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ORIGINAL ARTICLE

Exposure study to examine chemosensory effects of formaldehyde on hyposensitive and hypersensitive males

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Abstract
Objective Main objective of this study was to examine the chemosensory effects of formaldehyde on hyposensitive and hypersensitive males at concentrations relevant to the workplace. Attention focused on objective effects on and subjective symptoms of the mucous membranes of the eyes, the nose, the upper respiratory tract and olfactory function.
Methods Forty-one male volunteers were exposed for 5 days (4 h per day) in a randomised schedule to the control condition (0 ppm) and to formaldehyde concentrations of 0.5 and 0.7 ppm and to 0.3 ppm with peak exposures of 0.6 ppm, and to 0.4 ppm with peak exposures of 0.8 ppm, respectively. Peak exposures were carried out four times a day over a 15-min period of time. Subjective pain perception induced by nasal application of carbon dioxide served as indicator for sensitivity to sensory nasal irritation. The following parameters were examined before and after exposure: subjective rating of symptoms and complaints (Swedish Performance Evaluation System), conjunctival redness, eye-blinking frequency, self-reported tear film break-up time and nasal flow rates. In addition, the influence of personality factors on the volunteer's subjective scoring was examined (Positive And Negative Affect Schedule).
Results Formaldehyde exposures to 0.7 ppm for 4 h and to 0.4 ppm for 4 h with peaks of 0.8 ppm for 15 min caused no significant sensory irritation of the measured conjunctival and nasal parameters. No differences between hypo- and hypersensitive subjects were seen. Nevertheless, statistically significant differences were noted for olfactory symptoms, especially for the 'perception of impure air'. These subjective complaints were more pronounced in hypersensitive subjects.
Conclusions Formaldehyde concentrations of 0.7 ppm for 4 h and of 0.4 ppm for 4 h with peaks of 0.8 ppm for 15 min did not cause adverse effects related to irritation, and no differences between hypo- and hypersensitive subjects were observed.

Keywords Formaldehyde · Carbon dioxide · Sensitivity · Hypersensitive · Exposure · Chemosensory effect · Sensory irritation

Introduction

Gaseous formaldehyde (FA) is irritating to the eyes and respiratory tract by chemosensory effects and leads to reflex responses such as lacrimation, rhinorrhea, coughing, vasodilatation and changes in rate and depth of respiration (Paustenbach et al. 1997; Arts et al. 2006a, 2008). These typical effects were used to derive a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) for regulatory purposes. Based on the findings of controlled human studies, Paustenbach et al. proposed an occupational exposure limit (OEL) of 0.3 ppm

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over 8 h (8 h time-weighted average (TWA)) and a ceiling value (CV) of 1.0 ppm. They noted that below 1.0 ppm, if irritation occurs in some persons, the effects rapidly subside due to accommodation (Paustenbach et al. 1997).

Arts et al. (2006a) concluded that mild/slight eye irritation was observed at levels >1 ppm, and mild/slight respiratory tract irritation at levels >2 ppm. The time course of effects showed that all symptoms disappeared very quickly, underlining the mildness of the effects at low concentrations (Arts et al. 2006a).

In a previous controlled human exposure study, performed by our working group, we found eye irritation to be the most critical effect induced by FA. Statistically significant increases in eye-blinking frequency and conjunctival redness were observed at FA concentrations of 0.5 ppm for 4 h with peaks of 1.0 ppm for 15 min. From these results, we derived a no-observed-adverse-effect level (NOAEL) of 0.5 ppm FA without peaks and of 0.3 ppm with peaks of 0.6 ppm (Lang et al. 2008).

Considering this outcome, the Scientific Committee on Occupational Exposure Limits (SCOEL) concluded that the time-weighted average occupational exposure limit (TWA-OEL) of FA should be kept at or below the NOAEL for sensory eye irritancy. SCOEL therefore proposed an indicative occupational exposure limit (IOEL) of 0.2 ppm with peak excursions of 0.4 ppm and argued that 'this especially considers possible interindividual differences in susceptibility to irritation by formaldehyde, which may be expected based on the entire body of data' (SCOEL 2008).

To address the issue of interindividual susceptibility to chemosensory FA effects, mentioned by SCOEL, we performed the present study. Its objective was to evaluate irritant FA effects on male volunteers, which were defined as hypo- or hypersensitive against sensory irritation. Therefore, objective parameters (conjunctival redness, eye-blinking frequency, tear film break-up time, nasal flow rates) and subjective symptoms were assessed.

Another aim of this study was to examine potential genotoxic FA effects on human nasal mucosa and peripheral blood cells. These results were presented elsewhere and showed that FA neither has genotoxic effects nor induces relevant biological changes in gene expression (Zeller et al. 2011a, b).

Materials and methods

Study design and subjects

After written informed consent, 41 healthy, non-smoking male adults, aged 32 ± 9.9 years on average, were exposed for 4 h per day to four different gaseous FA concentrations plus the control condition of 0 ppm (0.01 ppm FA background level) in a repeated-measures crossover design. The sequence of exposures was determined by blinded randomisation. The trial was approved by the Ethics Committee of Heidelberg University and performed according to the Declaration of Helsinki. Exclusion criteria comprised (1) eye-blinking frequency >20/min, (2) allergy and/or skin diseases, (3) drug abuse or consumption of alcohol >50 g/day, (4) exposure to FA at workplace or at home, (5) diseases of the respiratory tract, metabolism or heart and (6) inadequate visus without visual aids.

