Available online at www.sciencedirect.com
ScienceDirect
Regulatory Toxicology and Pharmacology 50 (2008) 23–36
Regulatory Toxicology and Pharmacology
www.elsevier.com/locate/yrtph

Formaldehyde and chemosensory irritation in humans: A controlled human exposure study

Isabelle Lang, Thomas Bruckner, Gerhard Triebig *

Institute and Out-patient Clinic of Occupational and Social Medicine, University of Heidelberg, Vossstrasse 2, 69115 Heidelberg, Germany

Received 30 March 2007
Available online 5 September 2007

Abstract

Objectives: The objective of this study was to examine the possible occurrence of sensory irritation and subjective symptoms in human volunteers exposed to formaldehyde concentrations relevant to the workplace. The set up of the study included formaldehyde exposures with and without peaks, the presence and absence of a masking agent, and evaluation of the influence of personality factors.

Methods: Testing was conducted in 21 healthy volunteers (11 males and 10 females) over a 10-week period using a repeated measures design. Each subject was exposed for 4 h to each of the 10 exposure conditions on 10 consecutive working days. The 2-week exposure sequences were randomized, and the exposure to formaldehyde and the effect measurements were conducted in a double-blind fashion. During 4 of the 10 exposure sessions, 12–16 ppm ethyl acetate (EA) was used as a 'masking agent' for formaldehyde exposure. Measurements consisted of conjunctival redness, blinking frequency, nasal flow and resistance, pulmonary function, and reaction times. Also subjective ratings of discomfort as well as the influence of personality factors on the subjective scoring were examined. These were carried out pre-, during and/or post-exposure, and were used to evaluate the possible irritating effects of formaldehyde at these concentrations.

Results: The results indicated no significant treatment effects on nasal flow and resistance, pulmonary function, and reaction times. Blinking frequency and conjunctival redness, ranging from slight to moderate, were significantly increased by short-term peak exposures of 1.0 ppm that occurred at a baseline exposure of 0.5 ppm formaldehyde. Results of the subjective ratings indicated eye and olfactory symptoms at concentrations as low as 0.3 ppm. Nasal irritation was reported at concentration levels of 0.5 ppm plus peaks of 1.0 ppm as well as at levels of 0.3 and 0.5 ppm with co-exposure to EA. However, exposure to EA only was also perceived as irritating. In addition, volunteers who rated their personality as 'anxious' tended to report complaints at a higher intensity. When 'negative affectivity' was used as covariate, the level of 0.3 ppm was no longer an effect level but 0.5 ppm with peaks of 1.0 ppm was. Increased symptom scores were reversed 16 h after the end of the exposures.

Conclusions: The results of the present study indicated eye irritation as the most sensitive parameter. Minimal objective eye irritation was observed at a level of 0.5 ppm with peaks of 1 ppm. The subjective complaints of ocular and nasal irritation noted at lower levels were not paralleled by objective measurements of eye and nasal irritation and were strongly influenced by personality factors and smell. It was concluded that the no-observed-effect level for subjective and objective eye irritation due to formaldehyde exposure was 0.5 ppm in case of a constant exposure level and 0.3 ppm with peaks of 0.6 ppm in case of short-term peak exposures.

Keywords: Formaldehyde; Sensory irritation; Blinking frequency; Subjective ratings

1. Introduction

At ambient temperature, formaldehyde (CAS reg. no. 50-00-0; 1 ppm = 1.2 mg/m^3) is a flammable, colourless, reactive, and readily polymerized gas. Formaldehyde is irritating to the eyes and respiratory tract already at low concentrations, which is caused by a chemosensory effect, i.e. interaction with local nerve endings (nervus trigeminus) which is called trigeminal stimulation or sensory irritation (see reviews by Paustenbach et al., 1997; Arts et al., 2006a). Sensory irritation leads to reflex responses such as sneez-

* Corresponding author. Fax: +49 6221 562991.
E-mail address: GTriebig@med.uni-heidelberg.de (G. Triebig).

0273-2300/$ - see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.yrtph.2007.08.012

ing, lacrimation, rhinorrhea, coughing, vasodilatation and changes in the rate and depth of respiration resulting in a decrease in the total amount of inhaled material thus protecting the individual. In rodents, it leads to a reduction in breathing frequency (Alarie, 1973; Nielsen et al., 1999). The onset of the response is usually observed within a few seconds and when the respiration rate is low enough it will be characterized by a pause during the expiratory phase of respiration (Alarie, 1973). The sensory irritation potential of formaldehyde resulted in RD50 values of 3–5 ppm in various strains of mice, and 14 and 32 ppm in two strains of rats (summarized in Bos et al., 1992 and Schaper, 1993). It should be borne in mind that trigeminus stimulation will not necessarily be accompanied by cell or tissue damage. In contrast, respiratory tract irritation occurring at high concentrations is a localized pathophysiological response to a chemical involving local redness, swelling, pruritis or pain; these effects are comparable to those induced at pathophysiological skin or eye irritation (Arts et al., 2006b).

In humans, for various odorous irritating chemicals, substantial differences exist in the lowest concentration found to be irritating to eyes, nose or throat. These observations can generally not be explained by differences in sensitivity of the method, inherent variability in biological response, or fluctuations in the exposure concentrations. These chemicals usually have a strong odour, and at least part of the wide variation in the findings may be ascribed to insufficient distinction between olfactory and trigeminal stimulation in several of the studies (Arts et al., 2006b). Formaldehyde has a pungent, suffocating odour (NIOSH, 1997). Its odour is detected and/or recognized by most human beings at concentrations below 1 ppm (Arts et al., 2006a). The odour threshold for formaldehyde has been reported to be generally between 0.04 and 0.4 ppm (van Gemert, 2003). As indicated by Dalton, subjective reports of irritation at low levels that cannot be reconciled with objective measures should prompt a careful investigation into other factors (e.g. cognitive or emotional) that may be modulating the sensory response. Distinguishing between the exposure that elicits local effects of sensory irritation in the upper respiratory tract and the exposure that elicits self-reports of irritation should be a key component in establishing safe levels for exposed workers (Dalton, 2001, 2002, 2003).

Olfactory and trigeminal stimulation can be distinguished (Cain and Cometto-Muniz, 1995; Cain et al., 1983; Dalton, 1996). Various experimental techniques are used in both humans and laboratory animals to study chemical-induced irritation consisting of examinations of functional changes, e.g. alterations in breathing frequency and pattern, nasal, bronchial and pulmonary function parameters, nasal mucosal swelling, acoustic rhinomanometry, eye blinking frequency, tear film stability, and chemosensory evoked potentials (Kjaergaard and Hodgson, 2001; Arts et al., 2002a, 2006b); in addition, in humans, subjective measurements such as symptom questionnaires are used.

In Germany, the current TLV value (MAK value) as a 8-h time weighed average is set at 0.3 ppm formaldehyde with peak category II (DFG, 2006); this corresponds to an excursion factor of 2 (0.6 ppm) for four 15-min periods per working shift; the current Occupational Exposure Limit (OEL) for formaldehyde is 0.5 ppm.

The objective of the present study was to establish the possible occurrence of sensory irritation and subjective symptoms in human volunteers exposed to formaldehyde concentrations relevant to occupational exposure, viz. up to 0.5 ppm with peak exposures up to 1 ppm. The set up of the study included formaldehyde exposures with and without peaks and the presence and absence of a masking agent. Objective measurements of irritation such as conjunctival redness, blinking frequency, nasal resistance and flow, pulmonary function, and reaction times were used. Also subjective ratings on discomfort as well as the influence of personality factors on the subjective scoring were examined. These examinations were carried out prior to, during and after exposure, and were used to evaluate the possible irritating effects of formaldehyde at these concentrations.

2. Materials and methods

2.1. Study design

Testing was conducted with 21 subjects (11 males and 10 females) using a balanced design. Subjects satisfying with all study selection criteria were exposed to 10 different conditions (Table 1). The test sessions for each subject were carried out Monday through Friday during two consecutive weeks. Each subject was exposed for 4 h to each of the 10 exposure conditions; the sequence of the exposure conditions is outlined in Table 2. Assignment to the treatment sequence was at a random order. The exposure to the test material and the effect measurements were conducted in a double-blind fashion, i.e. neither the subject nor the investigator/assistant was aware of the exposure condition. The study was approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg.

2.2. Characterization of the test material and masking agent

The test material used was paraformaldehyde, supplied by Merck, Darmstadt, Germany. Since formaldehyde has a pungent, suffocating odour (NIOSH, 1997), exposed subjects are not blinded with respect to a control condition (i.e. no formaldehyde present). Therefore, the masking agent EA[1] was used in 4 out of 10 exposure conditions (Tables 1 and 2). EA was chosen as it has a characteristic typical intensive odour and was considered a good candidate to mask the odour of formaldehyde. In humans sensory irritation by EA was not observed at concentrations below 400 ppm (Nelson et al., 1943; Seeber et al., 2002). EA was obtained from Roth, Karlsruhe, Germany. It was simultaneously generated (in 4 out of 10 exposure conditions) with formaldehyde at a maximum target level of 20 ppm.

2.3. Subjects

Twenty-six subjects volunteered as subjects for participation in the present study. They were recruited via on-line advertisement in the employment offices in Heidelberg and Mannheim (D) as well as via leaflets

[1] *Abbreviations used:* EA, ethyl acetate; PANAS, Positive and Negative Affect Schedule; SPES, Swedish Performance Evaluation System.

Table 1
Various exposure conditions to formaldehyde

Scenario	Formaldehyde continuous exposure (ppm)	Formaldehdye peaks (ppm)	EA (ppm)
1	0	—	—
2	0.15	—	—
3	0.3	—	—
4	0.3	4 × 0.6	—
5	0.5	—	—
6	0.5	4 × 1.0	—
7	0	—	12–16
8	0.3	—	12–16
9	0.5	—	12–16
10	0.5	4 × 1.0	12–16

All exposures lasted 4 h.

on bulletin boards throughout the University of Heidelberg. For inclusion in the study, each subject had to meet the following criteria: (a) adults up to 40 years in good health, (b) adequate German language skills, and (c) written informed consent and voluntary participation. The exclusion criteria were: (1) age below 18 and over 40 years, (2) smoking (status was verified by cotinine test), (3) severe allergy and/or manifest skin diseases, (4) acute infection, (5) consumption of more than 50 g alcohol per day, (6) drug abuse, (7) use of contact lenses, (8) exposure to formaldehyde at the workplace or in the community, (9) acute or chronic diseases of the upper airways, lungs, heart or skin, and (10) (potential) pregnancy. The principles of informed consent in the current revision of the Declaration of Helsinki (Hong Kong, 1989) were implemented in the study.

