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Acute effects of an exposure to 100 ppm 1-methoxypropanol-2 on the upper airways of human subjects

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HIGHLIGHTS

- After a 4 h-exposure to 100 ppm 1-methoxypropanol-2, the olfactory threshold was elevated.
- Exposure did not cause clear-cut inflammatory effects on the upper airways.
- With respect to our findings the MAK value needs not to be changed.

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ABSTRACT

The German MAK value of 1-methoxypropanol-2 has been fixed at 100 ppm. The aim of this study was to evaluate possible acute effects of an exposure to 100 ppm 1-methoxypropanol-2 on the upper airways of human subjects. Twenty subjects were exposed in a crossover design to 100 ppm 1-methoxypropanol-2 and to air in an exposure chamber for 4 h. Subjective symptoms were assessed by questionnaire. Olfactory thresholds for n-butanol and mucociliary transport time were measured before and after exposure. Concentrations of interleukin 1β and interleukin 8 were determined in nasal secretions taken after exposure. mRNA levels of interleukins 1β, 6 and 8, tumor necrosis factor α, granulocyte-macrophage colony-stimulating factor, monocyte chemotactic protein 1, and cyclooxygenases 1 and 2 were measured in nasal epithelial cells, obtained after exposure. Possible effects were investigated by semiparametric and parametric cross-over analyses. Subjects did not have any subjective irritating symptoms. The olfactory threshold was slightly elevated following exposure to 1-methoxypropanol-2. Mucociliary transport time did not change. Neither concentrations of interleukins in nasal secretions nor mRNA levels except for interleukin 1β were higher after exposure to 1-methoxypropanol-2. In conclusion, the acute exposure to 100 ppm 1-methoxypropanol-2 did not cause clear-cut adverse effects in test subjects

1. Introduction

The use of glycol ethers is widespread in industry and consumer products. Because of their toxicity ethylene glycol ethers have extensively been replaced by propylene glycol ethers, among them mainly methoxypropanol (propylene glycol monomethyl ether, PGME) (Dentan et al., 2000). Measurements revealed concentrations greater than 50% in inks, varnishes, paints, solvents, diluents, pickling solutions, auxiliary materials, hardeners and other products (Dentan et al., 2000). Occupational exposure to methoxypropanol may occur by inhalation and skin exposure at workplaces where this compound is produced or used. There are two isomers of methoxypropanol with different toxicity, resulting in different threshold limit values. The German MAK value of 1-methoxypropanol-2 (Chemical Abstracts Service Registry Number (CASRN) 107-98-2) has been fixed at 100 ppm and that of 2-methoxypropanol-1 (CASRN 1589-47-5) at 5 ppm (Deutsche Forschungsgemeinschaft, 2012). In the past, technical 1-methoxypropanol-2 contained up to 5% of 2-methoxypropanol-1 (Deutsche Forschungsgemeinschaft, 1998). Consequently, the

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MAK value refers to technical 1-methoxypropanol-2 (Deutsche Forschungsgemeinschaft, 1998). The MAK value is derived from the no observed adverse effect level (NOAEL) of the respective substance. The MAK value of 1-methoxypropanol-2 has been fixed at 100 ppm to protect workers from irritating effects (Deutsche Forschungsgemeinschaft, 1998); systemic effects were reported only at considerably higher concentrations.

With regard to other organic solvents, proinflammatory effects and an impairment of mucociliary transport in the nose were reported after acute exposure with concentrations not exceeding the respective MAK values (Mann et al., 2002; Muttray et al., 1999, 2002). In these experimental studies, test subjects did not report any irritating effects in the upper airways. Inflammatory changes of the respiratory nasal epithelium can be assessed on protein level by the determination of cytokines in nasal secretion. In addition, the measurement of mRNA expression in tissue specimens from nasal respiratory mucosa offers a possibility to investigate a wider range of inflammatory mediators and key enzymes like cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). COX-1 and its inducible isoform COX-2 catalyze the conversion of arachidonic acid to prostaglandin endoperoxides, leading to the formation of prostaglandin and thromboxane mediators of inflammation. COX-2 mRNA can be greatly increased in the presence of inflammation (Smith and Dewitt, 1996). The increase of proinflammatory mediators and/or the impairment of mucociliary transport may explain the development of clinical rhinitis in subjects highly exposed to solvents (Muttray et al., 2002).