Nasal sensitivity to carbon dioxide (CO_2)

Nasal sensitivity to CO_2 irritation was used as surrogate for individual chemosensory sensitivity (Dunn et al. 1982; Hummel et al. 1996; Shusterman and Balmes 1997a, b), which was tested by a 2-s application of three defined CO_2 concentrations (40, 60 and 80 per cent by volume of CO_2) to volunteers' nasal mucosa by means of a gas delivery device. CO_2 gas and air (Guttroff GmbH, Wertheim, Germany) were supplied from compressed air cylinders (flow rate: CO_2, 5 l/min; air, 3 l/min) and conducted to subjects' nasal septum via an application hose. Prior to testing, participants were instructed in velopharyngeal closure (Kobal 1985) to seal off their nasal cavity from the mouth cavity and throat. During examination, this allows the participant to breathe through the mouth, without extracting the incoming CO_2–air mix from the nasal cavity and thereby reducing the concentration applied.

After the 2-s CO_2 application to the volunteer's nasal septum, a 30-s break was taken before applying the next concentration. Each concentration was offered three times in a randomised order per complete measurement cycle. During the entire examination, volunteers were blinded to the actual CO_2 concentrations applied.

To record participants' individual 'sensation of pain' or 'intensity of irritant effect' caused by the CO_2–air mixture applied, we used a visual analogue scale (VAS), the two endpoints of which were designated as 'none at all' and 'unbearable'. For assessment, the subject's markings on the VA scales were measured and the 'sensation of pain' expressed in millimetres (0–100 mm), documented for each individual measurement.

CO_2 sensitivity measurements were taken daily before and after exposure and, in addition, during three follow-up tests at approximately one-week intervals after the end of exposure. Measurements recorded at the last follow-up examination formed the basis for a breakdown of volunteers into different sensitivity groups. First, mean values of the actually measured 'sensation of pain' (marked on VAS) at concentrations of 40, 60 or 80 per cent by volume were

calculated for each participant. These three values were added to an individual 'CO_2 sum score' (individual 'CO_2 sensitivity score'), with a maximum possible range from 0 to 300 mm, and used for classification into hypo- and hypersensitive subjects depending on whether their value was below or above the median.

Generation and analysis of formaldehyde

Generation and analysis of FA has been described in detail elsewhere (Lang et al. 2008). Briefly, paraformaldehyde (Merck®, Darmstadt, Germany) was heated. FA vapours were introduced into the exposure chamber (about 30 m^3) and distributed by a ventilation system. Aimed-at and actual FA concentrations were determined by real-time monitoring (ANSYCO® formaldehyde monitor; type HCHO, model no. 4160-DSP, serial no. 821010, Analytische Systeme und Componenten GmbH, Karlsruhe, Germany) and verified by HPLC analysis using air samples (aldehyde sampler cartridges; WATERS® 'SEP-PAK XPoSure', product no: WAT047205, Milford, USA).

Formaldehyde exposure

Exposure took place on five consecutive days and to five different exposure conditions (Table 1). A maximum of four subjects were exposed per week, separated into two groups with exposures time-staggered by 1 h. During the 4-h daily exposure, subjects had to perform four cycle ergometer units at 80 watts for 15 min at predefined times. On days with exposure peaks, two of the four ergometric units were carried out during an exposure peak. Subjects were instructed not to eat shortly before an examination and during exposure, while drinking of non-sparkling mineral water (reclosable bottles) was allowed.

Table 1 Results of the measured formaldehyde concentrations in the exposure chamber (mean ± SD); peak exposures were 15 min each; air samples for HPLC analysis were taken both during baseline and peak exposures

Concentration/ Exposure condition	FA concentration (ppm) (target)	FA concentration (ppm) (real-time monitoring)	FA concentration (ppm) (HPLC)
A	0.0	0.01 ± 0.00[a]	0.01 ± 0.01[a]
B	0.3 + 4 × 0.6 (peaks)	0.33 ± 0.02	0.32 ± 0.02
C	0.4 + 4 × 0.8 (peaks)	0.44 ± 0.03	0.39 ± 0.05
D	0.5	0.51 ± 0.01	0.49 ± 0.06
E	0.7	0.71 ± 0.02	0.70 ± 0.03

[a] Background level

Order of daily examinations

The examinations were measurement of nasal flow rates and self-reported tear film break-up time (sBUT), detection of CO_2 sensitivity as well as determination of conjunctival redness (photo documentation), eye-blinking frequency (video recording), and recording of subjective symptoms and complaints (SPES questionnaire; short for Swedish Performance Evaluation System). Unless stated otherwise, our volunteers were examined approximately within 1 h before start and 1 h after the end of exposure each day. Apart from eye-blinking frequency recording and completion of the SPES questionnaire, which took place during the last 15 min of exposure *inside* the chamber, no examinations were carried out in the daily exposure phase.

Conjunctival redness

Photographs were taken by means of a digital camera (Fujifilm FinePix S1 Pro; Medical-Nikkor 120 mm f/4 IF Macro-lens with built-in ringflash) to document any dilatation of the conjunctival blood vessels indicative of irritant FA effects. A section of the participants' medial conjunctiva was photographed, and severity of conjunctival irritation was evaluated according to Lang et al. (2008).

Eye-blinking frequency

Participants' basal eye-blinking frequency was determined before registration for the study. Normal blinking frequency was set to ≤20/min. Recording was done in a modified technique based on the method described by Ziegler et al. (2008). Eye-blinking frequency was documented for approximately 5 min by means of a digital camcorder (JVC GR-D230E, serial no. 159P1566) directly before FA exposure *outside* the exposure chamber and within the last 15 min of exposure *inside* the chamber. During measurement, subjects focused their eyes on a marking on the opposite wall. Afterwards, processing of the 5-min recordings with video-editing software ('Cut Assistant' v.0.9.12.2) generated coherent video sequences of exactly 60 s length. Counting of eye blinks was done by two independent investigators blinded to the actual exposure concentrations.