Prior to the admission into the study, a check of the subject's general health status was performed. The pre-study screening of the selected subjects involved: (1) medical history and physical examination, (2) questionnaire to investigate positive and negative affectivity—PANAS (Positive and Negative Affectivity Schedule; Watson et al., 1988), (3) a multiple-choice vocabulary test, a test to determine the subject's verbal intelligence independent of social experiences (Lehrl et al., 1995), (4) reaction time, (5) pulmonary function and spirometry, (6) rhinomanometry, and (7) urine collection (cotinine test to exclude smokers). Also, subjects were informed about the possible health effects of formaldehyde, such as irritation of the airways and/or eyes, and the unpleasant odour. They also received a standardized schedule of the study plan, detailed information on the tests they had to undergo, and a leaflet describing how to behave in the exposure chamber. After allowed adequate reflection, the subjects agreed to participate in the study by written consent that included that subjects could withdraw from the study without a reason. This agreement also included a statement from the investigator, guaranteeing patient confidentiality, and anonymous analysis of the collected data.

The screening of the subjects took place approximately 1 week prior to the beginning of the experimental phase of the study. Twenty-six subjects were selected according to the criteria given above but five left the study prematurely due to various reasons. Out of these five subjects three subjects considered the health risk too high after one or two exposure days (formaldehyde concentrations of 0 and/or 0.3 ppm). One subject left after two exposure days for personal reasons, the fifth subject was excluded from the study after the seventh exposure because of headache and fever which were considered to be the symptoms of a flu-like infection.

On the last exposure day, a comprehensive series of measurements were carried out in all subjects (see further). In addition, the subjects returned for follow-up examinations one, two, and three weeks after the last exposure to complete the Swedish Performance Evaluation System (SPES) questionnaire (Iregren et al., 1996).

2.4. Exposure chamber and generation of the test atmosphere

The exposure was carried out in an exposure chamber with a volume of approximately 30 m^3 (length 4.3 m; width 2.9 m; height 2.4 m). An antechamber prevented a sharp reduction in the exposure chamber concentration when the examiner or subjects entered or left the exposure chamber as daily exposure start was not the same for each subject. The subjects entered and left the exposure chamber with one hour time shift, hence the maximum number of volunteers in a 2-week session was six but maximally four subjects were in the chamber at the same time. Three ventilators were used to assure a homogenous distribution of the formaldehyde (and EA) test atmosphere(s) generated under quasi static conditions. The homogeneity of the test atmospheres within the chamber was demonstrated in technical trial runs before the start of exposure of the volunteers. The temperature and relative humidity in the chamber were controlled and recorded hourly with a thermohygrometer (model 93353, Bioblock Scientific, Doornik, Belgium). The temperature was kept at 22 ± 3 °C and the relative humidity at 50 ± 10%. The formaldehyde test atmosphere was generated by vaporizing paraformaldehyde on a magnetic hot plate stirrer at 200 °C. Under these conditions paraformaldehyde depolymerizes quantitatively to monomeric formaldehyde and water vapor (Walker, 1975). The vapor was introduced into the exposure chamber by convection via a metallic duct so allowing adjusting the concentrations. During the exposure conditions containing peak exposures, the peaks were generated by additional evaporation of formaldehyde until the desired peak concentration was reached, followed by forced ventilation of the exposure chamber using an exhaust air system, until the desired basic level was reached again. Real-time monitoring of the formaldehyde concentrations was carried out using an Asynco® Formaldehyde Monitor (Interscan, HCHO, model no. 4160-DSP, serial no. 821010, Karlsruhe, Germany). In order to verify the results of the real-time monitoring, two air samples taken each exposure day were analyzed using dinitrophenylhydrazine and HPLC analysis

Table 2
Randomization of the various exposure conditions during the 10-week study period

Group	Week	Day 1	Day 2	Day 3	Day 4	Day 5
1	1	0.5	0.3	0.15	0.3 + EA	0.5 + P + EA
	2	0	0.5 + EA	0 + EA	0.5 + P	0.3 + P
2	3	0.15	0	0.3 + P	0.5	0 + EA
	4	0.5 + P + EA	0.3	0.5 + P	0.5 + EA	0.3 + EA
3	5	0	0.3 + P	0.3	0.5 + P	0.5
	6	0.3 + EA	0.5 + P + EA	0 + EA	0.5 + EA	0.15
4	7	0.3	0 + EA	0.15	0.5 + P	0.5 + EA
	8	0.5 + P + EA	0.5	0.3 + P	0	0.3 + EA
5	9	0.15	0.3 + EA	0.3 + P	0 + EA	0.3
	10	0	0.5 + EA	0.5 + P + EA	0.5 + P	0.5

All exposures lasted 4 h; EA, ethyl acetate 12–16 ppm; P, peaks; number and concentrations of peaks are indicated in Table 1. Each subject was exposed during a complete 2-week period.

according to DFG (1996). EA was also vaporized on a magnetic hot plate stirrer at 200 °C and introduced into the exposure chamber via a metallic duct so allowing adjusting the concentrations. The concentrations of EA were measured by real-time monitoring using photo ionization (MSA AUER PPM Photo Ionization Detector; model FZ 9944 107, Berlin, Germany). The PID was calibrated at the start of the study; during the course of the study the measurements remained fairly constant, proving that no change in adjustment did occur. A maximum target concentration of 20 ppm was chosen to sufficiently mask the characteristic odour of formaldehyde. Both analysers were checked for cross-sensitivity for either formaldehyde or EA.

2.5. Order of daily examinations

Each exposure session for subjects was carried out as follows: (1) Preliminary examination before the start of exposure, consisting of a SPES questionnaire and rhinomanometry. In addition, on day 1 pulmonary function was examined. (2) Exposure during 4 h to formaldehyde (or clean air) with or without EA. (3) Test cycle, performed at the start of exposure, and at 120 and 195 min of exposure. The cycle consisted of cycle ergometry during 15 min at 80 watts, SPES questionnaire (2 min), digital slit lamp photography (1 min), and video recording of blinking frequency (6 min). (4) Post-exposure tests consisted of SPES questionnaire, rhinomanometry, and reaction time measurements; these were carried out immediately after exposure stop.

On the last exposure day, after exposure had ended, the following measurements were carried out: physical examination (10 min) and pulmonary function (15 min).

2.6. Study parameters

2.6.1. Conjunctival redness

Digital slit lamp photographs of the eyes of the subjects were made three times during each day's exposure to investigate dilatation of the conjunctival blood vessels and an indication of irritation of the mucosal membranes of the eyes. A focusing mode was used to obtain photographs under standardized conditions. A section of the bulbar conjunctiva limited medially by the lacrimal caruncle was photographed, with the upper and lower eyelids as upper and lower boundaries of the section, respectively. As these boundaries were consistently selected for each subject, identical sections of the conjunctival mucosa were photographed. A standardized ophthalmologic grading scale of the CCLRU (Cornea and Contact Lens Research Unit) was used to minimize the influence of a subjective assessment of the degree of redness of the conjunctival mucosa (Wolffsohn, 2004). The severity of the conjunctival redness was ranked as follows: 1 = very slight, 2 = slight, 3 = moderate, and 4 = severe. For the evaluation of the results, the photographs were projected onto a 20-in. computer screen in random order, and the redness of the eyes was independently judged by two scorers unaware of the exposure concentration(s).

2.6.2. Blinking frequency

A new semi-automatic method was developed for the quantitative analysis of the blinking frequency (Ziegler et al., 2007). It was based on the method described by Tsubota et al. (1996). Blinking frequency was measured three times during each day's exposure using a high resolution camrecorder (JVC, model JY-HD 10). Subjects were asked to sit in an upright and relaxed position, and their heads were fixed in a rigid foamed plastic support. During measurements, the subject watched a documentary film on a video screen. Subjects were told not to speak during recording. The total recording period was 6 min, the subjects not being informed when the camera was running. After recording, the data were transferred to a computer. Blinking sequences were edited using HD Capture Utility 1.0 and MPEG Edit Studio Pro 1.0 software. The final results were film portions of exactly 90 s during which no movements of the subjects were noted. The blinks in these 90-s film portions were counted manually and used for statistical analysis. Counting was done in a randomized and blind fashion. All film portions were counted again at a different order; this procedure did not reveal any deviations from the first counting.

2.6.3. Nasal resistance and flow

The resistance in the nasal passages was measured using active anterior rhinomanometry (AAT; Jaeger Rhinoscreen®, Höchberg, Germany. The measurements were carried out in strict compliance with the criteria of the Committee for the standardization of rhinomanometry (Clement, 1984; Carney et al., 2000). This form of rhinomanometry is a standardized no-load current measurement, i.e. in the case of nasal breathing the energy to overcome the nasal resistance is produced by the respiratory muscles of the subject. The active cooperation of the subject is required (Naumann, 1990). The subject deeply inhales and exhales four or five times while sitting in an upright position. The flow rate and differential pressure between the nasal vestibule and the posterior naris are measured separately for each side using plugs. The nasal flow and the specific transnasal resistance are then calculated separately for each side; the total resistance is calculated from the individual measurements of both sides (Malm et al., 2000). The difference between each day's post-exposure and pre-exposure measurement was used as an indication for the presence of an effect.

2.6.4. Pulmonary function

The pulmonary function of the subjects was examined using a body plethysmograph with an integrated spirometer (Jaeger Bodyscreen®, Höchberg, Germany. The following parameters were measured: airway resistance (Rtot), Peak Expiratory Flow (PEF), Forced Expiratory Volume in 1 s (FEV1), and Maximum Midexpiratory Flow (MMEF). PEF measures the maximum flow rate during a forced expiratory manoeuvre, FEV1 measures the volume of air expired in 1 s at maximum expiration and MMEF measures the slope of the line between 25% and 75% of FVC (forced vital capacity). The pulmonary function was assessed during the initial examination, and on the first and last exposure day, prior to and immediately after exposure, respectively. The difference between the post-exposure and pre-exposure measurement was used as an indication for the presence of an effect: a positive value in airway resistance or a negative value in FEV1 would be indicative of airway obstruction. Due to technical reasons, total airway resistance was measured in 15 out of the 21 subjects only.