Our question was whether an acute exposure to 100 ppm 1-methoxypropanol-2 causes inflammatory changes in healthy subjects' nasal epithelium or impairs mucociliary transport. For ethical reasons, the MAK value was not exceeded.

2. Methods

2.1. Experimental design

Twenty healthy male subjects were exposed to 100 ppm 1-methoxypropanol-2 and to sham (air) according to a crossover design. The sequence of exposures was assigned by chance. The interval between the two exposure sessions was one week. Subjects were informed that they were exposed to an organic solvent in an exposure chamber twice at two separate days, at concentrations not exceeding the occupational threshold value. It was decided not to blind subjects as all relevant dependent variables, except for the olfactory threshold, were not influenced by the possible knowledge of exposure. Furthermore, the use of a masking odorant could have biased the determination of the olfactory threshold.

2.2. Subjects

Subjects were 20 male non-smoking healthy men. Median age was 25.7 (19–31 years). Participants were on no medication. A screening to exclude possible diseases included occupational and past-medical history, physical examination, electrocardiogram, spirometry, blood count, determination of γ-glutamyl transpeptidase, alanine aminotransferase, sedimentation rate, activated partial thromboplastin time (aPTT), prothrombin time, and urine analysis with a test strip (Combur 10®, Roche). Nasal or paranasal sinus diseases were examined by history, nasal endoscopy, A-scan-sonography of the paranasal sinuses, active anterior rhinomanometry, acustic rhinometry, measurement of mucociliary transport time, and determination of the olfactory threshold for n-butanol with the Sniffin' Sticks test (Hummel et al., 1997). Thresholds for n-butanol were within normal range (Hummel et al., 2007). Immediately before each exposure, a short medical check was performed. Prior to the study, written informed consent was obtained from every subject. The study was performed in accordance with the ethical principles of the Declaration of Helsinki and its latest amendments. The protocol was approved by the local ethics committee. Subjects were paid for participation.

2.3. Exposure

Exposure was carried out in an 18 m^3 chamber. Exposure time was four hours. Concentration of 1-methoxypropanol-2 ($\geq$ 99.5%, 2-methoxypropanol-1 $\leq$ 0.5%, Sigma–Aldrich Chemie GmbH, Steinheim, Germany) was monitored continuously by infrared-spectroscopy (Gasmet FT-IR, Temet Instruments, Oy, Finland). Mean 1-methoxypropanol-2 concentration was 100.3 ppm (±3.9 SD). Mean temperature was 21.0 °C (±0.2 SD) and 21.1 °C (±0.3 SD) for 1-methoxypropanol-2 and sham exposure, respectively. Humidity was 47.3% (±2.4 SD) and 46.5% (±0.3 SD), respectively. Air exchange was 6 times per hour.

2.4. Experimental procedure

Each experimental day started at 8:00 a.m. with the first subject. Four other subjects were tested consecutively, with intervals of 30 min in between. Upon arrival, each subject was medically assessed. All participants had to fill out a general questionnaire (e.g. intermediate health complaints) and the baseline Swedish Performance Evaluation System (SPES) questionnaire (Iregren et al., 1996) subsequently. Olfactory threshold for n-butanol and mucociliary transport time were measured. Then the subject entered the exposure chamber and was exposed to 1-methoxypropanol-2 or filtered air for 4 h, respectively. During exposure, follow-up SPES questionnaires were filled out after 2 min, 2 and 4 h. Subjects were continuously observed. They could read, communicate with each other, eat and drink, but were not allowed to sleep. We made sure that subjects did not ingest beverages and food, which might impair olfaction or irritate mucous membranes. Immediately after leaving the chamber, olfactory threshold and mucociliary transport time were measured once again in a neighbored quiet room. Afterwards, nasal secretions and epithelial cells were taken. The same procedure was repeated on the second experimental day. Finally subjects were asked by questionnaire, if exposure was higher at the first or the second day.