Tear film break-up time (sBUT)

To register 'self-reported tear film break-up time', participants were instructed to hold their eyes open as long as possible and to keep them fixed on a mark on the opposite wall. The time elapsed until the first closure of the subject's eyelid was measured by means of a stop watch (Johanson et al. 1995; Nihlen et al. 1998; Norback and Wieslander 2002).

Rhinomanometry

Anterior active rhinomanometry (AAR) was used to determine nasal resistance and flow rates in accordance with the criteria of the International Committee on Standardization of Rhinomanometry (ICSR) (Carney et al. 2000). For specific description of measurement and evaluation of AAR, see Lang et al. (2008).

Subjective symptoms (SPES questionnaire)

For detection of subjective symptoms and complaints, use was made of the German version (Seeber et al. 2002) of the validated SPES questionnaire (Gamberale 1989; Iregren et al. 1996). Data were gathered twice a day, namely prior to exposure and shortly before the end of exposure. The SPES questionnaire contained 31 questions about symptoms or states of irritation concerning different organ systems (e.g. nose and eyes) as listed in Online Resource 1. Ratings of symptoms' strength or severity were also documented using VAS from 0 to 100 mm (endpoints: 'not at all'—'very strong'; no subdivisions).

For statistical evaluation, the 31 individual symptoms were combined to form a 'sum score' and, additionally, organ-related subscores of symptoms concerning eye or nose irritation and olfactory symptoms were calculated (Ihrig et al. 2006).

Positive And Negative Affect Schedule (PANAS)

The PANAS questionnaire served to determine subjects' positive and negative personality patterns and traits (Watson et al. 1988; Krohne et al. 1996). As a self-description tool, PANAS contains 20 adjectives, ten of which define rather positive and another ten rather negative emotions and feelings (Online Resource 2). The PANAS questionnaire had to be answered by each participant once before start of exposure. For self-description, subjects had to choose among five different grades of each personality feature (very slightly/not at all, a little, moderately, quite a bit, extremely). Questionnaires were evaluated only at the end of the study, and both positive and negative affectivity determined for each subject.

Statistics

SAS version WIN 9.1 was used for statistical calculations. Categorical data were summarised by means of absolute and relative frequencies (counts and percentage). Quantitative data were summarised using the following summary statistics: number of observations, arithmetic mean, standard deviation (SD), minimum, median and maximum. To clearly demonstrate potential differences between effects, scaling was adapted and values outside the selected scale were omitted (marked by open squares).

Possible differences between the five exposure conditions were verified by covariance analysis methods (ANCOVA), and potential divergences between categorical variables calculated based on the chi-square test.

Differences in measurements before and after exposure were determined by one-sample *t*-test. The Spearman rank correlation coefficient and the corresponding *p*-values were calculated to demonstrate possible relations between the continuous variables or scores. The level of significance was set to 5%; alpha-adjustment was not done.

Results

Formaldehyde exposure

Table 1 contains mean FA concentrations and standard deviations. The concentrations determined by real-time monitoring and HPLC analysis, respectively, were in good agreement with each other and in line with target concentrations. A background level of 0.01 ppm FA was detected (Table 1).

Positive And Negative Affect Schedule (PANAS)

Subjects rated positive affectivity (mean ± SD, 3.3 ± 0.6; median, 3.5; range, 1.8–4.5) with higher scores than negative affectivity (mean ± SD, 1.5 ± 0.4; median, 1.4; range, 1.0–2.9).

To identify potential effects of individual personality patterns on our findings, supplementary statistical analyses of subjective symptoms (SPES) were undertaken considering negative affectivity as covariate. Since data evaluation revealed no significant influence of negative affectivity as covariate, the following results are based on statistical analyses irrespective of personality traits.

CO_2 sensitivity

According to the CO_2 sensitivity measurements, 20 hyposensitive and 21 hypersensitive subjects were divided by the median of 80.3 mm. On the 0- to 300-mm scale, participants' 'CO_2 sum score' ('CO_2 sensitivity score') ranged from 0 to 228 mm (mean ± SD, 82.9 ± 56.1 mm). Subjective CO_2 sensitivity was reproducible with a significant association between the first and the last measurement ($r = 0.39$, $p < 0.05$). No relationship was recognised between PANAS and subjective CO_2 sensitivity.

Conjunctival redness, eye-blinking frequency (EBF) and tear film break-up time (sBUT)

Conjunctival redness decreased or was constant in most participants. No concentration–effect relationship was found (Online Resource 3, displayed are percentage changes of conjunctival redness after exposure). After exposure to 0 ppm (concentration A), hyposensitive persons showed statistically significantly increased conjunctival redness (Online Resource 4), whereas hypersensitive volunteers demonstrated statistically significantly decreased conjunctival redness (Online Resource 5). However, this difference in control condition is to be regarded as an incidental observation and cannot be ascribed to formaldehyde.

