

Acute airway effects of airborne formaldehyde in sensitized and non-sensitized mice housed in a dry or humid environment

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ABSTRACT

We investigated the role of air humidity and allergic sensitization on the acute airway response to inhaled formaldehyde (FA) vapor. Mice were sensitized to the immunogen ovalbumin (OVA) by three intraperitoneal injections followed by two aerosol challenges, giving rise to allergic airway inflammation. Control mice were sham sensitized by saline injections and challenged by saline aerosols. Once sensitized, the mice were housed at high (85–89%) or low (<10%) relative humidity, respectively for 48 h prior to a 60-min exposure to either 0.4, 1.8 or about 5 ppm FA. Before, during and after exposure, breathing parameters were monitored. These included the specific markers of nose and lung irritations as well as the expiratory flow rate, the latter being a marker of airflow limitation.

The sensory irritation response in the upper airways was not affected by allergic inflammation or changes in humidity. At high relative humidity, the OVA-sensitized mice had a decreased expiratory airflow rate compared to the saline control mice after exposure to approximately 5 ppm FA. This is in accordance with the observations that asthmatics are more sensitive than non-asthmatics to higher concentrations of airway irritants including FA. In the dry environment, the opposite trend was seen; here, the saline control mice had a significantly decreased expiratory airflow rate compared to OVA-sensitized mice when exposed to 1.8 and 4 ppm FA. We speculate that increased mucus production in the OVA-sensitized mice has increased the “scrubber effect” in the nose, consequently protecting the conducting and lower airways.

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Introduction

High indoor air humidity and mold growth have been associated with upper and lower airway symptoms and with development and exacerbation of asthma (Mendell et al., 2011). However, little is known about the interplay between airway irritants and humidity. Dry eyes and dry airways are commonly reported symptoms in office environments and clinical studies indicate that low relative humidity (RH) has a strong influence by desiccation of eyes and possibly also the upper airways (Wolkoff and Kjærgaard, 2007). Two hypotheses have been suggested; the first proposes that exposure to low RH causes desiccation of eyes and mucous membranes, directly causing the irritation. The second proposes that exposure to low RH has no effects per se, but symptoms are caused by exacerbated sensitivity to sensory irritants due to a compromised mucus barrier. Thus, trigeminal stimulation by volatile compounds should be preceded by a destabilized eye tear film

which increases penetration of irritants and causes eye symptoms (Wolkoff et al., 2012). Whatever the mechanism, it may be speculated that a similar mechanism would apply for the airways.

Mice showed increased trigeminal stimulation in the upper airways by 45 min exposure to ammonia under dry (0% RH) compared to humid conditions (95% RH). The difference in responsiveness was apparent from the concentration of ammonia necessary to reduce the respiratory rate by 50% (RD₅₀), which was 582 and 732 mg/m³ in the dry and humid environments, respectively (Li and Pauluhn, 2010). Rats did not show a similar difference by exposure to dry versus humid ammonia; the RD₅₀ values were 972 and 905 mg/m³, respectively. Mice exposed to a reaction mixture of ozone and limonene showed statistically less sensory irritation at 32% RH than at 2% RH (Wilkins et al., 2003). A similar trend was observed by exposing male subjects to a similar reaction mixture under dry and humid conditions; the decrease in eye blink frequency, a proxy for trigeminal stimulation, was less pronounced at elevated RH, indicating an alleviating effect under humid conditions (Nøjgaard et al., 2005). Exposure of subjects to dry air showed detrimental effects on the upper airways by reduced saccharin clearance from the nose, especially among elderly (Sunwoo et al., 2006); the authors speculated that long-term dry air exposure deteriorates the function of cilia, important for clearance of the

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airways. Thus, exposure to dry air may condition the airways in becoming more susceptible to trigeminal stimulation by sensory irritants like formaldehyde (FA).