2.6.5. Reaction times

The reaction times of the subjects to stimuli were measured before and after exposure on each exposure day using the Vienna Test System (Wiener Testsystem) (Schuhfried and Prieler, 2001). In the present study, visual and acoustic stimuli in different configurations were used. The subjects had to press a button when a single stimulus (short appearance of yellow light or a beep tone) or a combination of these two stimuli appeared. Motor (movement time) and reactive (decision time) components of the reaction time can be distinguished using this test system, i.e. the decision time covers the duration from the appearance of the stimulus to the start of the subject's motor reaction by leaving the finger from its fixation point. The movement (motor) time represents the duration of movement of the finger from its fixation point to the response button.

2.6.6. Subjective ratings

To record the subject's physical symptoms and mental state, the validated SPES questionnaire was used (Gamberale, 1989; Iregren et al., 1996). This questionnaire was translated into German by Seeber et al. (2002). The questionnaires were filled out each day before exposure, at three time points during exposure, and a few minutes after leaving the exposure chamber. Filling out the same questionnaire several times per day for several days has not resulted in response fatigue (Seeber, 2007). The total score of all symptoms (see Table 3), and the subscores for irritation of the eyes, nasal irritations, and olfactory symptoms were evaluated. The SPES questionnaire also included a second part where the subjects rated their current complaints and well-being on a scale from 1 to 7 (Table 4).

Table 3
Listing of the individual organ systems with corresponding symptoms used in the SPES questionnaire

Subscore	Symptoms
Eyes	Tiring eyes Itchy eyes Burning eyes Irritation of the eyes Dry eyes Watery eyes Redness of the eyes
Nasal	Irritation of the nose Itchy nose Dry nose Running nose Burning nose
Olfactory	Perception of impure air Unpleasant smell Foul odour Stench
Respiratory	Pressure on chest Urge to cough Oppression to breath
Taste	Bad taste Unpleasant taste Foul taste
Unclassified	Unclear vision Throat irritation Skin irritation
Unspecific	Tiredness Headache Dizziness Malaise
Sham	Palpitations Double vision

These symptoms were scored on 6 levels: 0 = not at all, 1 = slight, 2 = somewhat, 3 = quite, 4 = strong, and 5 = very strong.

Table 4
Listings of ratings of complaints in the SPES questionnaire

Tension level	*Relaxed* Calm, collected, harmonious, well-adjusted	*Tense* Nervous, quickly excited, irritated, restlessness
Level of tiredness	*Awake* Active, alert, ready for action	*Tired* Lack of energy, weakness, exhaustion, sleepy
Complaints	*No* Complete physical well-being, no physical problems	*Strong* Physical feeling of being unwell, notable malaise
Level of discomfort	*Not uncomfortable* No negative impact on subject	*Very uncomfortable* Clear negative impact on subject, difficult to tolerate

These complaints were scored on an analogue scale from 1 to 7.

2.6.7. Personality factors

The PANAS questionnaire was used to investigate the personality traits of the subjects before the first exposure. The PANAS is a 20-item questionnaire in which a subject rates his/her emotional affectivity. Two dimensions of emotion, positive affectivity (PA) and negative affectivity (NA) are recorded and evaluated (Watson et al., 1988). Feelings and emotions were rated as indicated in Table 5.

2.7. Statistics

All statistical analyses were performed using SAS version WIN 9.1. If appropriate, data were visualized by means of box-whisker plots (see Fig. 1). Concentrations–response relationships were analyzed by repeated measures two-way analysis of variance (ANOVA) followed by post-hoc contrast test. In case of inhomogeneity of variances, Kruskall–Wallis was used followed by Mann–Whitney U test. Categorical variables were evaluated using Fisher's exact test. Differences before and after exposure were tested using Wilcoxon signed rank test. Changes in conjunctival redness between the 0 ppm scenario and the exposure levels were tested using McNemar's test of symmetry. The Spearman rank correlation coefficient and corresponding p-values were calculated to demonstrate possible differences between blinking frequency and the subjective rating of ocular irritation. Gender differences were tested using the Wilcoxon–Mann–Whitney U test. For all statistical tests $p < 0.05$ was considered statistically significant.

3. Results

3.1. Demographics

The mean age of the study subjects was 26.3 ± 5.6 years and ranged from 19 to 39 years. The group consisted of students ($n = 13$), employed persons ($n = 4$), and unemployed persons ($n = 4$).

There were no gender-related statistically significant differences except for eye redness at formaldehyde concentration levels of 0.15 and 0.3 ppm, and the rating of ocular irritations in the SPES questionnaire immediately after exposure to 0.5 ppm formaldehyde with peaks and co-exposure to EA (data not shown). Because there was no general or consistent influence of gender, results of males and females were combined.

3.2. Exposure

Mean formaldehyde exposure concentrations were calculated by averaging the daily analytical mean values of each exposure condition over the five exposure groups. Results of both real-time monitoring and HPLC measure-

Table 5
List of feelings and emotions evaluated in the PANAS questionnaire

Positive affectivity	Negative affectivity
Interested	Distressed
Excited	Upset
Strong	Guilty
Enthusiastic	Scared
Proud	Hostile
Alert	Irritable
Inspired	Ashamed
Determined	Nervous
Attentive	Jittery
Active	Afraid

Feelings and emotions were rated as follows: 1 = very slightly or not at all, 2 = a little, 3 = moderately, 4 = quite a bit, and 5 = extremely.

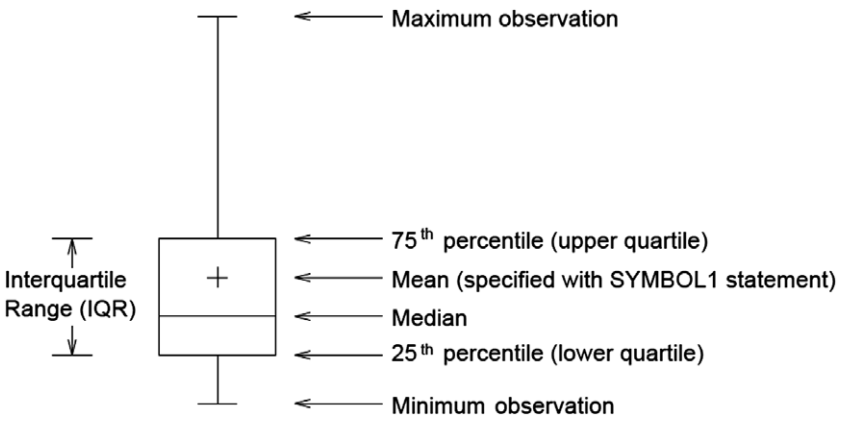

Fig. 1. Example of box-whisker plot.

ments were in good agreement (Table 6). On the days that the formaldehyde concentration should have been 0 ppm, in spite of intensive ventilation, nonetheless a minimal concentration of formaldehyde was detected that had lingered over from the previous exposure day. Mean EA (masking agent) concentrations were between 12 and 16 ppm, lower than the target concentration of 20 ppm (Table 6).

3.3. Study parameters

3.3.1. Conjunctival redness

In the present study, the degree of eye redness varied from slight (grade 2) to moderate (grade 3). As grade 2 indicates a normal to slight redness of the eyes only, grade 3 results are presented (Fig. 2). A statistically significant increase in moderate eye redness compared to the 0 ppm-control condition was observed in subjects exposed to 0.5 ppm formaldehyde with peaks of 1.0 ppm (without EA), 195 min after the start of exposure. Using the McNemar's symmetry test, it was shown that the time of day had no influence on the degree of redness, indicating that the degree of redness remained fairly constant throughout the course of a study day.

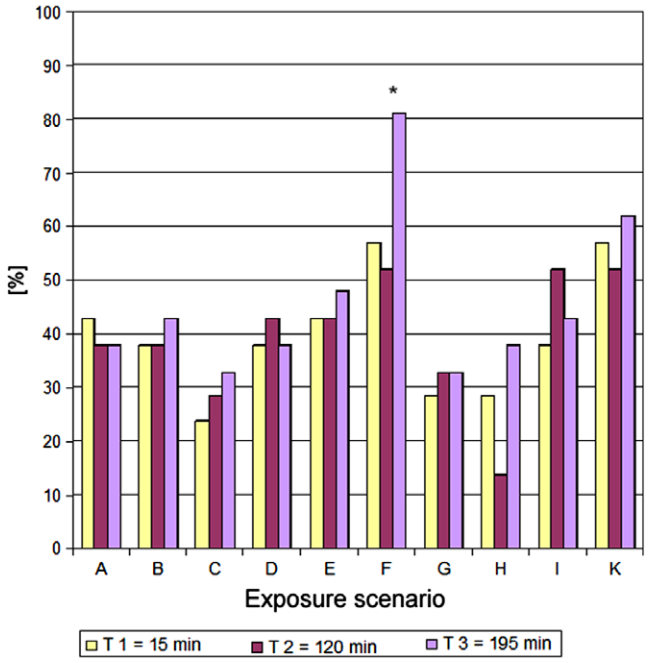

Fig. 2. Conjunctival redness in 21 subjects during exposure to different concentrations of formaldehyde with or without ethyl acetate (EA). Results are expressed as percentage of subjects showing moderate (grade 3) redness of the eyes. A = 0 ppm, B = 0.15 ppm, C = 0.3 ppm, D = 0.3 ppm + 4 peaks of 0.6 ppm, E = 0.5 ppm, F = 0.5 ppm + 4 peaks of 1.0 ppm, G = 0 ppm + EA, H = 0.3 ppm + EA, I = 0.5 ppm + EA, K = 0.5 ppm + 4 peaks of 1.0 ppm + EA. Statistics: McNemar's test of symmetry; $^{*}p < 0.05$.

Table 6
Measured formaldehyde and EA concentrations in the exposure chamber

Target formaldehyde concentration (ppm)	Formaldehyde concentration (real-time monitoring) (ppm)	Formaldehyde concentration (HPLC) (ppm)	EA concentration (real-time monitoring)
0	0.05 ± 0.05	0.02 ± 0.01	—
0.15	0.16 ± 0.00	0.14 ± 0.03	—
0.3	0.30 ± 0.01	0.28 ± 0.04	—
0.3 + 4 peaks at 0.6	0.30 ± 0.00	0.26 ± 0.04	—
0.5	0.50 ± 0.01	0.48 ± 0.03	—
0.5 + 4 peaks at 1.0	0.47 ± 0.02	0.46 ± 0.04	—
0 + EA	0.06 ± 0.01	0.04 ± 0.01	12.4 ± 2.7
0.3 + EA	0.30 ± 0.00	0.29 ± 0.03	15.4 ± 3.3
0.5 + EA	0.50 ± 0.00	0.49 ± 0.05	15.8 ± 1.8
0.5 + 4 peaks at 1.0 + EA	0.49 ± 0.02	0.45 ± 0.04	12.9 ± 4.3

Data shown as daily means ± SD ($n = 5$); air samples for HPLC analysis of formaldehyde concentrations were not taken during peak exposures, EA, ethyl acetate.