FA did not cause relevant changes in participants' EBF as demonstrated in Fig. 1. Results are presented as box–whisker plots showing mean differences (bullet/plus) between post- and pre-exposure measurements as well as upper and lower quartiles (25th and 75th percentile) and range. There was no consistent statistically significant change in EBF after exposure to the different FA concentrations (Fig. 1). In general, we observed a decrease in mean EBF after exposure. This decrease was statistically significant only for hypersensitives exposed to 0 ppm (concentration A). Neither statistically significant differences between hypo- and hypersensitives nor a concentration–effect relationship was found.

sBUTs were partly statistically significantly prolonged in hypo- and hypersensitive volunteers after exposure to different FA concentrations (Fig. 2). In addition, shortened mean sBUTs were recognised in hypersensitives compared to their hyposensitive counterparts at 0 ppm FA (concentration A), and in hyposensitive subjects after exposure to 0.3 ppm/0.6 ppm FA and to 0.7 ppm FA (concentrations B and E) when compared to the control condition. No clear concentration–effect relationship was noticed (Fig. 2).

As a main result of these examinations, it is concluded that no consistent FA-induced irritant effects on subjects' eyes could be observed.

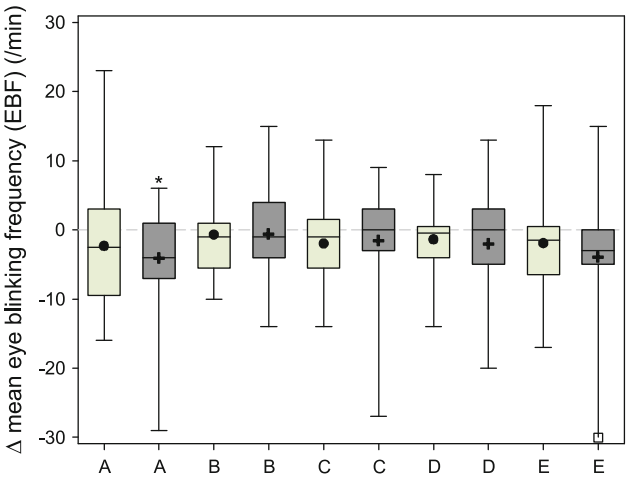

Fig. 1 Results of eye-blinking frequency (/min). Displayed are mean differences of EBF in hyposensitives (*bullet*)/hypersensitives (*plus*) at the end of exposure compared to pre-exposure; FA concentration: *A*: 0 ppm; *B*: 0.3 ppm/0.6 ppm peaks; *C*: 0.4 ppm/0.8 ppm peaks; *D*: 0.5 ppm; *E*: 0.7 ppm; $^{*}p < 0.05$ compared to pre-exposure. For additional data see Online Resource 6

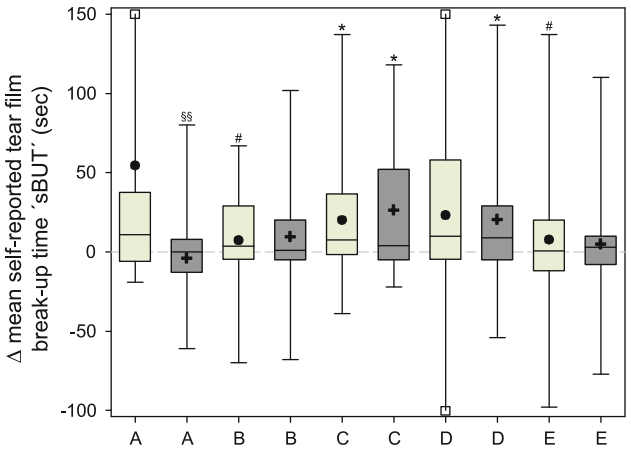

Fig. 2 Results of self-reported tear film break-up time (s). Displayed are mean differences of sBUT in hyposensitives (*bullet*)/hypersensitives (*plus*) after exposure compared to pre-exposure; FA concentration: *A*: 0 ppm; *B*: 0.3 ppm/0.6 ppm peaks; *C*: 0.4 ppm/0.8 ppm peaks; *D*: 0.5 ppm; *E*: 0.7 ppm; omitted outlier values (*open square*) are contained in Online Resource 7; $^{*}p < 0.05$ compared to pre-exposure; $^{\#}p < 0.05$ compared to control condition (0 ppm); $^{\S\S}p < 0.01$ compared to hyposensitives

Nasal flow

Mean nasal flow rates did not reveal uniform changes (Fig. 3). For example, hypersensitives exposed to 0.7 ppm FA (concentration E) had a statistically significantly higher mean nasal flow compared to pre-exposure values, while a statistically significantly higher mean nasal flow in hyposensitive persons was observed after exposure to 0.3 ppm/0.6 ppm peaks FA (concentration B) compared to 0 ppm (concentration A). Because the two sensitivity groups developed both an increased and decreased mean nasal flow and a consistent difference compared to the control condition was missing, no obvious FA-specific effect was detected.

Results of subjective symptoms (SPES questionnaire)

Since volunteers' subjective symptoms could not be ascribed to FA-specific effects, only results for the SPES 'sum score' and those SPES subscores and items of relevance to the objective parameters 'eyes', 'upper respiratory

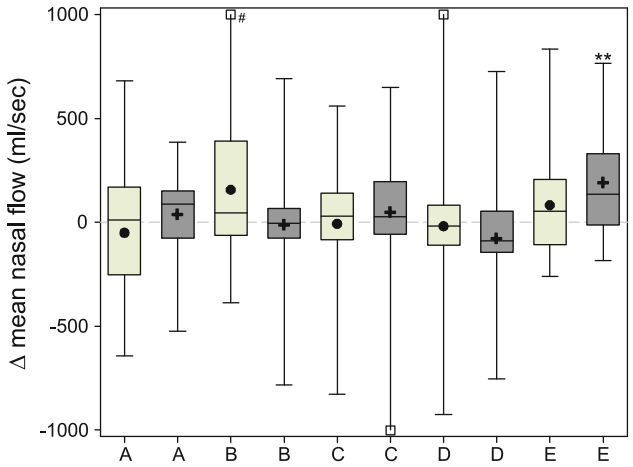

Fig. 3 Results of nasal flow measurement (millilitres/second). Displayed are mean differences of nasal flow rates in hyposensitives (*bullet*)/hypersensitives (plus) after exposure compared to pre-exposure; FA concentration: *A*: 0 ppm; *B*: 0.3 ppm/0.6 ppm peaks; *C*: 0.4 ppm/0.8 ppm peaks; *D*: 0.5 ppm; *E*: 0.7 ppm; omitted outlier values (*open square*) are contained in Online Resource 8; $^{**}p < 0.01$ compared to pre-exposure; $^{\#}p < 0.05$ compared to control condition (0 ppm)

