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Human exposure to acrolein: Time-dependence and individual variation in eye irritation

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ABSTRACT

The aim of the study was to examine the time dependence on sensory irritation detection following exposure to threshold levels of acrolein, in humans. The exposures occurred in an exposure chamber and the subjects were breathing fresh air through a mask that covered the nose and mouth. All participants participated in four exposure conditions, of which three consisted of a mixture of acrolein and heptane and one of only heptane. Exposure to acrolein at a concentration half of the TLV-C lead to sensory irritation. The perceived sensory irritation resulted in both increased detectability and sensory irritation after about 6.8 min of exposure in 58% of the participants. The study confirm the previously suggested LOAEL of about 0.34 mg/m^3 for eye irritation due to acrolein exposure. The sensory irritation was still significant 10 min after exposure. These results have implications for risk assessment and limit setting in occupational hygiene.

1. Introduction

The detection of chemicals in the environment is mediated by two separate, but interrelated, systems in humans: the olfactory and trigeminal systems. Stimulation of the olfactory system (cranial nerve I) results in sensations of smell, while stimulation of the trigeminal system (cranial nerve V) evokes reactions such as sneezing, watering of the eyes, irritation, and pain (Doty et al., 2004). For most chemicals, both nerves are activated, although at different concentrations. At low concentrations only odor is detected; as the concentration increases, sensory irritation is perceived. The difference between estimated sensory irritation thresholds and corresponding odor thresholds are generally orders of magnitude (Cometto-Muñiz et al., 2004). The chemicals identified in indoor air are usually present at concentrations below the sensory irritation threshold (as well as below occupational threshold limit values) (Brown, 1999; Korpi et al., 2009; Sunesson et al., 2006), but problems related to sensory irritation attributed to indoor air are nevertheless reported by about 4–7% of the population (Eriksson and Stenberg, 2006). There is convincing evidence demonstrating that if the ventilation rate increases, the number of reported symptoms decreases (Sundell et al., 2011). Volatile organic compounds (VOCs) can be ventilated, and despite the fact that they are typically present at low concentrations, they are a possible contributing factor to perceived sensory irritation in indoor air. Sensory irritation is therefore an important endpoint in the development of guidelines in both occupational and environmental toxicology.

The influence of time on sensory irritation is well documented and probably dependent on both the concentration and the compound. Perceived irritation has in some studies been found to increase during the first 20–40 min of exposure with no evidence of adaptation (Hempel-Jørgensen et al., 1999; Hudnell et al., 1992; Molhave et al., 1986). In other studies increased sensitivity were reported in the beginning of the exposure but then adaptation occurred after about 30 – 60 min (Cain et al., 1986; Ernstgård et al., 2006) or after repeated exposures during consecutive days (Dalton et al., 2006). However, in these studies, VOCs at relatively high concentrations with little relevance to actual indoor air exposure levels were used. Exposure studies using lower concentrations of VOCs did not report an effect of time (Cain et al., 2007; Claeson et al., 2009; Ernstgård et al., 2013). Temporal integration is often studied by brief exposures (up to 10 s) at concentrations above threshold and sensory irritation has been shown to be dependent on the total mass delivered to the site of action. According to Haber's rule (c x t = k) used in risk assessment, time and concentration is equally important to produce sensory irritation, but for longer durations, it has been demonstrated that concentration usually have a larger influence on sensory irritation than time (Shusterman et al., 2006).

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Studies investigating the effect of time on exposures at or below threshold levels are rare. Wise and colleagues investigated temporal integration at the threshold level for CO_2, NH_3, and ethanol (Wise, 2004; Wise et al., 2007, 2005). The same model of imperfect integration as identified for exposures above threshold could also be applied in subthreshold exposures, but the degree of integration could not be predicted (e.g., the slope). Certain reactive compounds, such as methylisothiocyanate (MITC), with known reactive properties towards specific receptors (e.g., TRPA1) show near perfect integration, where half of the concentration required the doubling of time (Cain et al., 2010).