3.3.2. Blinking frequency

Blinking frequencies varied considerably per subject without exposure (0 ppm) from 3 up to 120 blinks per 90 s (see Table 7). This corresponds to 2 and 80 blinks per minute. Throughout the day (data not shown), there were no increases in blinking frequency with time. As results did not differ if expressed as absolute values or as relative increases, absolute data were used and reported.

Table 7
Blinking frequency per 90 s in subjects during exposure ($t = 195$ min) to different concentrations of formaldehyde with or without EA

Formaldehyde concentration (ppm)	Mean blinking frequency ± SD	Median	Range
0	28.2 ± 30.2	20	3–120
0.15	31.2 ± 31.4	21	3–145
0.3	27.8 ± 24.7	21	4–118
0.3 + 4 peaks at 0.6	34.4 ± 23.6	27	2–92
0.5	29.2 ± 29.7	18	2–128
0.5 + 4 peaks at 1.0	46.3 ± 45.6*,#	37	2–200
0 + EA	28.6 ± 30.9	20	2–114
0.3 + EA	29.6 ± 24.0	24	3–95
0.5 + EA	34.5 ± 35.1	26	4–157
0.5 + 4 peaks at 1.0 + EA	45.2 ± 45.0*,#	30	5–166

Results are expressed in mean, median, and range of 21 subjects. Statistics: repeated measures ANOVA with contrasts.
* $p < 0.05$ compared to 0 ppm.
# $p < 0.05$ compared to 0 ppm + EA.

A statistically significant increase in blinking frequency was observed 195 min after the start of exposure in subjects exposed to 0.5 ppm formaldehyde with peaks of 1.0 ppm with or without EA compared to the 0 ppm-control condition with or without EA (Table 7).

3.3.3. Nasal resistance and flow

In general, mean total nasal resistance values had a positive value and mean total nasal flow values a negative value after exposure which indicated that subjects had slightly more difficulty in breathing through the nose when compared to before exposure. These differences were not statistically significant. These changes were seen in all exposure conditions, 0 ppm control included (data not shown) and therefore these were considered not to be related to formaldehyde or EA exposure.

3.3.4. Pulmonary function

There were no statistically significant differences in pulmonary function between the baseline measurements on day 1 and the post-exposure measurements on day 10 (data not shown), indicating that exposure to formaldehyde (and EA) did not induce pulmonary function changes.

3.3.5. Reaction times

Decision reaction time upon a visual stimulus was significantly increased in subjects exposed to 0.3 ppm with or without co-exposure to EA (Fig. 3); the motor reaction time had not changed (data not shown). Also, the reaction time upon an acoustic stimulus had increased in subjects exposed to 0.3 ppm without co-exposure to EA (Fig. 4); the motor reaction time had not changed (data not shown). Finally, the decision reaction time upon a combined visual/auditory stimulus had increased in subjects exposed to 0.3 ppm without co-exposure to EA (Fig. 5); and again, the motor reaction time had not changed (data not shown). As effects were observed in subjects exposed to 0.3 ppm only and due to high variability, the slight change in decision reaction time was considered to be an incidental finding.

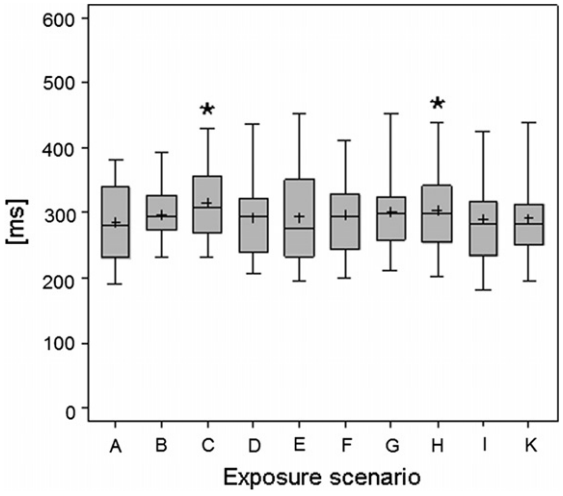

Fig. 3. Decision reaction time upon a visual stimulus after exposure to different concentrations of formaldehyde with or without ethyl acetate (EA). Results are expressed in a box-whisker plot. A = 0 ppm, B = 0.15 ppm, C = 0.3 ppm, D = 0.3 ppm + 4 peaks of 0.6 ppm, E = 0.5 ppm, F = 0.5 ppm + 4 peaks of 1.0 ppm, G = 0 ppm + EA, H = 0.3 ppm + EA, I = 0.5 ppm + EA, K = 0.5 ppm + 4 peaks of 1.0 ppm + EA. Statistics: repeated measures ANOVA with contrasts; $^*p < 0.05$.

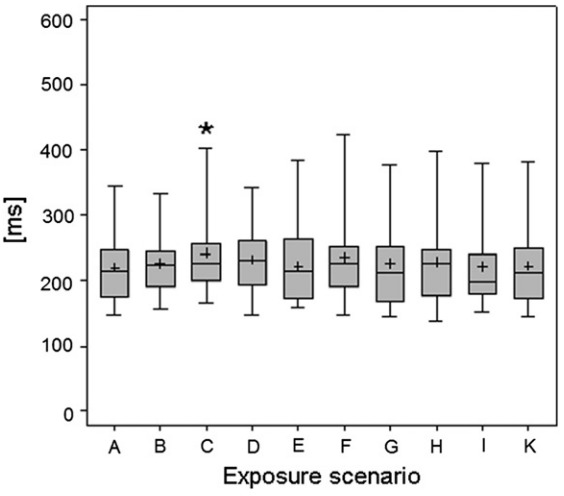

Fig. 4. Decision reaction time upon an acoustic stimulus after exposure to different concentrations of formaldehyde with or without ethyl acetate (EA). Results are expressed in a box-whisker plot. A = 0 ppm, B = 0.15 ppm, C = 0.3 ppm, D = 0.3 ppm + 4 peaks of 0.6 ppm, E = 0.5 ppm, F = 0.5 ppm + 4 peaks of 1.0 ppm, G = 0 ppm + EA, H = 0.3 ppm + EA, I = 0.5 ppm + EA, K = 0.5 ppm + 4 peaks of 1.0 ppm + EA. Statistics: repeated measures ANOVA with contrasts; $^*p < 0.05$.

3.3.6. Subjective ratings

As symptom scores were highest for all subjects after 195 min of exposure, these scores are reported. The mean total symptom score ranged from about 3 to 8 (at a scale up to 35) and had slightly but significantly increased in sub-

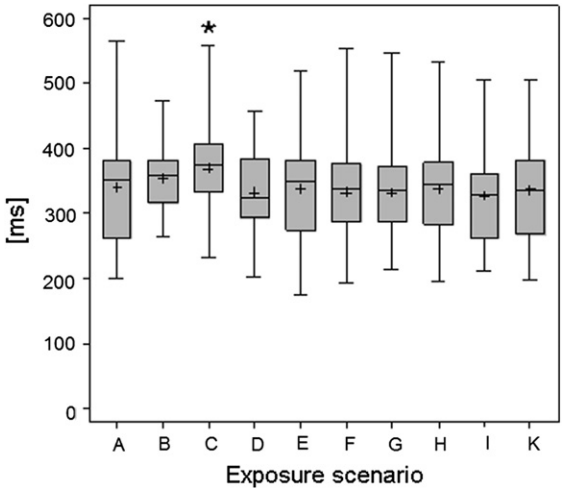

Fig. 5. Decision reaction time upon a combined visual/auditory stimulus after exposure to different concentrations of formaldehyde with or without ethyl acetate (EA). Results are expressed in a box-whisker plot. A = 0 ppm, B = 0.15 ppm, C = 0.3 ppm, D = 0.3 ppm + 4 peaks of 0.6 ppm, E = 0.5 ppm, F = 0.5 ppm + 4 peaks of 1.0 ppm, G = 0 ppm + EA, H = 0.3 ppm + EA, I = 0.5 ppm + EA, K = 0.5 ppm + 4 peaks of 1.0 ppm + EA. Statistics: repeated measures ANOVA with contrasts; $^{*}p < 0.05$.

jects exposed to 0.5 ppm with peaks of 1.0 ppm with and without co-exposure to EA. The total symptom score at 0 ppm formaldehyde with co-exposure to EA was almost similar to that at exposure to 0.5 ppm formaldehyde with peaks of 1.0 ppm (Fig. 6).

Subjective ratings of eye irritation were clearly different from the 0 ppm-control condition. At exposure concentrations as low as 0.3 ppm subjects rated eye irritation as significantly higher than at 0 ppm. However, also the 0 ppm + EA condition was significantly more irritating to the eyes than the 0 ppm without EA. At exposure concentrations of 0.3 ppm with peaks of 0.6 ppm, and at 0.5 ppm with peaks of 1.0 ppm with and without EA, but not at 0.5 ppm without peaks, eye irritation was rated significantly higher than at the 0 ppm + EA control condition. Nevertheless, eye irritation was on average rated less than 2, indicating a score of less than 'somewhat' (Fig. 7).

Nasal irritation was reported to be significantly higher in subjects exposed to 0.5 ppm with peaks of 1.0 ppm with or without EA compared to either the 0 ppm condition or the 0 ppm + EA condition. In contrast, the exposure conditions of 0.3 ppm + EA or 0.5 ppm + EA, although resulting in significantly higher ratings than at the 0 ppm condition, did not result in significantly higher ratings than at 0 ppm + EA (Fig. 8), indicating that the subjects did not differentiate between the irritation caused by formaldehyde and the perception of the EA odour. The nasal irritation rating ranged from about 0.5 to 2, therefore a mean maximum score of 'somewhat' was reached.

Scores of olfactory symptoms were significantly increased at concentrations of 0.3 ppm formaldehyde and up, and were higher when peak exposure was present when compared to the 0 ppm-control condition. Co-exposure to EA clearly increased the olfactory symptom ratings. Only the 0.5 ppm with peaks + EA condition was significantly higher than the 0 ppm + EA control condition. Overall mean symptom ratings were not higher than the level of 'somewhat' (Fig. 9).

Respiratory irritation ratings were significantly increased at concentrations of 0.3 ppm (without peaks), at 0.5 ppm with peaks, and at all formaldehyde levels with co-exposure to EA. These ratings were not increased at the level of 0.3 ppm with peaks or 0.5 ppm without peaks. Moreover, mean symptom ratings were between 0 and 1, in other words between 'not at all' and 'hardly' (Fig. 10).