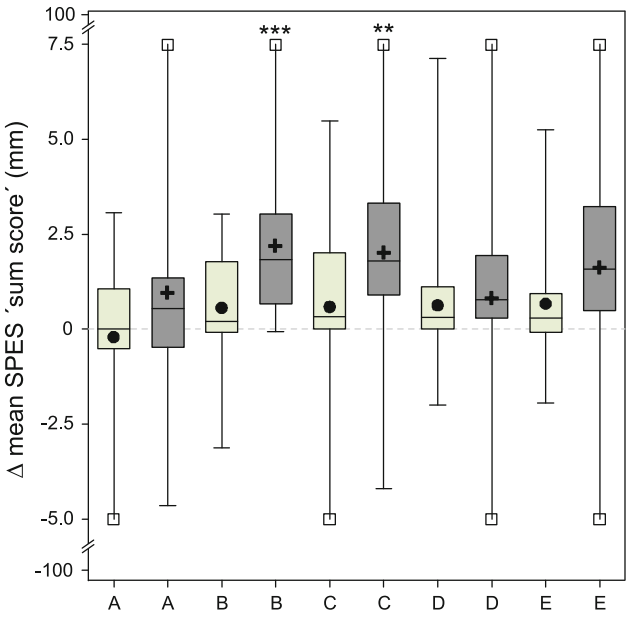

Fig. 4 Results of SPES 'sum score' (millimetres). Displayed are mean differences of SPES 'sum score' in hyposensitives (*bullet*)/hypersensitives (*plus*) after exposure compared to pre-exposure; FA concentration: *A*: 0 ppm; *B*: 0.3 ppm/0.6 ppm peaks; *C*: 0.4 ppm/0.8 ppm peaks; *D*: 0.5 ppm; *E*: 0.7 ppm; omitted outlier values (*open square*) are contained in Online Resource 9; $^{**}p < 0.01$; $^{***}p < 0.001$ compared to pre-exposure

tract' and, in addition, the subscore 'olfactory function' are represented.

SPES 'sum score'

The mean SPES 'sum score' (Fig. 4) was found to be increased after exposure in all participants, except for hyposensitives at 0 ppm (concentration A). This increase in subjective symptoms was statistically significant for hypersensitive volunteers after exposure both to 0.3 ppm/0.6 ppm peaks FA (concentration B) and to 0.4 ppm/0.8 ppm peaks FA (concentration C), as compared to pre-exposure values. Additionally, a comparison between hypo- and hypersensitives under the same exposure condition consistently yielded numerically higher mean post-exposure symptom or complaint levels for hypersensitives than for their hyposensitive counterparts, even when subjects were exposed to 0 ppm FA.

SPES 'eye irritation'

Subscore 'eye irritation' subsumed the items 'tiring eyes', 'itchy eyes', 'burning eyes', 'irritation of the eyes', 'dry eyes', 'watery eyes' and 'redness of the eyes' (Online Resource 1).

Mean 'eye irritation scores' basically increased numerically after exposure. Only hyposensitive subjects exposed to both 0.5 ppm FA (concentration D) and 0 ppm FA (concentration A) reported a marginal decline in mean eye irritation symptoms (Online Resource 10). However, we neither found statistically significant changes nor a concentration–effect relationship. It is to be noticed that mean differences between reported post- and pre-exposure symptom scores in subjective eye complaints were found within only a small range (−0.2 to 2.1 mm on a 100-mm VAS) (Online Resource 10).

SPES 'nasal irritation'

Subscore 'nasal irritation' included the items 'irritation of the nose', 'itchy nose', 'dry nose', 'running nose' and 'burning nose' (Online Resource 1).

Numerically increased and reduced mean 'nasal irritation' ratings were observed in both hypo- and hypersensitive volunteers (mean differences between post- and pre-exposure symptom scores, −1.13 to 0.73 mm; VAS, 100 mm). However, a clear concentration–effect relationship was missing; neither did we find any statistically significant differences between hypo- and hypersensitives at the FA concentrations tested, including control condition (Online Resource 11).

SPES 'olfactory symptoms'

The following SPES items were combined to form the subscore 'olfactory symptoms': 'perception of impure air',