The exposure of asthmatic subjects to low (<1 ppm) FA at ambient RH did not show exacerbation of lung functions (Golden, 2011; Wolkoff and Nielsen, 2010). However, increased water loss by dry conditions has been shown to influence lung functions, including decreased forced expiratory volume in 1 s (FEV₁) (McFadden et al., 1999) indicating an increased airway resistance.

Our objective was to determine the respiratory tract effects of exposure to airborne particle-free FA in dry and humid atmospheres and to study the role of allergic sensitization on airway responsiveness to FA exposure using a mouse inhalation model. Effects of FA were studied at all three levels of the airways i.e. the upper, conducting and pulmonary levels. FA was considered to be an appropriate model compound since at low concentrations the effect is primarily at the eyes and upper airways (Nielsen et al., 1999; World Health Organisation, 2010), but at high concentrations sufficient FA levels can penetrate beyond the upper airways and affect the lungs (Nielsen et al., 1999). To our knowledge, the influence of humidity on FA exposure has not been investigated previously.

Method/materials

Chicken egg ovalbumin (CAS 9006-59-1) (OVA) was grade V (purity ≥ 98%) from Sigma-Aldrich, St. Louis, MO, USA. Aluminum hydroxide (Al(OH)₃) adjuvant was from Alhydrogel, Brenntag Biosector, Denmark. Formaldehyde was supplied by Kin-Tek (TX, USA) as a certified Trace Source™ permeation tube with paraformaldehyde.

Animals. Inbred BALB/cA male mice were purchased from Taconic, Denmark, and were housed in polypropylene cages (380 × 220 × 150 mm) with pinewood sawdust bedding (Lignocel S8, Brogaarden, Denmark). The photoperiod was from 6 a.m. to 6 p.m., and the temperature and relative humidity (RH) in the animal room were 22 ± 2 °C and 30–50%, respectively. The cages were sanitized twice weekly. Food (Altromin no. 1324, Altromin, Lage, Germany) and tap water were available ad libitum. Treatment of the animals adhered to procedures approved by the Animal Experiment Inspectorate, Denmark with permission numbers 2006/561-1123 and 2011/561-1990.

Sensitization. Mice (n = 30) were immunized to OVA by intraperitoneal (i.p.) injections of 1 µg OVA in combination with 270 µg Al(OH)₃ in 100 µL 0.9% saline on day 0. Mice were boosted i.p. on days 14 and 21 with 0.1 µg OVA in 100 µL 0.9% saline. Finally, the animals were exposed 20 min to an aerosol of 0.2% OVA on days 28 and 29 using a Pari Star nebulizer (PARI GmbH, Starnberg, Germany), which mainly delivers respirable particles as specified (Hansen et al., 2007b). For the non-sensitized control mice (n = 30), saline (0.9%) was used for the three i.p. injections and the aerosol exposures. Afterwards, on days 29 and 30, the sensitized and non-sensitized mice were housed at either low (<10) or high (85–89) % RH. On day 31, the mice were exposed to FA.

Generation and monitoring of formaldehyde concentration and ultrafine particles. Formaldehyde was generated from a Kin-Tek (TX, USA) gas standard generator (Model 491MB) by use of a permeation tube and dry air, and led to a 24 L exposure chamber (Larsen and Nielsen, 2012). The airflow rates in the chamber were set between 18.8 and 23.2 L/min. The chamber exposure concentrations of FA were monitored pre- and post-FA exposures and every 10 min during exposures by 10 min air sampling of 4.4 L on dinitrophenylhydrazine (DNPH) sampling cartridges (LpDNPH S10, Supelco, Bellefonte, PA). Humidification of the sampling air to 50% relative humidity by a sparger with clean water was placed in front of the cartridge under the dry conditions. The cartridges were eluted within 1 h after sampling and

analyzed immediately thereafter by HPLC using a diode array detector using a standard mix ((Supelco, Bellefonte, PA) Carbonyl-DNPH Mix 1) for six-point calibration ($r^2 > 0.999$). The FA concentrations are reported as the mean of five samples. The chamber background air was <0.1 and <0.2 ppm at dry and humid conditions, respectively. Target concentrations were 0.4, 1.8 and 7 ppm, respectively. The FA concentrations measured in the animal exposure chamber were similar under dry and humid environments both at the low (0.42 ± 0.01 ppm) and medium (1.8 ± 0.09 ppm) FA levels. At the highest FA level, the concentrations were 4.0 ppm and 5.7 ppm in the dry and humid environments, respectively.

