH) and limonene-1,2-diol (LMN-1,2-OH), as well as unsaturated carboxylic acids such as perillic acid (PA) (Kodama et al., 1976; Poon et al., 1996; Zhang et al., 1999). For αPN, mono-hydroxylated structures such as *cis*- and *trans*-verbenol (cVER and tVER) are predominant metabolites (Eriksson and Levin, 1990; Ishida et al., 1981; Southwell et al., 1980), however for LMN, *cis*- and *trans*-carveol (cCAR and tCAR) as well as perillyl alcohol (POH) are minor ones (Fig. 1) (Schmidt et al., 2013). Unlike LMN and αPN, the human metabolism of CRN is still poorly explored. However, our recent studies revealed that 3-caren-10-carboxylic acid (CRN-10-COOH) is a relevant human metabolite of CRN in vivo (Fig. 1; Schmidt et al., 2015).

Nevertheless, biomonitoring of MTps is a hitherto barely explored area of research. Solely three pilot studies applied αPN biomonitoring on post-shift urine samples of workers in Swedish and Finish saw mills. The studies showed strong and significant correlations of αPN or total MTps in indoor air with urinary verbenols, indicating that MTp biomonitoring may be successfully used for internal MTp exposure assessment (Eriksson et al., 1996; Liljelind et al., 2003; Rosenberg et al., 2002). However, there are no studies correlating indoor MTp levels with biomonitoring data of the general population.

Because of the lack of knowledge on the internal MTp exposure of the general population and particularly on the association between indoor air pollution and internal exposure, we took the opportunity for the comparative analyses of MTp air monitoring in German daycare centers (DCCs) and a human biomonitoring of the visiting children as part of the LUPE 3 study. This pilot study was used as a first approach to check if the association between MTp indoor air pollution and internal exposure assumed by other working groups is also detectable in cases of environmental background exposure.

2. Material and methods

2.1. Chemicals and consumables

The reference substances (1*R*)-(+)-α-pinene (αPN; Fig. 1), (1*S*)-(+)-3-carene (CRN; Fig. 1), (4*R*)-(+)-limonene (LMN; Fig. 1), and the enantiomeric references (*S*)-*cis*-verbenol [(1*S*,2*S*,5*S*)-*cis*-verbenol; cVER; Fig. 1], (1*R*)-(−)-myrtenol [(1*R*,5*S*)-myrtenol; MYR; Fig. 1], (−)-carveol [separable mixture of *cis*-(1*R*,5*R*)- and *trans*-(1*S*,5*R*)-isomers; cCAR and tCAR; Fig. 1], (*S*)-(−)-perillyl alcohol (POH; Fig. 1), (*S*)-(−)-perillic acid (PA; Fig. 1), (1*S*,2*S*,4*R*)-(+)-limonene-1,2-diol (LMN-1,2-OH; Fig. 1) each at highest purity available (≥96%), as well as *N*,*O*-bis(trimethylsilyl) trifluoroacetamide with 1% trimethyl chlorosilane (BSTFA, ≥98.5%), and *N*-(trimethylsilyl) imidazole (TSIM, ≥98%) were purchased from Sigma-Aldrich (Steinheim, Germany). The enantiomeric reference substances and internal standards (IStds): (1*R*,2*S*,5*R*)-*trans*-verbenol (tVER; Fig. 1), D_3-(1*R*,2*S*,5*R*)-*trans*-verbenol (D_3-tVER),

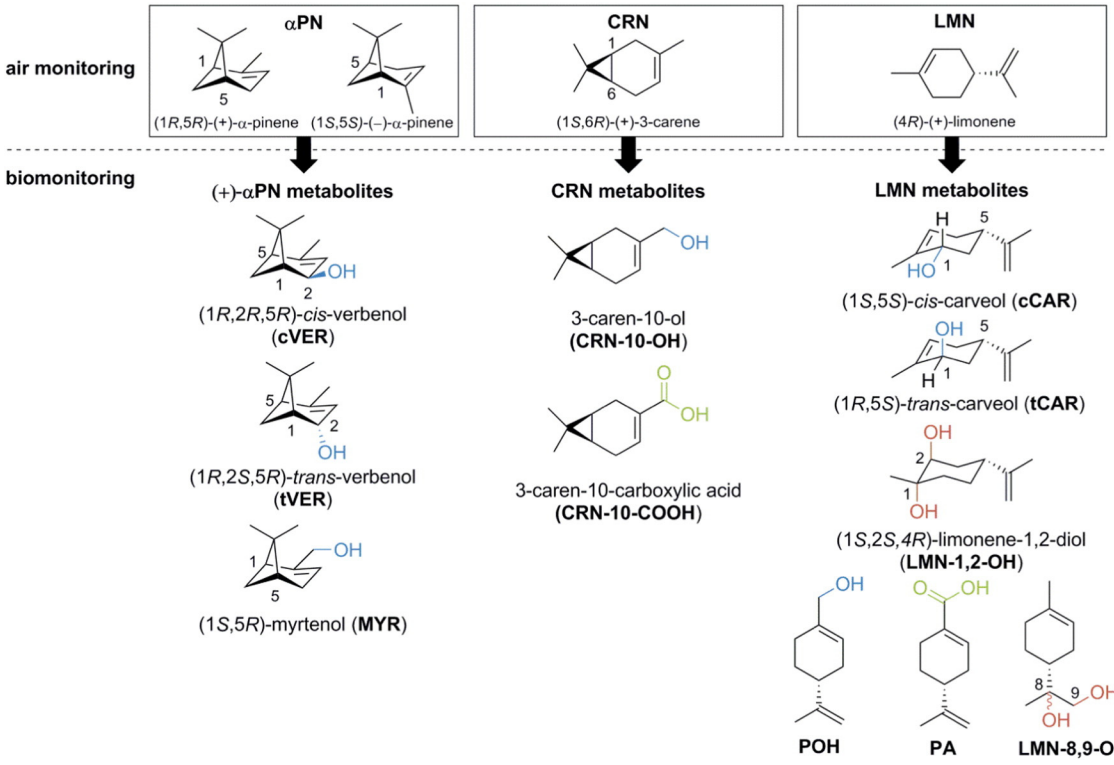

Fig. 1. Structures of the precursor monoterpenes in indoor air [α-pinene (αPN), 3-carene (CRN), and (4*R*)-limonene (LMN)] and their corresponding oxidative human metabolites analyzed in urine [αPN: *trans*-verbenol (tVER), *cis*-verbenol (cVER), and myrtenol (MYR); 3-carene: 3-caren-10-ol (CRN-10-OH) and 3-caren-10-carboxylic acid (CRN-10-COOH); as well as (4*R*)-limonene: *cis*-carveol (cCAR), *trans*-carveol (tCAR), perillyl alcohol (POH), perillic acid (PA), limonene-1,2-diol (LMN-1,2-OH), and limonene-8,9-diol (LMN-8,9-OH)]. Structures of enantiomeric references partially used for quantification are not shown.