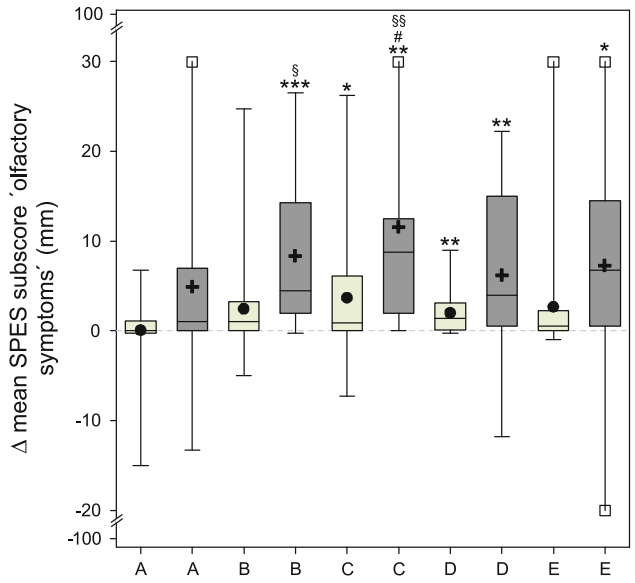

Fig. 5 Results of SPES subscore 'olfactory symptoms' (millimetres). Displayed are mean differences of 'olfactory symptoms' in hyposensitives (*bullet*)/hypersensitives (*plus*) after exposure compared to pre-exposure; FA concentration: *A*: 0 ppm; *B*: 0.3 ppm/0.6 ppm peaks; *C*: 0.4 ppm/0.8 ppm peaks; *D*: 0.5 ppm; *E*: 0.7 ppm; omitted outlier values (*open square*) are contained in Online Resource 12; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared to pre-exposure; $^{\#}p < 0.05$ compared to control condition (0 ppm); $^{\S}p < 0.05$; $^{\S\S}p < 0.01$ compared to hyposensitives

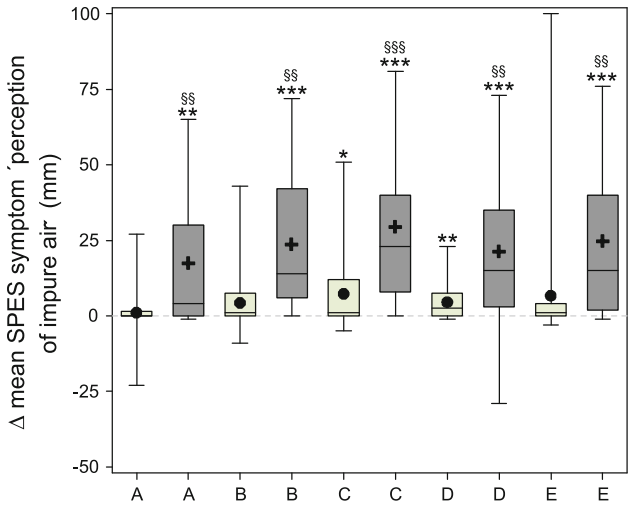

Fig. 6 Results of SPES item 'perception of impure air' (millimetres). Displayed are mean differences of 'perception of impure air' in hyposensitives (*bullet*)/hypersensitives (*plus*) after exposure compared to pre-exposure; FA concentration: *A*: 0 ppm; *B*: 0.3 ppm/0.6 ppm peaks; *C*: 0.4 ppm/0.8 ppm peaks; *D*: 0.5 ppm; *E*: 0.7 ppm; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared to pre-exposure; $^{\S\S}p < 0.01$; $^{\S\S\S}p < 0.001$ compared to hyposensitives. For additional data see Online Resource 13

'unpleasant smell', 'foul odour' and 'stench' (Online Resource 1).

We detected a general rise in mean olfactory complaints at all concentrations (A–E) compared with pre-exposure examination (Fig. 5). This increase was found to be statistically significant in hypersensitives at concentrations ≥0.3 ppm/0.6 ppm peaks FA, and in hyposensitives exposed to both 0.4 ppm/0.8 ppm peaks FA (concentration C) and 0.5 ppm FA (concentration D).

Increased mean olfactory symptoms after exposure to FA (concentrations B–E) were noticed in comparison with the control condition (concentration A) and reached statistical significance in hypersensitive volunteers exposed to 0.4 ppm/0.8 ppm peaks FA (concentration C). However, no concentration–effect relationship was found (Fig. 5).

In addition, hypersensitive participants reported higher mean olfactory complaints than their hyposensitive counterparts. This difference between the two sensitivity groups was statistically significant after exposure to both 0.3 ppm/0.6 ppm peaks FA (concentration B) and to 0.4 ppm/0.8 ppm peaks FA (concentration C) (Fig. 5).

SPES item 'perception of impure air'

A detailed analysis of items forming the subscore 'olfactory symptoms' showed that the parameter 'perception of impure air' (Fig. 6) was the only subjective symptom for which participants documented consistently higher post-exposure values at all exposure conditions (concentrations A–E). These increases were statistically significant for hypersensitive subjects at all five conditions, even after exposure to 0 ppm FA. Hyposensitive persons indicated more symptoms after exposure to 0.3 ppm/0.6 ppm peaks FA (concentration C) and to 0.5 ppm FA (concentration D). Beyond this, hypersensitives consistently scored statistically significantly higher ratings than hyposensitives at all FA concentrations including control condition. Although we observed increasing symptom scores with higher FA levels, there was no significant difference in reported complaints in comparison with those after exposure to 0 ppm (concentration A), as shown in Fig. 6.

Discussion

To our knowledge, this is the first study that examined FA-induced effects on healthy males with different 'sensitivity' to chemical irritation. Depending on their perception ('pain') of nasally applied CO_2, participants were divided into 'hyposensitives' and 'hypersensitives'. CO_2 is known to irritate the mucous membranes (Shusterman and Balmes 1997a, b; Hummel and Livermore 2002) and to cause concentration-dependent stinging and painful sensations (Garcia Medina and Cain 1982; Hummel et al. 1996; Iannilli et al. 2008), which show only a low intra-individual

variability (Anton et al. 1992). These effects are usually mediated via the trigeminal nerve, which is distinct from activation of specialised chemical senses like olfaction or taste (Alarie 1973; Cometto-Muniz and Noriega 1985; Doty et al. 2004).