For the low RH experiments, medicinal grade air (2% RH) was used upstream to dilute the FA, while air passed through a heated sparger (30 °C) containing clean water in experiments with elevated RH (85–89%) at 22.5–23.3 °C measured in the chamber. Humidity and temperature were measured with a calibrated Testo 650 humidity/temperature instrument (Testo GmbH & Co., D-79849 Lenzkirch).

Measurement of ultrafine particles in the center of the exposure chamber was carried out without animals at 4.8 ppm FA and 80% RH with a particle condensation counter from TSI (MN, USA) Model 3007 (particle size: 0.01 to >1.0 µm) for 160 min. The measurement showed that the background level of less than 40 particles/cm³ remained constant after the FA gas generator was turned on (data not presented). This shows that the generator delivered gaseous FA.

Bioassay. The respiratory effects were studied in a mouse bioassay (Larsen et al., 2000). The bioassay allows the detection of respiratory effects on the upper airway (sensory) irritation as well as effects in the conducting airways and at the alveolar level (Alarie, 1998).

Respiratory parameters. Different types of effects from the respiratory system can be studied simultaneously via continuous computerized monitoring of the breathing pattern of unanesthetized mice (for details, see Boylestein et al., 1995, 1996; Vijayaraghavan et al., 1993, 1994). Brief descriptions of parameters of interest for the present study are given below. For a thorough description of all respiratory parameters, cf. Nielsen et al. (2005).

Sensory irritation pattern. In humans, chemicals stimulating the trigeminal nerve endings of the upper respiratory tract cause irritation that may increase to burning and painful sensations, termed 'sensory irritation' (Alarie, 1973). Sensory irritants decrease the respiratory rate (f, breaths per min) in mice due to a reflex causing a break at the end of the inspiratory phase. The increase in the time of break (TB, s) and the decrease in f, both show concentration–effect relationships and both can be used to quantify sensory irritation effects, although TB is the more specific of the two parameters (Alarie, 1998).

Airflow limitation. Airflow limitation, also known as bronchoconstriction, may be a result of constriction or inflammation of the conducting airways. Narrowing of the bronchi causes increased resistance that reduces the expiratory flow (VD, mL/s).

Pulmonary irritation. Pulmonary irritation is due to stimulation of vagal nerve endings at the alveolar level. Pulmonary irritation may be quantified by "time of a pause" (TP, s), which is characterized by a short break at the end of expiration. The duration of the TP increases with increasing exposure concentration and thus TP is the specific parameter to quantify this effect (Boylestein et al., 1995, 1996; Vijayaraghavan et al., 1993, 1994).

Exposure conditions and data acquisition. Five OVA- and five saline-sensitized mice, were simultaneously exposed head-only at each exposure concentration. Briefly, mice were inserted into body plethysmographs that were connected to the exposure chamber. The respiratory parameters were obtained for each mouse from a Fleisch

pneumotachograph connected to each head-out plethysmograph that allows continuously monitoring of the parameters (Boylestein et al., 1995, 1996; Larsen et al., 2004; Vijayaraghavan et al., 1993, 1994). The exposures were preceded by a period that allowed the mice to adapt to the plethysmographs. Then, a 15 min period was used to establish baseline (control) values of the respiratory parameters. This period was followed by a 60 min exposure period and a 30 min recovery period. From the baseline period, the mean value for each parameter was calculated for each animal and made equal to 100%. Values obtained during exposure and recovery were expressed as “% of baseline level”. The Notocord Hem (Notocord Systems SAS, Croissy-Sur-Seine, France) data acquisition software was used to collect respiratory parameters and the acquisition conditions were as previously described (Larsen et al., 2004).