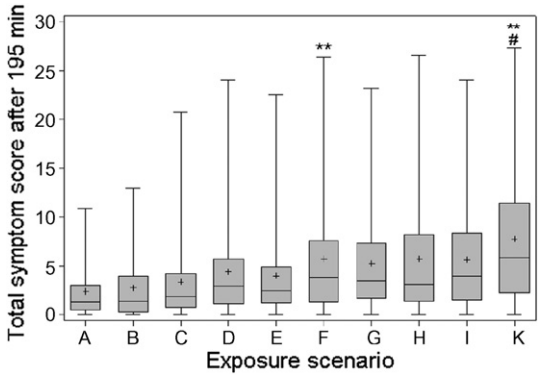

Fig. 6. Total symptom score recorded during exposure ($t = 195$ min) to different concentrations of formaldehyde with or without ethyl acetate (EA). Results are expressed in a box-whisker plot. A = 0 ppm, B = 0.15 ppm, C = 0.3 ppm, D = 0.3 ppm + 4 peaks of 0.6 ppm, E = 0.5 ppm, F = 0.5 ppm + 4 peaks of 1.0 ppm, G = 0 ppm + EA, H = 0.3 ppm + EA, I = 0.5 ppm + EA, K = 0.5 ppm + 4 peaks of 1.0 ppm + EA. Statistics: repeated measures ANOVA with contrasts; $^{**}p < 0.01$ compared to 0 ppm; $^{\#}p < 0.05$ compared to 0 ppm + EA.

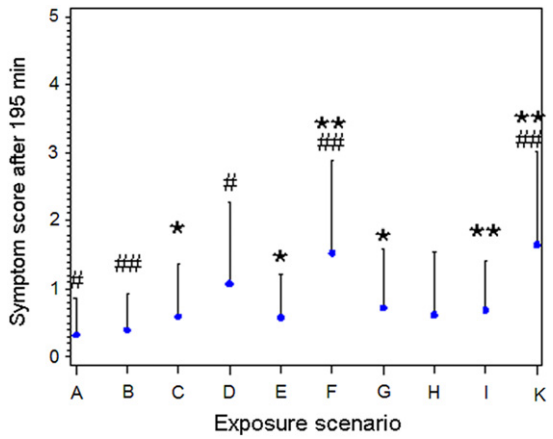

Fig. 7. Symptom score for eye irritation recorded during exposure ($t = 195$ min) to different concentrations of formaldehyde with or without ethyl acetate (EA). Results are expressed in means ± SD. A = 0 ppm, B = 0.15 ppm, C = 0.3 ppm, D = 0.3 ppm + 4 peaks of 0.6 ppm, E = 0.5 ppm, F = 0.5 ppm + 4 peaks of 1.0 ppm, G = 0 ppm + EA, H = 0.3 ppm + EA, I = 0.5 ppm + EA, K = 0.5 ppm + 4 peaks of 1.0 ppm + EA. Statistics: repeated measures ANOVA with contrasts; $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to 0 ppm; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to 0 ppm + EA.

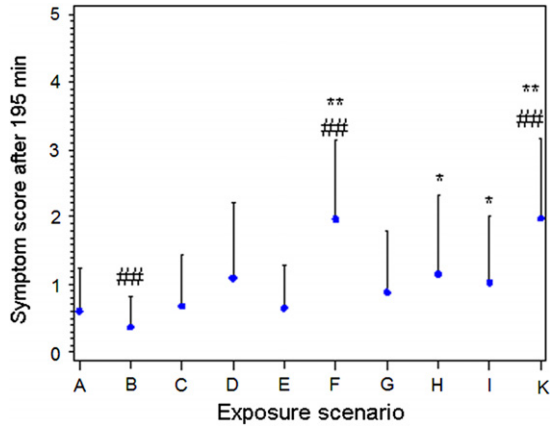

Fig. 8. Symptom score for nasal irritation recorded during exposure ($t = 195$ min) to different concentrations of formaldehyde with or without ethyl acetate (EA). Results are expressed in means ± SD. A = 0 ppm, B = 0.15 ppm, C = 0.3 ppm, D = 0.3 ppm + 4 peaks of 0.6 ppm, E = 0.5 ppm, F = 0.5 ppm + 4 peaks of 1.0 ppm, G = 0 ppm + EA, H = 0.3 ppm + EA, I = 0.5 ppm + EA, K = 0.5 ppm + 4 peaks of 1.0 ppm + EA. Statistics: repeated measures ANOVA with contrasts; $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to 0 ppm; $^{\#\#}p < 0.01$ compared to 0 ppm + EA.

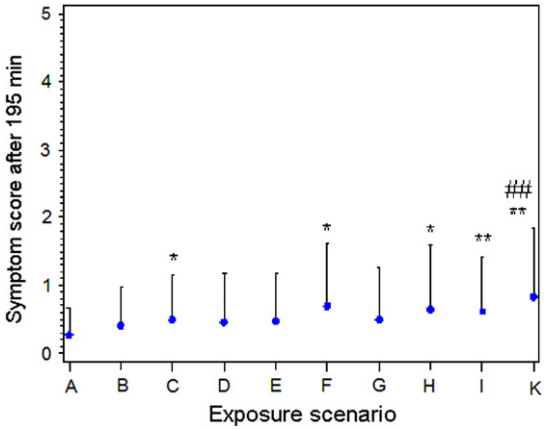

Fig. 10. Respiratory symptom score recorded during exposure ($t = 195$ min) to different concentrations of formaldehyde with or without ethyl acetate (EA). Results are expressed in means ± SD. A = 0 ppm, B = 0.15 ppm, C = 0.3 ppm, D = 0.3 ppm + 4 peaks of 0.6 ppm, E = 0.5 ppm, F = 0.5 ppm + 4 peaks of 1.0 ppm, G = 0 ppm + EA, H = 0.3 ppm + EA, I = 0.5 ppm + EA, K = 0.5 ppm + 4 peaks of 1.0 ppm + EA. Statistics: repeated measures ANOVA with contrasts; $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to 0 ppm; $^{\#\#}p < 0.01$ compared to 0 ppm + EA.

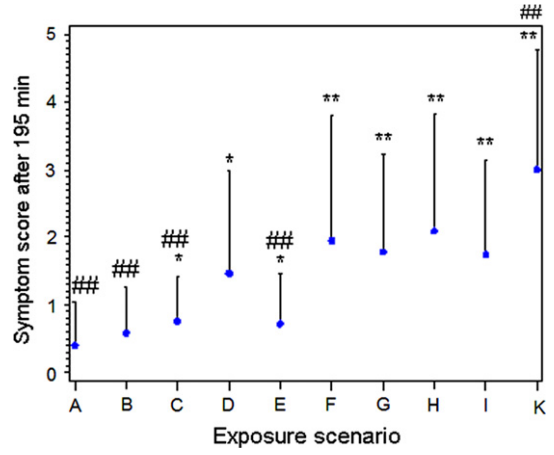

Fig. 9. Olfactory symptom score recorded during exposure ($t = 195$ min) to different concentrations of formaldehyde with or without ethyl acetate (EA). Results are expressed in means ± SD. A = 0 ppm, B = 0.15 ppm, C = 0.3 ppm, D = 0.3 ppm + 4 peaks of 0.6 ppm, E = 0.5 ppm, F = 0.5 ppm + 4 peaks of 1.0 ppm, G = 0 ppm + EA, H = 0.3 ppm + EA, I = 0.5 ppm + EA, K = 0.5 ppm + 4 peaks of 1.0 ppm + EA. Statistics: repeated measures ANOVA with contrasts; $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to 0 ppm; $^{\#\#}p < 0.01$ compared to 0 ppm + EA.

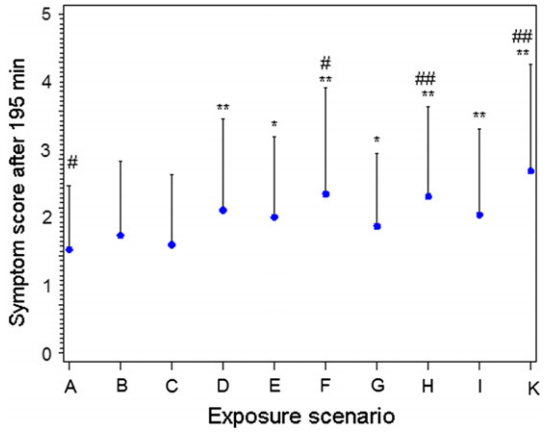

Fig. 11. Annoyance ratings recorded during exposure ($t = 195$ min) to different concentrations of formaldehyde with or without ethyl acetate (EA). Results are expressed in means ± SD. A = 0 ppm, B = 0.15 ppm, C = 0.3 ppm, D = 0.3 ppm + 4 peaks of 0.6 ppm, E = 0.5 ppm, F = 0.5 ppm + 4 peaks of 1.0 ppm, G = 0 ppm + EA, H = 0.3 ppm + EA, I = 0.5 ppm + EA, K = 0.5 ppm + 4 peaks of 1.0 ppm + EA. Statistics: repeated measures ANOVA with contrasts; $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to 0 ppm; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to 0 ppm + EA.

With regard to complaints and well-being, the mean total score did not show significant differences between the various exposure conditions; there was, however, a large inter-individual variability. Ratings varied between 'hardly' and 'somewhat' (data not shown). The mean rating of annoyance increased with elevated formaldehyde concentrations, it had also increased at all levels with co-exposure to EA, including the 0 ppm + EA control condition (Fig. 11), indicating that EA was the main cause of these symptoms. Mean annoyance ratings varied from 2 to 3.5 (on a scale from 1 to 7).