Acrolein (2-propenal), another known TRPA1 agonist, is a highly reactive VOC present in cigarette smoke, smoke from fires, automobile exhaust, and smog. Emissions from certain building materials also contain acrolein. The compound is found in both outdoor and indoor air, but it is present at higher concentrations indoors (Seaman et al., 2009). Indoor air concentration ranges from <0.05–29 $\mu g/m^3$, although in restaurant kitchens and bakeries, higher levels have been measured (0.02–0.6 mg/m^3) (Faroon et al., 2008). Acrolein has an acrid, pungent odor, with sensory irritating effects on the mucous membranes, especially in the eyes (Beauchamp et al., 1985). It has been shown to exacerbate asthma in children and it is also suspected to contribute to other chronic airway diseases (Bein and Leikauf, 2011; Woodruff et al., 2007). Acrolein causes sensory irritation by reacting with the TRPA1 channel, a channel known to be activated by a wide variety of environmental irritants that share a special electrophilic group (Bautista et al., 2006). The reactive group forms reversible covalent bonds with cysteine residues and, therefore, activation is expected to be time dependent, as more energy is required to break the bond than to make it. Sensory irritation through such covalent bonds seems to be a unique feature of the TRPA1 channel, and this modification can lead to irritation at low levels of exposure, and possibly to time-dependent amplification of sensory irritation (Bessac and Jordt, 2008). The reaction is also likely to be highly dependent on the chemical environment surrounding the channel. Moreover, most TRPA1 agonists react with cellular and extracellular glutathione, a compound that acts to remove potentially harmful substances from the body. The concentration of glutathione is therefore crucial, since when all available glutathione is depleted, sensory irritation will likely increase, leading to a cumulative effect of the environmental irritant reacting with the TRPA1 channel (Bessac and Jordt, 2008; Ganea and Harding, 2006; Hinman et al., 2006; Macpherson et al., 2007). Knowledge about the reactivity towards such special receptors makes it plausible that acrolein and other compounds containing the same functional group (α,β-unsaturated aldehydes) would react differently when compared to other compounds (Cain et al., 2010). Therefore methods, such as continuous exposure in an exposure chamber, which takes time-dependence into account should be used when investigating sensory irritation detection thresholds to compounds like acrolein.

In addition to exposure level and duration, the intensity of the reported sensory irritation is dependent on a number of non-sensory factors (Brüning et al., 2014), such as earlier experiences and/or negative information about a compound (Andersson et al., 2013; Dalton, 1996). Self-reported stress and negative affect have been proposed to exacerbate the reports of sensory irritation from some exposures but not from others (Andersson et al., 2013; Dalton and Jaén, 2010; Mueller et al., 2013; Smeets and Dalton, 2005). Women generally report more sensory irritation than do men (Cometto-Muniz and Noriega, 1985; Olofsson and Nordin, 2004; Shusterman et al., 2003); however, the difference might not be that women are more sensitive per se (Hummel et al., 2003; Mattes and DiMeglio, 2001). Rather, women, compared to men, perceive weak concentrations as more irritating and seem to use different strategies when detecting possible health hazards (higher false-alarm rate) (Claeson and Nordin, 2011). Inter-individual differences in sensory irritation thresholds have been reported in earlier studies and are concluded to originate mainly from input from the olfactory system (Dalton et al., 2000; van Thriel et al., 2008) or from methodological differences between studies (Cain and Schmidt, 2009)

The main objective of this study was to examine the time dependence of sensory irritation detection following exposure to threshold levels of the TRPA1 agonist, acrolein, in humans. The focus of the study was on the detection of sensory irritation, and not to evoke health symptoms; therefore, only the eye – which is considered to be most sensitive towards acrolein – was investigated (Beauchamp et al., 1985; Gomes et al., 2001). The eye-only exposures were also performed to avoid any bias from olfaction. Concentrations at or below previously reported sensory irritation thresholds that were initially too low to evoke sensory irritation in the eye, but that might do so in exposures of up to 60 min, were used. Detection of sensory irritation was measured with confidence ratings which in earlier studies have shown to correlate well with actual detection (Cain et al., 2007). Data on perceived intensity was also collected using magnitude estimation during exposure. Objective measurements of eye irritation, such as blink frequency and self-reported tear-film break-up time (BUT[s]), were also used. The second objective was to study inter-individual differences in sensory irritation detection and perceived intensity during exposure to acrolein.