Assessment of airway inflammation. Bronchoalveolar lavage (BAL) was performed to assess whether the exposures caused inflammation in the lungs (Brewer et al., 1999; Henderson, 1984). At the end of exposure, the lungs of each mouse were flushed four times with 0.8 mL of saline (0.9%) using a tracheal cannula and the recovered fluid was pooled; the total number of cells and the differential counts were determined as previously described (Hansen et al., 2007a; Larsen et al., 2004).

Statistics. Comparisons of number of inflammatory cells in BAL (Fig. 1) were done by means of the Mann–Whitney’s *U*-test. Effects of FA exposure on the breathing parameters TB (Fig. 2), VD (Fig. 3) and TP (Fig. 4) were compared by calculating the area under the time-response curve (AUC) for each mouse. AUC was calculated using the entire 60-min exposure period, except for TB. Since TB was maximum within the first 20 min of the exposure period (Fig. 2), the response was quantified by calculating the AUC using only this part of the exposure period. The AUCs ($n = 5/\text{group}$) from different groups were compared by means of the Mann–Whitney’s *U*-test. Mann–Whitney’s *U*-test was used for comparison of cell number in BAL fluids. *p*-Values < 0.05 were considered statistically significant.

Results

Lung inflammation

No effect was seen on FA exposure and humidity on the degree of lung inflammation (data not shown). Only the sensitization procedure increased the number of BAL cells and consequently, all OVA-groups were averaged as were the saline groups. As shown in Fig. 1 the sensitization procedure with three i.p. OVA injections followed by two OVA nebulizations successfully induced allergic airway

inflammation as apparent from the significantly increased number of eosinophils ($p = 0.005$), lymphocytes ($p = 0.005$), neutrophils ($p = 0.013$) and macrophages ($p = 0.013$) in the BAL fluid.

Airway effects of FA in sensitized mice

Humid environment. In the humid environment, little effect of allergic sensitization was seen in the upper respiratory tract, as suggested by the superimposed TB responses (Fig. 2, left panel); TB is a specific marker of nose irritation. As typical for nose irritation, the maximum response was reached within the first 20 min of exposure period and thereafter gradually reduced during the remaining part of the period. After cessation of exposure (at time = 60 min), the TB response rapidly approached the baseline level. No apparent difference was revealed by comparison of the AUCs from the OVA and saline sensitized mice, respectively. Similar results were seen at the two lowest FA concentrations (data not shown).

In contrast to the upper airway effects, sensitization to OVA amplified the effect of FA in the conducting airways at the highest FA concentration. Thus, VD decreased more ($p = 0.012$) in the sensitized mice compared with the controls (Fig. 3C), but not at lower concentrations (Figs. 3A and B). The stronger response from the lungs at the higher FA concentrations was also to some extent reflected by a marginally elongated TP, a specific marker of pulmonary irritation, although this prolongation did not reach statistical significance ($p = 0.14$), when compared to the corresponding saline control group (Fig. 4, left panel).

Dry environment. In the dry environment, saline control mice had a high, short-lasting peak in TB, suggesting a vigorous, transient nose irritation (Fig. 2, right panel). However, no statistically significant difference ($p = 0.31$) was seen in the AUCs for the exposure period of 0–20 min between the saline control and OVA-sensitized mice.

OVA-sensitization reduced the airway responsiveness to FA in the conducting airways compared to the saline control mice. Thus, both at the medium and high FA exposure concentrations, the saline sensitized control mice showed a more pronounced reduction in VD compared to the OVA-sensitized mice (Figs. 3E and F), although the difference only reached statistical significance at the highest FA level ($p = 0.022$). The difference in airflow limitation, was supported by the significantly ($p = 0.026$) elongated TP response (Fig. 4, right panel) in the OVA-sensitized mice compared to the control mice at the highest FA exposure level. The TP elongation developed gradually during the initial part of the exposure period and reached a maximum response after about 20–40 min of exposure and thereafter declined. Little effect was seen on TP at lower concentrations (data not shown).