2.5. Subjective ratings of exposure level

A self-assessment questionnaire was used to distinguish whether or not subjects were aware of the exposure condition (1-methoxypropanol-2 vs. sham). The wording was: "How strongly did you perceive today's solvent concentration in the chamber?" The subjects rated their perceived level of exposure on an ordinal scale from 1 (no test substance detectable) to 10 (very high concentration) after each experimental day. Then, subjects marked the degree of certainty of their exposure estimates on an ordinal scale from 1 (not at all) to 10 (absolutely sure). At the end of the second experimental day, subjects were asked on which day the concentration was higher by filling out an analogous questionnaire. Staff rated the level of exposure after each experimental session by questionnaire on an ordinal scale from 1 to 10.

2.6. Assessment of acute symptoms

The questionnaire addressing acute symptoms (see Section 3.2) was derived from the Swedish Performance Evaluation System (SPES) (Iregren et al., 1996) and comprises 17 items (Seeber et al., 1994) with an ordinal scale of 0 (equals no complaints at all) to 5 (equals maximum discomfort, pain or symptoms). The subjects were familiarized with the questionnaire before the first experimental day.

2.7. Rhinological and laboratory examinations

The olfactory threshold for n-butanol was assessed with the Sniffin' Sticks test (Hummel et al., 1997, 2007). Subjects were instructed to abstain from sharply flavored food, onions, garlic, perfume, and shaving lotions preceding the day of the experiment. They were blindfolded with a sleeping mask to prevent visual identification of odorant containing sticks. The detection threshold for n-butanol was assessed by the initially ascending single-staircase method. The single staircase started with the lowest concentration of n-butanol. In each trial, subjects had to identify the odorant out of three stimuli (the odorant and two blanks). Concentrations were increased with a ratio of 1:2, until correct detection occurred on two consecutive trials. If an incorrect response was given during any trial, the concentration of n-butanol was increased one step. If a correct response was given, the staircase was reversed and subsequently moved downward. The mean of the four staircase reversal points after the third staircase reversal was defined as threshold measure (the higher the score, the higher the subject's sensitivity). Subjects perceived no feedback on their decisions (Muttray et al., 2004). Mucociliary transport time was measured by placing saccharine particles on the floor of the nose just below the head of the inferior concha and measuring the time required for the subject to detect a strong sweet taste. Nasal secretions were collected with absorbent foam rubber samplers (Muttray et al., 1999), centrifuged and deep frozen at −80 °C. Interleukin 1β (IL-1β) was analyzed with a commercial ELISA QuantiGlo® human IL-1β (R&D Systems, Wiesbaden, FRG). For quantitative determination of interleukin 8 (IL-8) in nasal secretions a sandwich enzyme immunoassay with absorption-based detection (DuoSet® ELISA Development System human IL-8, R&D Systems) was used. Results were expressed as the means of duplicate measurements. To harvest epithelial cells for mRNA isolation and Quantitative Polymerase Chain Reaction (Q-PCR), biopsies were taken from the nasal floor using a small currette. Samples were immediately transferred in RNAlater solution (Applied Biosystems, Darmstadt, FRG) and stored at −20 °C until RNA-isolation. mRNA levels of IL-1β, interleukin 6 (IL-6), IL-8, tumor necrosis factor α (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF), monocyte chemotactic protein 1 (MCP-1), cyclooxygenase 1 (Cox-1), and cyclooxygenase 2 (Cox-2) were determined. Two μg of total RNA were used from each specimen for cDNA synthesis using standard protocols (Gosepath et al., 2006). Obtained cDNAs were stored at −20 °C. Each sample was diluted 1:5 with