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Sensory irritation of vapours of formic, acetic, propionic and butyric acid

Gunnar Damgaard Nielsen

National Research Centre for the Working Environment, Lersø Parkallé 105, DK-2100, Copenhagen Ø, Denmark

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ABSTRACT

In mice, inhalation of formic, acetic, propionic and butyric acid caused a rapid decrease in the respiratory rate, which decreased to a stable level during the remaining part of the 30 min exposure period; this was due to sensory irritation. The concentration decreasing the respiratory rate (RD) by 50% (RD50) was 438, 308, 386 and 285 ppm, respectively, which allowed an adequate prediction of the Threshold Limit Values. In mice inhaling through a tracheal cannula, bypassing the trigeminal nerves, caused a slower decrease in respiratory rate due to pulmonary irritation. In the low concentration range, the pulmonary irritation response was less pronounced than the sensory irritation response. As the response in the normal (non-cannulated) mice was not influenced by pulmonary irritation, sensory irritation is the key effect, presumably due to the scrubbing effect of the upper airways, preventing access to the lungs. The activated receptors were in a non-lipophilic (hydrophilic) environment, from where the receptors may be activated by means of liberated protons. At the RD0, formic acid may, at least partly, activates ASIC, TRPV1 and TRPA1 receptors, whereas acetic, propionic and butyric acid may activate ASIC and TRPA1 receptors, based on the estimated pH in the mucus layer.

1. Introduction

Sensory irritation of eyes and upper airways is typically known from exposures to tear gases and high concentrations of industrial chemicals as formaldehyde, hydrogen chloride, sulphur dioxide and ammonia, causing painful, burning, stinging and itching sensations (c.f. Doty et al., 2004; Nielsen and Wolkoff, 2017). Sensory irritation is a neurogenic effect caused by activation of C and A_δ fibers (Doty et al., 2004), which mediate pain sensations (Julius and Basbaum, 2001). Sensory irritation is the critical effect at setting of about 40% of the occupational exposure limits (Brüning et al., 2014; Nielsen and Wolkoff, 2017) and thus, it constitute a highly important endpoint (Brüning et al., 2014). Additionally, sensory irritation is the critical effect at the setting of the indoor air guideline for formaldehyde (WHO, 2010) and it is a critical effect at setting of outdoor air standards (Kuwabara et al., 2007).

Limited *in vivo* data are available on sensory irritation of short-chained organic acids and many of the data are from brief exposure studies as summarized below. In cats, trigeminal nerves innervate the cornea, among others, by thin myelinated polymodal sensory nerve (A_δ) fibers. By acetic acid stimulation (pH: 4.5–6.0), the A_δ fibers responded by a fast insertion of discharge spikes that reached a maximum after 3 s and then desensitized completely or partially, where a low-frequency activity remained until the test solution was washed away 30–60 s later. The receptive site was extracellular as citric acid had the same excitatory effect as acetic acid at a similar pH. Repeated stimulation elicited a response that tended to build up more slowly and lasted longer (Belmonte et al., 1991). In rats, 50-min exposure to acetic acid caused an increase in nasal vasodilation, at least partly, due to activation of the nasal sensory nerves (Stanek et al., 2001). Also in rats, 10 s inhalation-exposure to vapours of propionic acid activated the trigeminal nerves (Silver and Moulton, 1982). Furthermore, flushing the rat nose with formic, acetic and propionic acid caused activation of the trigeminal nerves in a concentration-dependent manner (Wang et al., 2011). In mice, a 10-min inhalation-exposure caused a decrease in respiratory rate due to activation of the trigeminal nerves (Symanowicz et al., 2004).

In humans, the nasal lateralization threshold for formic and acetic acid was 57 and 40 ppm, respectively (Van Thriel et al., 2006), which is an objective measure of sensory irritation at sniff exposure conditions. Furthermore, a 2-sec stimulation with acetic acid vapour caused a stinging and burning sensation in the nose where the former sensation was the most prominent. Additionally, repeated 2-sec exposures with 45 s in between stimuli caused desensitization (Jacquot et al., 2005). A 10-sec inhalation of acetic acid vapour caused a concentration-dependent increase in irritation and odour sensations, an increase in the break before exhalation, a decrease in tidal volume, but no change in the nasal cross-sectional area (Warren et al., 1992, 1994); the decrease in tidal volume occurred at > 10 ppm acetic acid in the air and had a close correlation to the nasal irritation (Warren et al., 1994).

E-mail address: gdn@nrcwe.dk.

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The nasal lateralization threshold for propionic acid was 38 ppm in humans (Van Thriel et al., 2006). In anosmics (individuals without odour perception), the sensory irritation threshold was 25 ppm in one study (Warren et al., 1994) whereas a lower value (11 ppm) was reported in another study (Kendal-Reed et al., 1998). By 15-sec inhalation of propionic acid in the concentration range from 0.16 to 59 ppm, the reported odour and nasal irritation intensity increased monotonously in normal subjects. In anosmics, nasal irritation occurred at 59 ppm, but not at 8 ppm. The duration of a respiration decreased (considered a trigeminal effect) at 59 ppm but not at 8 ppm in normal and anosmic subjects. Analysis of the first 2 s of the exposure period showed a 14% decrease in airway ventilation at 8 ppm with a further decrease at 59 ppm in the normal individuals. In anosmics, a 19% decrease was observed at 59 ppm, but no decrease was observed at 8 ppm. In the normal individuals, the ventilation response was considered a mixed olfactory and trigeminal response (Walker et al., 2001).