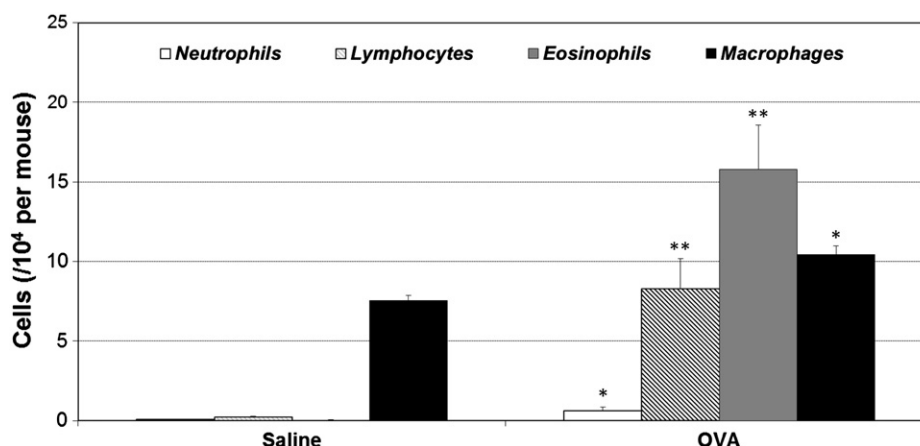


Fig. 1. Inflammatory cells in bronchoalveolar lavage (BAL) fluid. Mice were sensitized to ovalbumin (OVA, $n = 30$) or sham sensitized to saline (NaCl, $n = 30$). A significantly higher number in the OVA group compared to the saline group is indicated by * ($p < 0.05$) or ** ($p < 0.01$).

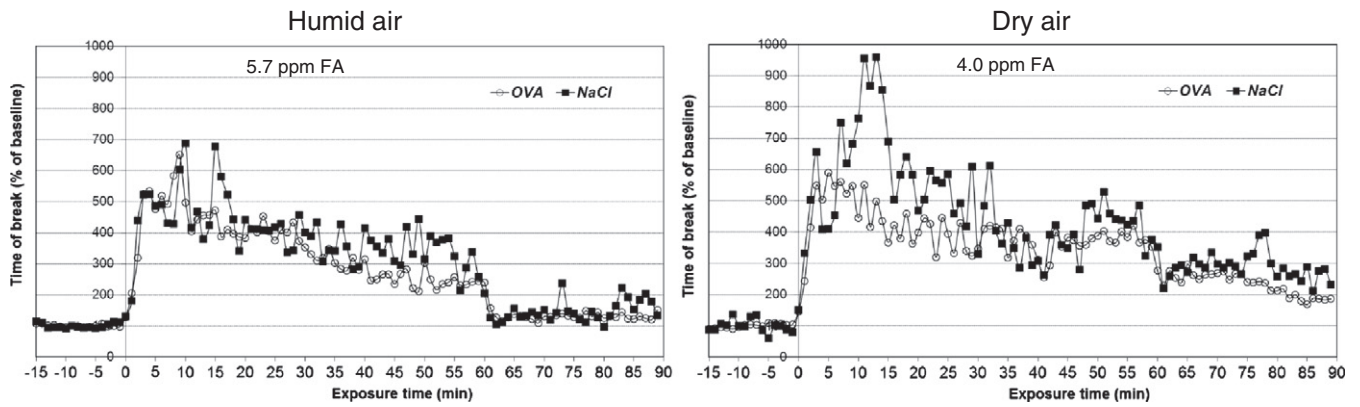


Fig. 2. Effect of formaldehyde exposure on the time of break, a specific marker of sensory (nose) irritation. Mice ($n = 5/\text{group}$) were sensitized to ovalbumin (OVA) or sham sensitized to saline (NaCl). Mice were then housed in environment with either 85–89% relative humidity (“humid air”, left column) or <10% relative humidity (“dry air”, right column) for 48 h. Afterwards, mice were placed in plethysmographs and exposed to clean air to obtain respiratory baseline level. The baseline period was followed by a 60-min exposure to 4.8 (4–5.7) ppm gaseous formaldehyde. Finally, mice were allowed to inhale clean air for 30 min (recovery period).