3.3.7. Personality factors

The subjects reported an overall higher score for positive affectivity (mean ± SD: 3.6 ± 0.4, median: 3.7, range: 2.8–4.5) than for negative affectivity (mean ± SD: 1.9 ± 0.7, median: 1.7, range: 1.1–3.5). When negative affectivity was used as covariate in the evaluation of the subjective symptom scores, many changes or differences were no longer statistically significant; symptoms that were still statistically significantly different from the 0 ppm control condition are indicated in Table 8. From this Table it can be seen that (a) significant differences only persisted at the

Table 8
Significant changes measured using the SPES questionnaire with negative affectivity ratings as covariate

Symptoms	Formaldehyde concentration (ppm)	Time of observation
Eye irritation	0.5 + EA	195 min of exposure
	0.5 + peaks of 1.0 + EA	
	0.5 + peaks of 1.0	Directly after exposure
	0.5 + peaks of 1.0 + EA	
Nasal irritation	0.5 + peaks of 1.0	195 min of exposure
	0.5 + peaks of 1.0 + EA	
Olfactory	0 + EA	15 min of exposure
	0.5 + EA	
	0.5 + peaks of 1.0 + EA	
	0.3 + EA	120 min of exposure
	0.5 + EA	
	0.5 + peaks of 1.0 + EA	
	0.5 + EA	195 min of exposure
	0.5 + peaks of 1.0 + EA	
	0.5 + EA	Directly after exposure
	0.5 + peaks of 1.0 + EA	

highest exposure concentration of 0.5 ppm formaldehyde, indicating that at lower formaldehyde concentrations personality factors had a stronger influence on subjectively perceived symptoms than at higher concentrations, and (b) the co-exposure to EA had a very strong influence on olfactory symptoms due to its intensive odour, or in other words, EA at 12–16 ppm had a stronger smell than formaldehyde at 0.3 or 0.5 ppm.

4. Discussions and conclusion

Sensory irritation induced by formaldehyde in humans at low concentrations mainly consists of eye and nasal irritation. Several volunteer studies have been carried out to examine these effects (see reviews by Paustenbach et al., 1997 and Arts et al., 2006a). In all studies, except for two studies in which eye blinking frequency was additionally measured (Weber-Tschopp et al., 1977; Yang et al., 2001), subjective methods (questionnaires) were used to examine eye or nasal irritation. In these studies using subjective methods, it appeared that both eye irritation as well as nasal irritation was reported in healthy volunteers at levels below 1 ppm, and that eye irritation was observed at a slightly lower level than nasal irritation (Paustenbach et al., 1997; Arts et al., 2006a). Based on these studies, Appel et al. (2006) concluded that a slight sensory irritation response could be observed at concentrations of 0.2–0.3 ppm.

The present study was aimed at establishing the possible occurrence of sensory irritation in human volunteers exposed to formaldehyde using objective methods and to correlate this with subjective symptoms. Subjects were exposed to concentrations relevant to the workplace, viz. up to 0.5 ppm with peak exposures up to 1 ppm. The levels were chosen in line with the current German MAK value of 0.3 ppm with peak category II (an 8-h time weighed average of 0.3 ppm with four 15-min periods of 0.6 ppm per working shift; DFG, 2006) and the current Occupational Exposure Limit (OEL) of 0.5 ppm.

The set up of the study included a combination of several items that, as far as known, have not been tested in a single study: (a) formaldehyde exposures with and without peaks, (b) the presence and absence of a masking agent, viz. EA, (c) objective and subjective measurements of irritation, and (d) using the 'negative affectivity' of the volunteers as covariate. The results of this study demonstrated that eye irritation was indeed the most critical effect. A significant increase in eye blinking frequency was observed at 0.5 ppm formaldehyde with peaks of 1.0 ppm. It was noted that blinking frequency showed a considerable inter-individual variability, ranging from 2 to 80 blinks per minute, with a mean value of about 13 blinks per minute. The latter value was in line with those reported by others (Monster et al., 1978; Al-Abdulmunem, 1999; Doughty, 2001; Wolkoff et al., 2003, 2005; Nojgaard et al., 2005; Ziegler, 2007). Two subjects had high blinking frequencies of 69 and 80 per minute. However this is to our opinion not abnormal because in the literature high blinking frequencies up to 60 per minute were regarded as normal. Nakamori et al. (1997) described 60 blinks per minute, Monster et al. (1978) a frequency up to 50 per minute, Bentivoglio et al. (1996) described a range up to 48 blinks per 60 s and Ziegler et al. (submitted for publication) observed 68 blinks per minute. Furthermore it is to stress that these two persons were in good health condition and had no obvious eye diseases. The blinking reflex involves the short and rapid closure of the eyelids as a response to external stimuli. These may be auditory, cognitive, or visual but most of all a reflex response due to trigeminal nerve stimulation. The blinking frequency may not only be influenced by extrinsic factors such as a direct stimulation of the nerve endings by debris or by ocular dehydration due to evaporation of the tear film, but also by factors such as temperature and ambient humidity (Acosta et al., 1999). Nevertheless, measurement of blinking frequency has been used as a sensitive indicator of irritation effects of exposure to vapors (Norn, 1992; Hempel-Jorgensen et al., 1998; Walker et al., 2001; Norbäck and Wieslander, 2002; Emmen et al., 2003; Kleno and Wolkoff, 2004; Kiesswetter et al., 2005; Nojgaard et al., 2005). Weber-Tschopp et al. (1977) observed an increase in eye blinking frequency at a concentration of 1.7 ppm formaldehyde.

In addition to measurement of blinking frequency, conjunctival redness was used as a second objective parameter. Ocular redness was investigated in two other studies using other compounds (Emmen et al., 2003; Ziegler et al., 2007). In the present study, an increase in conjunctival redness was also observed at 0.5 ppm formaldehyde with peaks of 1.0 ppm. This indicates that (a) an increase in ocular redness was associated with an increase in eye blinking frequency, and (b) because such changes were not observed at 0.5 ppm without peaks, nor at 0.3 ppm with peaks of

0.6 ppm, peak exposures of 1.0 ppm seem to have been responsible for the observed effects.

The subjective rating of eye irritation in the present study was significantly higher at concentrations of 0.3 ppm and above. It should, however, be noted that (a) the 0 ppm + EA condition was also classified as more irritating to the eyes than the 0 ppm condition without EA, (b) increased eye irritation was seen at both 0.3 and 0.5 ppm with peaks but not at 0.5 ppm without peaks, and (c) eye irritation was on average rated less than 2, indicating a score of less than 'somewhat'. These results indicate that the intensive odour of EA (the odour threshold of EA was reported to be generally between 0.8 and 14 ppm, with levels as low as 0.1 ppm (van Gemert, 2003)) may have prompted the volunteers to report irritation of the eyes, that formaldehyde peaks may have had a significant influence, and finally, although significant increases were reported in the severity of eye irritation, the degree still was minimal (less than somewhat). In the present study, there was a statistically significant correlation between the subjectively experienced irritation and the blinking frequency at the highest level tested (0.5 ppm with peaks) in the presence of EA (Fig. 12). The correlation was only slightly positive when EA was not present; a significant degree was not obtained (Fig. 13). These positive correlations seem to indicate that the ratings of symptoms of eye irritation by the subjects could be interpreted as valid.

These results were fully in line with several other studies, i.e. increases in eye irritation were observed at increasing formaldehyde concentrations at comparable levels (Schuck et al., 1966; Bender et al., 1983; Andersen and Mølhave, 1983; Kulle et al., 1987; Kulle, 1993), and the degree of eye irritation was minimal. In these studies, the severity of the eye symptoms at formaldehyde concentrations $\leqslant$1 ppm were rated between none and slight/mild; the response slight/mild was generally reported to be 'present but not annoying'. Arts et al. (2006a) therefore concluded that the eye irritation ratings at formaldehyde levels below 1 ppm should be translated into perception or awareness rather than an annoying ocular irritation.

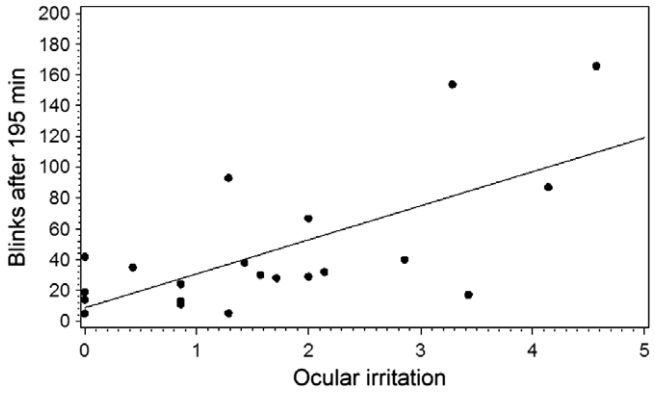

Fig. 12. Correlation between subjectively rated eye irritation and the number of blinks in 90 s in subjects exposed to 0.5 ppm formaldehyde with peaks of 1.0 ppm and co-exposure to ethyl acetate ($\rho = 0.54$; $y = 22.12x + 8.62$; $p = 0.01$). After using the three sigma criteria, the two highest values were no outliers. A = 0 ppm, B = 0.15 ppm, C = 0.3 ppm, D = 0.3 ppm + 4 peaks of 0.6 ppm, E = 0.5 ppm, F = 0.5 ppm + 4 peaks of 1.0 ppm, G = 0 ppm + EA, H = 0.3 ppm + EA, I = 0.5 ppm + EA, K = 0.5 ppm + 4 peaks of 1.0 ppm + EA.

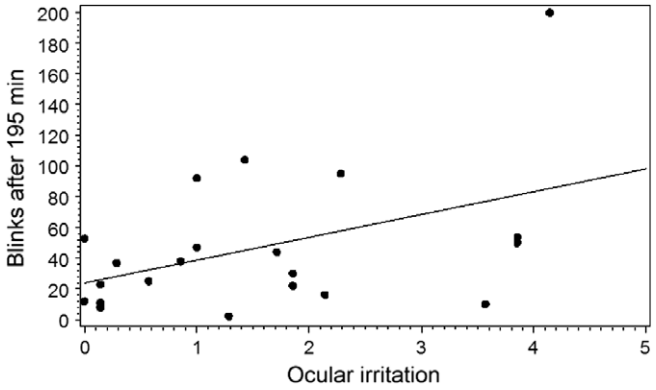

Fig. 13. Correlation between subjectively rated eye irritation and the number of blinks in 90 s in subjects exposed to 0.5 ppm formaldehyde with peaks of 1.0 ppm ($\rho = 0.36$; $y = 14.94x + 23.47$; $p = 0.10$). The highest value in this graph is an outlier (after the three sigma criteria). Without this value, the correlation is ρ 0.26 with a p-value of 0.26.

Because the perception of sensory irritation is highly influenced by personal factors such as anxiety, expectations, and attitudes towards health risks (Seeber et al., 2000) and subjects who indicate 'anxiety' as a characteristic of their personality tend to evaluate their complaints at an increased rate (Seeber et al., 2000; Dalton, 2003; Ihrig et al., 2006), in the present study, 'negative affectivity' from the PANAS questionnaire was used as a covariate in the evaluation of the subjective symptom scores. In doing so, significant differences in perceived eye irritation persisted at the highest exposure concentration of 0.5 ppm formaldehyde, almost exclusively with peaks. This indicated that at lower formaldehyde concentrations 'negative affectivity' had a stronger influence on subjectively perceived symptoms than at higher concentrations which resulted in over-interpretation of the symptoms ratings at these lower concentrations. In addition, the co-exposure to EA had a very strong influence on these symptoms due to its intensive odour (Table 8).