Sensitivity of subjects with seasonal allergic rhinitis was investigated by comparison with non-allergic subjects. The exposure was with 15 ppm acetic acid for 15 min. The allergic subjects developed an increase in nasal airway resistance (mean ~30%), whereas the non-allergic subjects showed no significant change. The irritation symptom score was similar in the two groups and the rating was about "slight" (Shusterman et al., 2005), suggesting a modest influence of rhinitis.

Overall, the short exposures are suggesting a lowest No Observed Adverse Effect Concentration (NOAEC) of about 10 ppm by the acids. However, as they don't account for a potential time-effect over longer exposures (Doty et al., 2004), these studies are not sufficient for setting environmental, indoor or occupational exposure limits or guidelines. Our previous study (Nielsen et al., 1996b) together with the controlled chamber studies (Ernstgård et al., 2006; Hey et al., 2009; Pacharra et al., 2016) can qualify setting of the exposure-limits for the acids. The experimental data from our previous study is presented below. It is an inhalation study in mice conducted with the purpose to evaluate the potency of vapours of the acids as sensory and pulmonary irritants as well as for selected systemic effects. The study was conducted twenty two years ago and only described in a short Danish research report by the funding agency (Nielsen et al., 1996b). The method followed the Standard ASTM method E981-84 (ASTM, 1984; Nielsen et al., 1996a). We are not aware of that similar data have been published for formic, propionic and butyric acid since our study in 1996. In this article, the interpretation of the data have been updated in the frame of the current knowledge to provide risk assessment relevant data and it is attempted to establish biological activation mechanisms for airborne exposures to the acids.

2. Materials and methods

2.1. Chemicals

Formic acid (≥98%), acetic acid (≥99.8%), propionic acid (99%) were from Merck and butyric acid (99%) was from Fluka. Physicochemical parameters are listed in Table 1.

2.2. Animals and housing

Male Ssc: CF-1 mice were obtained from Statens Seruminstitut, Denmark. The mice were placed in polycarbonate cages with sawdust bedding. Food (Altromin nr. 1324) and tap water were available ad libitum. The light:dark cycle was 12:12 h.

For exposure of mice via a tracheal cannula, mice were anesthetized with 50 mg/kg body weight of sodium pentobarbital i.p. A tracheal cannula was inserted, secured by a suture, and the skin incision was closed with cyanoacrylate glue. Each mouse was inserted in a plethysmograph and was allowed to recover before the exposure (Nielsen et al., 1996b). Each exposure used a new group of 4 mice.

The study was in accordance with the permission by the Danish Animal Inspectorate.

Table 1
Physicochemical properties.

Substance	Molecular weight[a]	Solubility in water g/l (mol/l)	pKa[c]	Log L^{w}[d]	Log Po/w[e]	Log Po/g[f]
Formic acid	46.03	Miscible[a]	3.77	5.10	−0.54	4.56
Acetic acid	60.05	Miscible[a]	4.76	4.91	−0.17	4.74
Propionic acid	74.08	~300 (4.0)[b]	4.88	4.74	0.33	5.07
Butyric acid	88.11	~80 (0.9)[b]	4.82	4.66	0.79	5.45

[a] Budavari et al. (1996).
[b] From Di Carlo (1990).
[c] From Takahashi et al. (1971).
[d] The Ostwald solubility coefficient in water (L^{w}) at 298 K. The value for formic acid was derived from Clegg and Brimblecombe (1990). Other values are from Abraham et al. (1994).
[e] Log octanol-water partition coefficients from Sangster (1989).
[f] Calculated log octanol-gas partition coefficient: log Po/g = log Po/w + log L^{w}.

2.3. Generation of gas-air mixtures

A dynamic exposure system was used. The gas-air mixtures were generated by means of an aerosol generator and the aerosols were mixed with dilution air; the aerosol generator was heated to secure complete evaporation of the acids. The acids were feed into the aerosol generator by means of a motor driven pump (ASTM, 1984). The gas-air mixture was led to a 3.3-l whole glass exposure chamber with four attached plethysmographs for accommodation of mice (ASTM, 1984). The nominal concentration in ppm (ml gas per m^3 gas-air mixture) was obtained from the evaporated amount of acid and the gas-air flow through the chamber, which was monitored by Fischer & Porter precision flowmeters; the flows varied from 18.6 to 26.6 l/min. The nominal concentration (C ppm) was calculated by means of the equation:

$$C = [V \cdot \varrho \cdot 24.45 \cdot 10^{6}]/[M \cdot F]$$

The infusion rate into the aerosol generator is V (ml/min), the density of the liquid acid is ϱ (g/ml), 24.45 is the molar volume (l/mol) of an ideal gas at 25 °C, M is the molecular weight (g/mol), and F (l/min) is the airflow through the exposure system. Additionally, the chamber concentrations were monitored continuously by infrared spectroscopy (ASTM, 1984; Nielsen et al., 1996a). The difference between the nominal and the monitored exposure concentrations was normally less than 10%.

2.4. Exposure conditions

Each mouse was inserted into a body plethysmograph, which was attached to the exposure chamber. The head of the mice protruded into the exposure chamber. Four plethysmographs were attached to the chamber. The respiratory rate and the relative tidal volume of each mouse were obtained continuously from the attached pressure transducer to each plethysmograph. Data were recorded on a dynograph and collected by a computer and mean values of each 1-min period was used in the data analyses. After normal mice being inserted into the plethysmographs, a period for settling down (~10 min) of the mice was used. After that, a pre-exposure baseline period of 10 min was recorded, which was followed by an exposure period (30 min) and a 20-min recovery period (ASTM, 1984; Nielsen et al., 1996b).

2.5. Evaluation of respiratory effects

In mice, substances that activate the trigeminal nerves in the upper