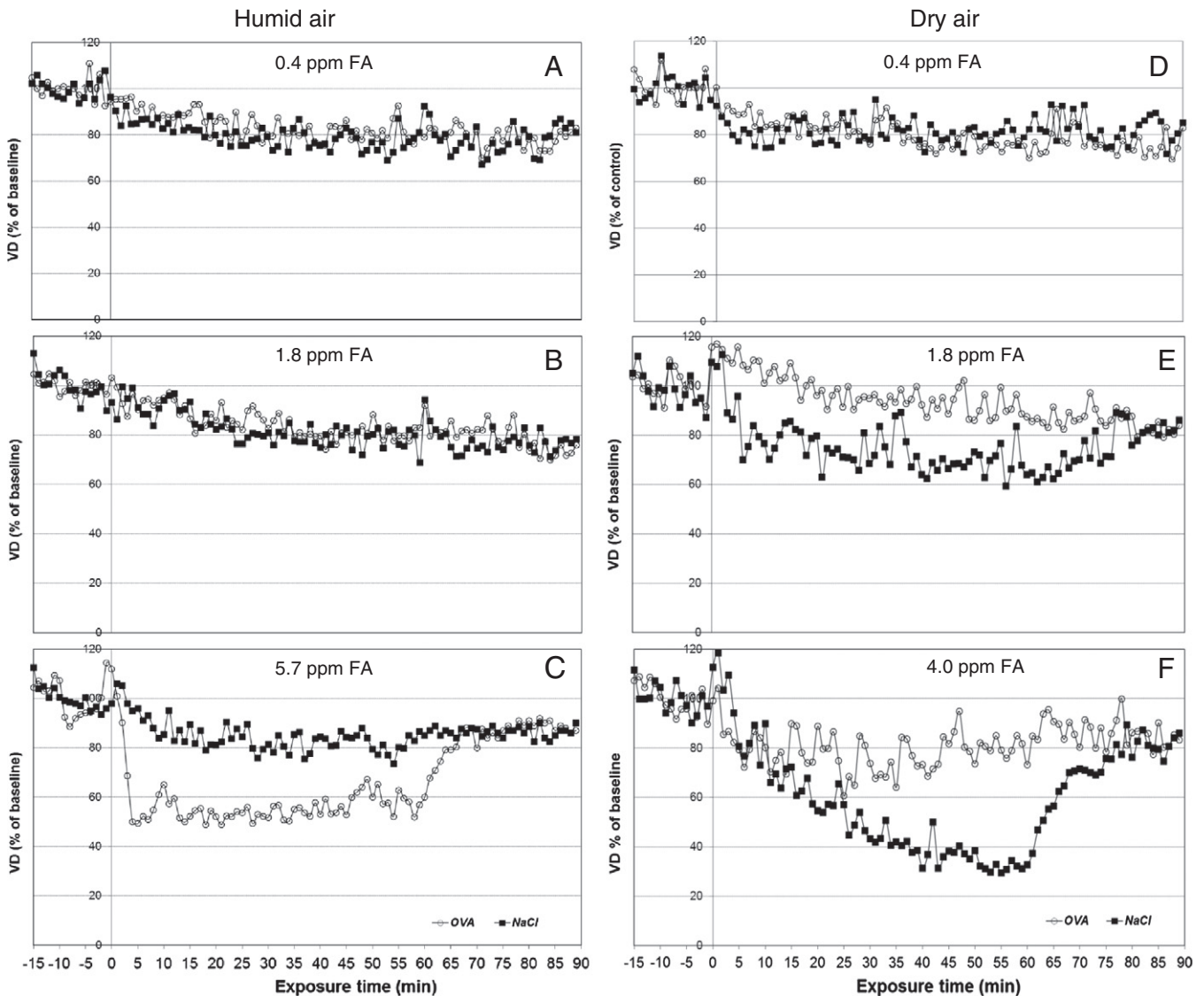


Fig. 3. Effect of formaldehyde exposure on the expiratory flow rate. Mice ($n = 5/\text{group}$) were sensitized to ovalbumin (OVA) or sham sensitized to saline (NaCl). Mice were then housed in environment with either 85–89% relative humidity (“humid air”, left column) or <10% relative humidity (“dry air”, right column) for 48 h. After the acclimatization, mice were placed in plethysmographs and exposed to clean air to obtain respiratory baseline level. The baseline period was followed by a 60-min exposure to 0.42 ppm (panels A and D), 1.8 ppm (panels B and E), 4.0 ppm (panel F) or 5.7 ppm (panel C) gaseous formaldehyde. Finally, mice were allowed to inhale clean air for 30 min (recovery period).

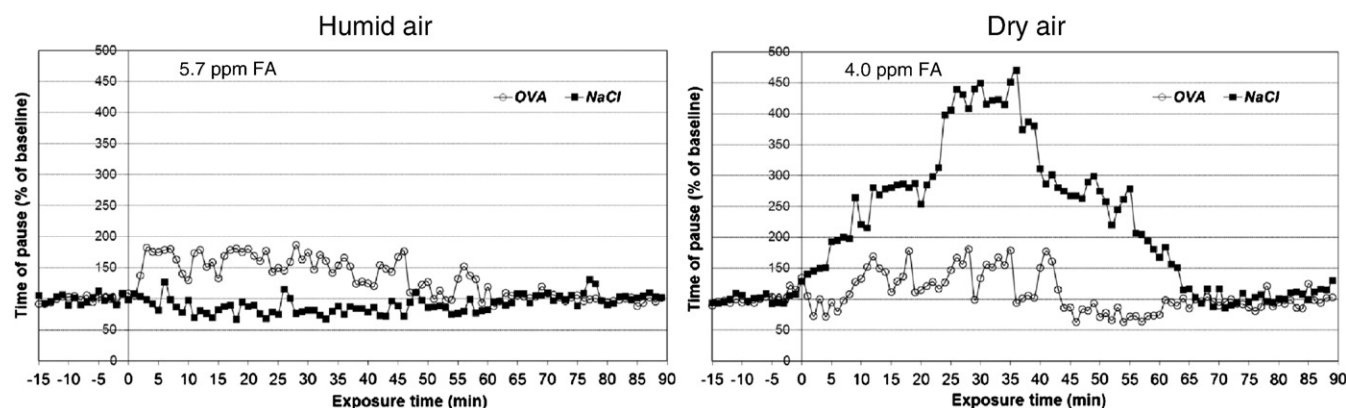


Fig. 4. Effect of formaldehyde exposure on the time of pause, a specific marker of lung irritation. Mice ($n = 5/\text{group}$) were sensitized to ovalbumin (OVA) or sham sensitized to saline (NaCl). Mice were then housed in an environment with either 85–89% relative humidity (“humid air”, left column) or <10% relative humidity (“dry air”, right column) for 48 h. After the acclimatization, mice were placed in plethysmographs and exposed to clean air to obtain respiratory baseline level. The baseline period was followed by a 60-min exposure to gaseous formaldehyde. Finally, mice were allowed to inhale clean air for 30 min (recovery period).

Effect of relative air humidity on airway response to FA

Saline control mice. No effect of humidity was apparent on nasal irritation in the non-sensitized mice even at high concentrations of FA (Fig. 2).