CO_2 is odourless and stimulates the trigeminal nerve. It has therefore been used as 'nasal irritant' in former studies (Garcia Medina and Cain 1982; Stevens and Cain 1986). CO_2 is assumed to act by dissociation to hydrogen protons (H^+) and in consequence by changing nasal mucosal pH (Shusterman and Avila 2003). Since the common receptor TRPV1 (transient receptor potential vallinoid-1) is activated both by low extracellular pH and FA (Wolkoff and Nielsen 2010), we concluded that CO_2 seems to be suited for examining individual sensitivity to chemical irritants like FA.

Previous studies on FA-induced sensory irritation in humans mainly used subjective methods (questionnaire) to examine eye or nasal irritation (see reviews by Paustenbach et al. 1997; Arts et al. 2006a, b). In addition to these subjective methods, we tested objective parameters in the present study.

Conjunctival redness, as a sensitive marker for irritation, did neither change significantly with FA exposure of up to 0.8 ppm (peak). This result is in line with recent findings of our working group, which revealed an increase in eye redness only at peak FA concentrations as high as 1.0 ppm superimposed on 0.5 ppm FA (Lang et al. 2008).

Eye-blinking frequency, as a more sensitive objective indicator of exposure to irritating concentrations, also often serves to document chemosensory irritant effects on the eyes (e.g. Weber-Tschopp et al. 1977; Emmen et al. 2003; Kiesswetter et al. 2005; Nojgaard et al. 2005; Kleinbeck et al. 2008; van Thriel et al. 2010). Baseline eye-blinking frequency ranges between approximately two and 20 blinks/min (Monster et al. 1978; Wolkoff et al. 2003, 2005). However, higher eye-blinking frequencies of up to 80 blinks/min in healthy persons have been reported (Monster et al. 1978; Nakamori et al. 1997; Lang et al. 2008; Ziegler et al. 2008). To avoid confounders due to high basal eye-blinking frequency, we excluded persons with more than 20 blinks/min. After exposure, a general reduction in eye-blinking frequency was observed in hypo- and hypersensitive subjects, but no increase, as would have been expected from irritation caused by FA. This finding is in line with recent studies performed by Weber-Tschopp et al. (1977) and our working group (Lang et al. 2008), which showed an increase in EBF only at FA concentrations as high as 1.7 and 0.5 ppm with 1.0 ppm peak FA, respectively.

Self-reported tear film break-up time, another common parameter to validate eye irritation (e.g. Nihlen et al. 1998; Wieslander et al. 1999; Norback and Wieslander 2002), is reported to be well correlated with the fluorescein method for detection of tear film break-up time (Wyon and Wyon 1987). Increased eye irritation causes earlier eyelid closure and, hence, shorter sBUT (Wilson et al. 1975; Basu et al. 1978; Wolkoff et al. 2003; Baudouin et al. 2010). As sBUTs were mainly prolonged in our study population after FA exposure, and since FA-caused differences of sBUT between hypo- and hypersensitive subjects did not occur, we could not identify a relevant FA-specific effect on sBUT.

There were no effects on nasal flow rates in correlation with increasing FA concentrations. 'Nasal stuffiness' is a common complaint of individuals exposed to irritating chemicals (Doty et al. 2004), and an increased nasal resistance and/or a reduced nasal flow rate is therefore to be expected as a response to FA. In contrast to this, we found a statistically significant increase rather than a decline in nasal flow rates in hypersensitive volunteers after exposure to 0.7 ppm FA. From this, we conclude that FA concentrations of up to 0.7 ppm (constant exposure) and to 0.8 ppm (peak exposure), respectively, do not cause FA-specific changes in nasal flow rates, which is in agreement with the findings of Kulle et al. (1987) who reported an increase in nasal resistance only after exposure to concentrations of 3.0 ppm FA but not to a level of 1.0 or 2.0 ppm FA. Evaluation of nasal resistance was also performed by Andersen and Mølhave (1983). In their study, subjects were exposed to concentrations of 0.24, 0.41, 0.81 and 1.62 ppm FA, no statistically significant FA-induced changes in nasal resistance were found.

The SPES sum score did not correlate with FA concentrations; therefore, no concentration–effect relationship could be demonstrated. For symptom subscores 'eye irritation' and 'nasal irritation', no statistically significant pre- and end-of-exposure differences were found. These results for subjective symptoms of eye and nose irritation were in agreement with our objective examinations of conjunctival redness, EBF, sBUT and nasal flow rates, which did not show consistent significant differences or a concentration–effect relationship, either. In contrast, olfactory symptoms increased statistically significantly upon FA exposure. In addition, symptom levels of hypersensitive persons turned out to be higher than those of hyposensitive subjects, reaching statistical significance at exposure concentrations of 0.3 ppm/0.6 ppm peaks FA and of 0.4 ppm/0.8 ppm peaks FA (concentrations B and C). This outcome is attributable to the post-exposure rise in the SPES item 'perception of impure air'. However, this increase cannot be ascribed to FA only, since a statistically significant difference in symptom scores between FA exposures and control condition was missing, and, in addition, hypersensitive subjects reported statistically significantly higher complaints even after exposure to 0 ppm. Therefore, we

concluded that the increase in olfactory symptoms is induced mainly by a displeasing ambient smell. It suggests an impairment of well-being caused by situational and climatical conditions in the exposure chamber.

In summary, none of the subjective or objective parameters indicative of irritations were affected by the FA exposure concentrations tested.