Objective measurements of functional nasal parameters at exposure levels up to 0.5 ppm (with and without peaks) did not result in any significant changes which was in line with observations by Kulle et al. (1987) and Kulle (1993). They found an increase in nasal resistance at a concentration of 3 ppm but not at 1 or 2 ppm. Subjective measurements revealed that nasal irritation was reported to be significantly higher in subjects exposed to 0.5 ppm with peaks of 1.0 ppm with or without EA. At lower concentrations, subjects could not differentiate between the irritation caused by formaldehyde and the perception of the EA odour. The nasal irritation was rated from about 0.5 to 2, therefore a mean maximum score of 'somewhat' was reached. Using the 'negative affectivity' score as a covariate, significant differences in perceived nasal irritation per-

sisted at the concentration of 0.5 ppm formaldehyde with peaks.

In addition, significant changes in lung function were absent in the present study. A small, statistically insignificant, decrease in lung function was reported in asthmatic volunteers at a level of 3 ppm formaldehyde (Sauder et al., 1987). A slight reduction in FEV1 was found at levels of 1–1.24 ppm (Khamaonkar and Fulare, 1983; Akbar-Khanzadek and Mlynek, 1997). Other authors, however, did not observe such changes in asthmatic volunteers at levels of 2 ppm (Witek et al., 1987) nor at 0.7 ppm (Harving et al., 1990), nor in healthy volunteers at levels of 1 ppm (Day et al., 1984), at 2 ppm (Schachter et al., 1986), or at 3 ppm (Sauder et al., 1986). Ezratty et al. (2007) exposed 12 subjects, with intermittent asthma and allergy to pollen, to 0.4 ppm formaldehyde for 60 min. Exposure to formaldehyde had no significant deleterious effect on airway allergen responsiveness of patients with intermittent asthma; in contrast, they found a trend toward a protective effect.

Subjective ratings of respiratory irritation were increased in the present study at several formaldehyde levels but a consistent increase was not observed. Moreover, mean symptom ratings were between 0 and 1, in other words between 'not at all' and 'hardly'. Using the 'negative affectivity' score as a covariate, significant differences in perceived respiratory irritation were absent.

A perception of smell was reported at a level as low as 0.3 ppm formaldehyde which was in line with the observation that odour detection thresholds of formaldehyde are generally between 0.04 and 0.4 ppm (van Gemert, 2003). Co-exposure to EA clearly increased the olfactory symptom ratings but overall mean symptom ratings were not higher than the level of 'somewhat'. Using the 'negative affectivity' score as a covariate, significant differences in olfactory symptoms were only present in the case of co-exposure to EA generally in combination with peak exposure (Table 8), indicating that the formaldehyde peaks were of influence but also that subjects could not easily differentiate between odour and irritation, or in other words, were not sufficiently able to differentiate between stimulation of the nervus olfactorius and nervus trigeminus. This also indicates that conclusions about irritation in the presence of olfactory stimuli should be interpreted with care. This was in line with other studies showing that the mere presence of smell could cause a perception of a sensory effect (Dalton, 1996, 1999; Dalton and Wysocki, 1996; Dalton et al., 2000). In addition, inter-individual differences in sensitivity to the perception of unpleasant odours may play a significant role (Dalton, 1999; Dalton et al., 2000).

Overall, from the results of the present study it can be concluded that eye irritation was the most critical effect induced by formaldehyde based on significant increases in both eye blinking frequency and conjunctival redness at a concentration of 0.5 ppm with peaks of 1.0 ppm. Subjective measurements of irritation revealed that eye and respiratory irritation and olfactory symptoms were recorded at formaldehyde levels as low as 0.3 ppm. However, taking into account the odour of formaldehyde, the influence of the presence of EA with its characteristic odour, and the use of 'negative affectivity' as covariate in the statistical analyses, only the concentration of 0.5 ppm with peaks of 1.0 ppm was considered an effect level. It was, in addition, considered a Lowest-Observed-Effect Level (LOEL) because of its low irritation score (always less than 'somewhat') and complete reversibility of the symptom scores 16 h after exposure. Based on the absolute levels of the responses it can even be argued whether the classification of the symptoms, viz. less than 'somewhat', is adverse indeed. But the objectively measured eye irritation shows that reflex mechanisms are induced at 0.5 ppm with peaks of 1.0 ppm resulting in increased eye blinking and increased vasodilatation. Again, these effects in themselves are not regarded as adverse but are induced to protect the individual.

In conclusion, concentration levels of 0.5 ppm formaldehyde without peaks, and 0.3 ppm with peaks of 0.6 ppm were considered to be No-Observed-Adverse-Effect Levels (NOAELs). These results were in line with those of Paustenbach et al. (1997) stating that: 'for most persons eye irritation clearly due to formaldehyde does not occur until at least 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, and those of Arts et al. (2006a) who 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 shows, as in most experimental studies (reviewed by Arts et al., 2006a) and in the occupational study reported by Ryan et al. (2003) that all symptoms disappeared very quickly, underlining the mildness of the effects at these low concentrations.

Acknowledgments

The authors gratefully acknowledge the volunteers for their participation, the assistance of Gudula Christ and Stefan Knohl in the examinations, and Heidi Ludwig, Thomas Krczal and Holger Zimmer for the excellent performance of the chemical analyses. They also thank Sylvana Müller for independent quality assurance of the study, Heinz-Peter Gelbke and Armin Gamer for study advice, and Josje Arts, TNO Toxicology and Applied Pharmacology, Zeist, The Netherlands, and her colleagues Hans Muijser and Jan Lammers, for preparation of this manuscript. They also thank the FormaCare sector group of CEFIC, Brussels, Belgium for the financial contribution to perform this study.

References

Acosta, M.C., Gallar, J., Belmonte, C., 1999. The influence of eye solutions on blinking and ocular comfort at rest and during work at video display terminals. Exp. Eye Res. 68, 663–669.

Akbar-Khanzadek, F., Mlynek, J.S., 1997. Changes in respiratory function after one and three hours of exposure to formaldehyde in non-smoking subjects. Occup. Environ. Med. 54, 296–300.

Al-Abdulmunem, M., 1999. Relation between tear breakup time and spontaneous blink rate. Int. Lens. Clin. 26, 117–120.

Alarie, Y., 1973. Sensory irritation of the upper airways by airborne chemicals. Toxicol. Appl. Pharmacol. 24, 279–297.

Andersen, I., Mølhave, L., 1983. Controlled human studies with formaldehyde. In: Gibson, E. (Ed.), Formaldehyde Toxicity. Hemisphere, Washington, DC, pp. 154–165.

Appel, K.E., Bernauer, U., Herbst, U., Madle, S., Schulte, A., Richter-Reichhelm, H.B., Gundert-Remy, U., 2006. Is there a save level for formaldehyde? Analysis of the data from studies on the carcinogenic effect of formaldehyde Umweltmed. Forsch. Prax. 11, 347–361 (in German language).

Arts, J.H.E., Mojet, J., van Gemert, L.J., Emmen, H.H., Lammers, J.H.C.M., Marquart, J., Woutersen, R.A., Feron, V.J., 2002. An analysis of human responses to the irritancy of acetone vapors. Crit. Rev. Toxicol. 32, 43–66.

Arts, J.H.E., Rennen, M.A.J., de Heer, C., 2006a. Inhaled formaldehyde: evaluation of sensory irritation in relation to carcinogenicity. Regul. Toxicol. Pharmacol. 44, 144–160.

Arts, J.H.E., de Heer, C., Woutersen, R.A., 2006b. Local effects in the respiratory tract: relevance of subjectively measured irritation for setting occupational exposure limits. Int. Arch. Occup. Environ. Health 79, 283–298.

Bender, J.B., Mullin, L.S., Graepel, G.J., Wilson, W.E., 1983. Eye irritation response to formaldehyde. Am. Ind. Hyg. Assoc. J. 44, 463–465.

Bentivoglio, A.R., Bressman, S.B., Cassetta, E., Carretta, D., Tonali, P., Albanese, A., 1996. Analysis of blink rate patterns in normal subjects. Mov. Disord. 12 (6), 1028–1034.

Bos, P.J.M., Zwart, A., Reuzel, P.G.J., Bragt, P.C., 1992. Evaluation of the sensory irritation test for the assessment of occupational health risk. CRC Crit. Rev. Toxicol. 21, 423–450.

Cain, W.S., Gent, J., Catalanotto, F.A., Goodspeed, R.B., 1983. Clinical evaluation of olfaction. Am. J. Otolaryngol. 4, 252–256.

Cain, W.S., Cometto-Muñiz, J.E., 1995. Irritation and odor as indicators of indoor air pollution. Occup. Med. 10, 133–145.

Carney, A.S., Bateman, N.D., Jones, N.S., 2000. Reliable and reproducible anterior active rhinomanometry for the assessment of unilateral nasal resistance. Clin. Otolaryngol. Allied Sci. 25, 499–503.

Clement, P.A., 1984. Committee report on standardization of rhinomanometry. Rhinology 22, 151–155.

Dalton, P., 1996. Odor perception and beliefs about risk. Chem. Senses 21, 447–458.

Dalton, P., Wysocki, CJ., 1996. The nature and duration of adaptation following long-term odor exposure. Percept. Psychophys. 58 (5), 781–792.

Dalton, P., 1999. Cognitive influences on health symptoms from acute chemical exposure. Health Psychol. 18, 579–590.

Dalton, P.H., Dilks, D.D., Banton, M.I., 2000. Evaluation of odor and sensory irritation thresholds for methyl isobutyl ketone in humans. Am. Ind. Health Assoc. J. 61, 340–350.

Dalton, P., 2001. Evaluating the human response to sensory irritation: implications for setting occupational exposure limits. AIHAJ 62, 723–729.

Dalton, P., 2002. Odor, irritation and perception of health risk. Int. Arch. Occup. Environ. Health 75, 283–290.

Dalton, P., 2003. Upper airway irritation, odor perception and health risk due to airborne chemicals. Toxicol. Lett., 239–248.

Day, J.H., Lees, R.E.M., Clark, R.H., Pattee, P.L., 1984. Respiratory response to formaldehyde and off-gas urea formaldehyde foam insulation. Can. Med. Assoc. J. 131, 1061–1065.