However, the humidity had an impact on the conducting airways at FA > 1.8 ppm. Hence, mice housed in a dry environment had a more pronounced decrease in VD at the highest FA concentration ($p = 0.012$, Figs. 3C and F) compared to mice housed in the humid environment. The amplifying effect of a dry environment on lower airway effects was also apparent from the TP; it showed that the highest FA concentration induced a stronger pulmonary irritation in the dry environment compared to the humid environment ($p = 0.043$, Fig. 4).

OVA sensitized mice. No effect of air humidity was seen for the OVA sensitized mice, neither on TB (Fig. 2), VD (Fig. 3) or TP (Fig. 4).

Discussion

In the present study, the acute airway effects of FA were evaluated in a mouse inhalation model. We studied the roles of allergic airway inflammation and RH on the airway responsiveness to inhaled FA.

Neither the allergic airway inflammation nor the RH changed the sensory (nasal) irritation response to FA in the mice to a statistically significant degree. Neither RH nor airway allergy was observed to influence the sensitivity of sensory irritation to FA at the European Union occupational exposure limit value (0.2 ppm) (<http://ec.europa.eu/social/main.jsp?catId=153&langId=en&intPageId=684>) and the WHO indoor air guideline value (0.1 mg/m³) (WHO, 2010). In contrast, both allergic sensitization and RH changed the effect of FA in the conducting airways at concentrations ≥ 1.8 ppm FA.

In the humid environment, allergic sensitization increased the effect in the conducting airways as apparent from the decrease in the expiratory flow rate (VD). The increase in airway reactivity to FA among the sensitized mice is in line with the general observation that in addition to a specific hypersensitivity to an allergen, allergic airway inflammation is also associated with a “non-specific” hyper-sensitivity to common airway irritants (Raeburn and Webber, 1994). Hence, it has previously been shown that mice sensitized to OVA, using a sensitization protocol similar to the one used in the present study, were hyperreactive to the lung irritant ozone, compared to saline control mice (Larsen et al., 2010). Although the physico-chemical properties of ozone (a deep lung irritant) differ from those of FA, it can reach the lower respiratory tract if the concentrations are sufficiently high. Both FA (Raeburn and Webber, 1994) and ozone (Taylor-Clark and

Undem, 2010) stimulate the transient receptor potential ankyrin 1 (TRPA1). Stimulation of this receptor may lead to the release of e.g. substance P, which is a potent bronchoconstrictor (Raeburn and Webber, 1994). OVA sensitization upregulated substance P in guinea-pigs compared to controls. If a similar upregulation occurs in mice, this could at least in part account for the amplified airflow limitation seen in the OVA-sensitized mice at the high exposure concentration at the humid air condition.

In the dry environment, non-sensitized mice had a more pronounced effect in the conducting airways when exposed to FA than mice housed in the humid environment. Since FA is a highly water soluble and reactive substance, it will be deposited mainly in the mucus in the upper airways (WHO, 2010), which will lead to the retention of the FA before it enters the lower airways. This phenomenon is sometimes referred to as the “scrubber effect”. It is tempting to speculate that very dry environment desiccates the airway mucus, thus reducing the upper airway scrubber capacity, allowing a greater proportion of FA to reach the lower airways. The sensitization protocol we used is known to increase the formation of mucus in the airways (Larsen et al., 2010). It could be speculated that the sensitized mice – due to their higher amount of airway mucus – are less sensitive to the desiccative effect of dry air and are therefore able to maintain a higher scrubber capacity even in the very dry environment.

Conclusion

The present study showed that FA-induced airway irritation in mice is influenced both by air humidity and by allergic airway inflammation. However, these differences in responsiveness to the airway irritant were seen only at high irritant exposure levels (≥ 1.8 ppm). Thus, for risk assessment of FA exposure at the occupational exposure limit or at the indoor guideline levels, our study did not find interaction between FA effects and humidity or allergic airway inflammation at 0.42 ppm FA.

Conflict of interest

The authors declare no conflicts of interest.

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