Our result is in accordance with the outcome of those studies that also examined FA-induced irritations on eyes and nose with objective methods (e.g. Weber-Tschopp et al. 1977; Kulle et al. 1987; Yang et al. 2001; Lang et al. 2008). Regarding subjective methods, our findings are in agreement with most published studies, too, as reviewed by Paustenbach et al. (1997) and Arts et al. (2006a). Paustenbach et al. (1997) concluded that 'for most persons eye irritation clearly due to FA does not occur until 1.0 ppm' and 'that moderate to severe eye, nose and throat irritation does not occur for most persons until airborne concentrations exceed 2.0–3.0 ppm'. In addition, Arts et al. (2006a) stated that minimal eye and nasal irritation is only found at FA levels of $\geq$1.0 ppm and $\geq$2.0 ppm, respectively.

In a previously published study performed by Lang et al. (2008), we found a significant increase in eye-blinking frequency, conjunctival redness and subjective nasal irritation only at a concentration of 0.5 ppm with 1.0 ppm peak FA, considering this concentration as LOEL (Lang et al. 2008). In addition, an increase in the subjective symptoms of eye irritation and olfactory function at concentrations upward of 0.3 ppm FA was observed (Lang et al. 2008). This divergent outcome of both studies may be explained by several methodical differences in study set-up, conditions and volunteers, which will be discussed in the following.

The mean PANAS score for negative affectivity, obtained in the previous study, was significantly higher than in our present trial (1.9 ± 0.6 vs. 1.5 ± 0.4, $p < 0.02$). Since it is obvious that personality factors like expectations, anxiety or attitudes towards health risks strongly influence the perception of sensory irritation and may lead subjects to report symptoms at an increased rate (Seeber et al. 2000; Dalton 2003; Ihrig et al. 2006), in the previous work the personality factor (negative affectivity) was introduced as a statistical covariate for subjective symptom evaluation (Lang et al. 2008). In doing so, we arrived at the conclusion that only a concentration of 0.5 ppm with 1.0 ppm peak FA is to be considered as LOEL for objective effects not influenced by personality traits (Lang et al. 2008). Furthermore, we defined concentrations of 0.5 ppm FA without peaks and of 0.3 ppm with 0.6 ppm peaks FA, respectively, as NOAEL (Lang et al. 2008). In addition to the above-mentioned inter-study differences in personality traits, the two studies differed in terms of the maximum FA concentration (0.7 vs. 0.5 ppm FA as continuous concentration; 0.8 vs. 1.0 ppm FA peak) as well as time-points and number of examinations (2 vs. 5). Furthermore, the present study used VAS that are assumed to be more sensitive (Joyce et al. 1975) than the previously applied numerical rating scales (NRS) and are known to generate lower values than NRS (Price et al. 1994; Breivik et al. 2000; Holdgate et al. 2003).

Taking into account these inter-study differences, we conclude that the findings of our both studies do not contradict each other.

Conclusions

Formaldehyde exposures to 0.7 ppm for 4 h and to 0.4 ppm for 4 h with peaks of 0.8 ppm for 15 min, respectively, are not associated with chemosensory effects on hypo- and hypersensitive males. Therefore, a NOAEL of 0.7 ppm as constant exposure and of 0.4 ppm with peaks of 0.8 ppm can be derived from this study. These results should be taken into consideration whenever occupational exposure limits (OELs) for chemosensory irritation caused by formaldehyde vapours are set.

The results of the previous study performed by our working group (Lang et al. 2008) were discussed by SCOEL when it derived an OEL (occupational exposure limit) for FA (SCOEL 2008). The committee concluded that 'the TWA-OEL of formaldehyde should be set at or below the NOAEL for sensory irritancy of the eye'. SCOEL further argued that 'in view of the limited number of persons that can be examined in a laboratory study (21 persons in the study by Lang et al. 2008), the exclusion of particularly sensitive persons with negative affectivity appears to be problematic'. It therefore proposed a formaldehyde 8-h TWA of 0.2 ppm, assuming this limit 'especially considers possible interindividual differences in susceptibility to irritation by formaldehyde, which may be expected based on the entire body of data'. The committee also concluded that 'short-term irritation may be prevented by a 15-min STEL of 0.4 ppm', presuming 'this STEL is set below the threshold for objective eye irritation, as outlined by Lang et al. (2008)' (SCOEL 2008).

We disagree with SCOEL's interpretation of results obtained in our previous study. The statement of SCOEL that 'particularly sensitive persons with negative affectivity' were excluded is not correct. As a matter of fact, in our previous study, neither persons especially sensitive to chemicals nor those with negative affectivity were excluded. Lang et al. (2008) used the negative affectivity measured for *all* volunteers as a covariate to examine whether personal traits influence the outcome of subjective symptoms and complaints reported. This does not imply that subjects were excluded from analysis or even from the study.

In general, the PANAS questionnaire is used to characterise subjects' affective-state dimensions of mood. Watson et al., who developed the PANAS questionnaire, define 'positive affectivity' (PA) as 'the extent to which a person feels enthusiastic, active and alert', whereas 'negative affectivity' (NA) describes a 'general dimension of subjective distress and unpleasurable engagement that subsumes a variety of aversive mood states, including anger, contempt, disgust, guilt, fear and nervousness' (Watson et al. 1988; Watson 1988). Clearly, such personality traits may strongly influence subjective reporting on sensory symptoms. However, it is not correct to equal positive or negative personality traits with sensitivity towards irritant chemicals. Since no distinction was made between hyper- and hyposensitive subjects in our previous study, SCOEL's interpretation, besides other factors, caused us to conduct the present trial in volunteers stratified according to their chemosensitivity.

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Conflict of interest The authors declare that they have no conflict of interest.

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