DFG, 1996. Deutsche Forschungsgemeinschaft. Analytische Methoden zur Prüfung gesundheitsschädlicher Arbeitsstoffe Band 1. ©1996 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

DFG, 2006. Deutsche Forschungsgemeinschaft. MAK-und BAT-Werte-Liste. ©2006 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. ISBN: 3-527-31356-7.

Doughty, M.J., 2001. Consideration of three types of spontaneous eyeblink activity in normal humans: during reading and video display terminal use, in primary gaze, and while in conversation. Optom. Vis. Sci. 78, 712–725.

Emmen, H.H., Muijser, H., Arts, J.H.E., Prinsen, M.K., 2003. Human volunteer study with PGME: eye irritation during vapour exposure. Toxicol. Lett. 140–141, 249–259.

Ezratty, V., Bonay, M., Neukirch, C., Orset-Guillossou, G., Dehoux, M., Koscielny, S., Cabanes, P.-A., Lambrozo, J., Aubier, M., 2007. Effect of formaldehyde on asthmatic responses to inhaled allergen challenge. Environ. Health Perspect. 115, 210–214.

Gamberale, F., 1989. Critical issues in the study of the acute effects of solvent exposure. Neurotoxicol. Teratol. 11, 565–570.

Harving, H., Korsgaard, J., Pedersen, O.F., Mølhave, L., Dahl, R., 1990. Pulmonary function and bronchial reactivity in asthmatics during low-level formaldehyde exposure. Lung 168, 15–21.

Hempel-Jorgensen, A., Kjaergaard, S.K., Mølhave, L., 1998. Cytological changes and conjunctival hyperemia in relation to sensory eye irritation. Int. Occup. Environ. Health 71, 225–235.

Ihrig, A., Hoffmann, J., Triebig, G., 2006. Examination of the influence of personal traits and habituation on the reporting of complaints at experimental exposure to ammonia. Int. Arch. Occup. Environ. Health 79, 332–338.

Iregren, A., Gamberale, F., Kjellberg, A., 1996. SPES: a psychological test system to diagnose environmental hazards. Swedish Performance Evaluation System. Neurotoxicol. Teratol. 18, 485–491.

Khamaonkar, M.B., Fulare, M.B., 1983. Pulmonary effects of formaldehyde exposure—an environmental epidemiology study. Indian J. Chest. Dis. Allied Sci. 33, 9–13.

Kiesswetter, E., van Thriel, C., Schäper, M., Blaszkewicz, M., Seeber, A., 2005. Eye blinks as an indicator for sensory irritation during constant and peak exposures to 2-ethylhexanol. Environ. Toxicol. Pharmacol. 19, 531–541.

Kjaergaard, S.K., Hodgson, M., 2001. The assessment of irritation using clinical methods and questionnaires. AIHAJ 62, 711–716.

Kleno, J., Wolkoff, P., 2004. Changes in eye blink frequency as a measure of trigeminal stimulation by exposure to limonene oxidation products, isoprene oxidation products and nitrate radicals. Int. Arch. Occup. Environ. Health 77, 235–243.

Kulle, T.J., Sauder, L.R., Hebel, J.R., Green, D.J., Chatham, M.D., 1987. Formaldehyde dose–response in healthy nonsmokers. J. Air Pollut. Control Assoc. 37, 919–924.

Kulle, T.J., 1993. Acute odor and irritation response in healthy nonsmokers with formaldehyde exposure. Inhal. Toxicol. 5, 323–332.

Lehrl, S., Triebig, G., Fischer, B., 1995. Multiple choice vocabulary test MWT as a valid and short test to estimate premorbid intelligence. Acta Neurol. Scand. 91, 335–345.

Malm, L., Gerth van Wijk, R., Bachert, C., 2000. Guidelines for nasal provocations with aspects in nasal patency, airflow, and airflow resistance. International Committee on Objective Assessment of the Nasal Airways, International Rhinologic Society. Rhinology 38, 1–6.

Monster, A.W., Chan, H.C., O'Connor, D., 1978. Long-term trends in human eye blink rate. Biotelem. Pat. Monit. 5, 206–222.

Nakamori, K., Odawara, M., Nakajima, T., Mizutani, T., Tsubota, K., 1997. Blinking is controlled primarily by ocular surface conditions. Am. J. Ophtalmol. 124, 24–30.

Naumann, H.H., 1990. Nase, Nasennebenhöhlen und Nasenrachen. In: Differentialdiagnostik in der Hals-Nasen-Ohrenheilkunde. Georg Thieme Verlag Stuttgart, New York, pp. 176–230.

Nelson, K.W., Ege Jr., J.F., Ross, M., Woodman, L.E., Silverman, L., 1943. Sensory response to certain industrial solvent vapors. J. Ind. Hyg. Toxicol. 25, 282–285.

Nielsen, G.D., Hougaard, K.S., Larsen, S.T., Wolkoff, P., Clausen, P.A., Wilkins, C.K., Alarie, Y., 1999. Acute airway effects of formaldehyde and ozone in BALB/c mice. Hum. Exp. Toxicol. 18, 400–409.

National Institute for Occupational Safety and Health, NIOSH, 1997. Pocket Guide to Chemical Hazards. National Institute for Occupational Safety and Health, Washington DC.

Nojgaard, J.K., Christensen, K.B., Wolkoff, P., 2005. The effects on human eye blink frequency of exposure to limonene oxidation products and methacrolein. Toxicol. Lett. 156, 241–251.

Norbäck, D., Wieslander, G., 2002. Biomarkers and chemosensory irritations. Int. Arch. Occup. Environ. Health 75, 298–304.

Norn, M.S., 1992. Diagnosis of dry eye. In: Lemp, M.A., Marquardt, R. (Eds.), The Dry Eye—A Comprehensive Guide. Springer-Verlag, Berlin, p. 134.

Paustenbach, D., Alarie, Y., Kulle, T., Smith, R., Swenberg, J., Witschi, H., Horowitz, S.B., 1997. A recommended occupational exposure limit for formaldehyde based on irritation. J. Toxicol. Environ. Health 50, 217–263.

Ryan, T.J., Burroughs, G.E., Taylor, K., Kovein, R.J., 2003. Video exposure assessments demonstrate excessive laboratory formaldehyde exposures. Appl. Occup. Environ. Hyg. 18, 450–457.

Sauder, L.R., Chatham, M.D., Green, D.J., Kulle, T.J., 1986. Acute pulmonary response to formaldehyde exposure in healthy subjects. J. Occup. Med. 28, 420–424.

Sauder, L.R., Green, D.J., Chatham, M.D., Kulle, T.J., 1987. Acute pulmonary response of asthmatics to 3.0 ppm formaldehyde. Toxicol. Ind. Health 3, 569–578.

Schachter, E.N., Witek, T.J., Brody, D.J., Tosun, T., Beck, G.J., Leaderer, B.P., 1986. A study of respiratory effects from exposure to 2 ppm formaldehyde in healthy subjects. Arch. Environ. Health 41, 229–239.

Schaper, M., 1993. Development of a database for sensory irritants and its use in establishing occupational exposure limits. Am. Ind. Hyg. Assoc. J. 54, 488–544.

Schuck, E.A., Stephens, E.R., Middleton, J.T., 1966. Eye irritation response at low concentrations of irritants. Arch. Environ. Health 13, 570–575.

Schuhfried, G., Prieler, J., 2001. Wiener Reaktionstest Version 27.00. Handanweisung.

Seeber, A., Demes, P., Golka, K., Kiesswetter, E., Schäper, M., van Thriel, C., Zupanic, M., 2000. Subjective symptoms due to solvents mixtures, dioxin, and toluene: impact of exposure versus personality factors. Neurotoxicology 21, 677–684.

Seeber, A., van Thriel, C., Haumann, K., Kiesswetter, E., Blaszkewicz, M., Golka, K., 2002. Psychological reactions related to chemosensory irritation. Int. Arch. Occup. Environ. Health 75, 314–325.

Seeber, A., 2007. Personal communication.

Tsubota, K., Hata, S., Okusawa, Y., Egami, F., Ohtsuki, T., Nakamori, K., 1996. Quantitative videographic analysis of blinking in normal subjects and patients with dry eye. Arch. Ophthalmol. 114 (6), 715–720.

van Gemert, L.J., 2003. Compilations of odour threshold values in air and water. Leffingwell & Associates; www.leffingwell.com/bacis2.htm.

Walker, J.F., 1975. Formaldehyde, third ed. American Chemical Society Monograph Series No. 159 ISBN 0-88275-218-9, 148–150.

Walker, J.C., Kendal-Reed, M., Utell, M.J., Cain, W.S., 2001. Human breathing and eye blink rate responses to airborne chemicals. Environ. Health Perspect. 109 (Suppl. 4), 507–512.

Watson, D., Clark, L.A., Tellegen, A., 1988. Development and validation of brief measures of positive and negative affect: the PANAS scales. J. Pers. Soc. Psychol. 54, 1061–1070.

Weber-Tschopp, A., Fischer, T., Gandjean, E., 1977. Reizwirkungen des Formaldehyds (HCHO) auf den Menschen. Irritating effects of formaldehyde in humans. Int. Arch. Occup. Environ. Health 39, 207–218.

Witek Jr., T.J., Schachter, E.N., Tosun, T., Beck, G.J., Leaderer, B.P., 1987. An evaluation of respiratory effects following exposure to 2.0 ppm formaldehyde in asthmatics: lung function, symptoms, and airway reactivity. Arch. Environ. Health 42, 230–237.

Wolffsohn, J.S., 2004. Incremental nature of anterior eye grading scales determined by objective image analysis. Br. J. Ophthalmol. 88, 1434–1438.

Wolkoff, P., Skov, P., Franck, C., Petersen, L.N., 2003. Eye irritation and environmental factors in the office environment-hypotheses, causes and a physiological model. Scand. J. Work Environ. Health 29, 407–409.

Wolkoff, P., Nojgaard, J.K., Troiano, P., Piccoli, B., 2005. Eye complaints in the office environment: precorneal tear film integrity influenced by eye blinking efficiency. Occup. Environ. Med. 62, 4–12.

Yang, X., Zhang, Y.-P., Chem, D., Chen, W.-G., Wang, R., 2001. Eye irritation caused by formaldehyde as an indoor air pollution—a controlled human exposure experiment. Biomed. Environ. Sci. 4, 229–236.

Ziegler, A.E., Zimmer, H., Triebig, G., 2007. Exposure study on chemosensory effects of ε-caprolactam in the low concentration range. Int. Arch. Occup. Environ. Health, in press.