2. Material and methods

2.1. Subjects

Twenty-six non-smoking individuals (18 women and 8 men) were recruited by an advertisement in the local newspaper and through billboard advertisement. All participants considered themselves to be healthy and are further described in Table 1. Smoking and pregnancy constituted the exclusion criteria. Subjects normally wearing contact lenses (n = 2) were asked not to wear them during the exposures. The subjects also filled out the Chemical Sensitivity Scale (CSS) (Nordin, 2003) and the Perceived Stress Questionnaire (PSQ) (Levenstein et al., 1993). The CSS is a questionnaire that is used to assess the affective reactions and behavioral disruptions resulting from odorous/pungent substances, and the PSQ quantifies the extent to which individuals subjectively perceived stress during the previous 4 weeks. The study was conducted in accordance with the Declaration of Helsinki, and the subjects provided their informed consent. The protocol was approved by the Ethics Committee of Umeå University (Dnr: 2012-112–31 M).

2.2. Exposure chamber

Exposure occurred in an exposure chamber ($1.5 \times 0.9 \times 2.0$ m). The mean temperature during exposure was $21\,^{\circ}C \pm 1\,^{\circ}C$ and the mean relative humidity (RH) was $18\% \pm 3\%$, which was slightly higher than the outside RH at the time of exposure. There were no significant differences in either RH ($P = 0.68$) or temperature ($P = 0.24$) between the exposures. Carbon-filtered air entered the chamber through an inlet at floor level and exited in the ceiling; the air exchange rate was set to 7.5 times/hour (approximately 330 L/minute). A metered amount of stimulus material was continuously pumped (by a syringe pump) through a nebulizer (OneNeb, Agilent Technologies). The aerosol from the nebulizer was mixed with air (4 L/minute) in an evaporation chamber with a volume of ~1 L. The air mixture was then further diluted and transported to the exposure chamber. In order to perform eye-only exposure, the subjects used a fresh air mask covering the nose and mouth.

Table 1
Descriptive data of the participants.

	Responders (n = 15)	Non-responders (n = 11)	P-value[e]
Sex (n; women/men)	12/3	6/5	
Age (years; mean ± SD)	32.9 ± 14.8	32.0 ± 12.0	Ns
Do you use:			
glasses?	6	3	Ns
lenses?	2	0	Ns
Perceived Stress Questionnaire[a], PSQ (mean ± SD)	0.30 (0.15)	0.24 (0.09)	Ns
Chemical Sensititvity Scale[b], CSS (mean ± SD)	59.7 (13.6)	58.8 (20.8)	Ns
Perceived general health, % (n)			
Very good or excellent	53.3 (8)	91.0 (10)	Ns
Good	40.0 (6)	9.0 (1)	
Poor or fairly good	6.7 (1)	0.0 (0)	
Reported no of symptoms[c], mean (±SD)?			
Airway, mucosae and skin, out of 13	0.3 (0.5)	0.5 (0.5)	Ns
Gastrointestinal, out of 2	0.6 (0.5)	0.2 (0.4)	<0.05
Head related, out of 2	0.5 (0.5)	0.1 (0.3)	<0.05
Cognitive and affective, out of 10	0.4 (0.5)	0.3 (0.5)	Ns
Diagnosis[d] (n)			
Asthma/allergy	0	0	
High blood-pressure	2	1	
Depression	2	0	
Chronic sinusitis	0	1	
Migraine	0	0	
Irritable Bowel Syndrome, IBS	1	0	
Chronic fatigue syndrome	2	0	

[a] Levenstein et al., 1993.
[b] Nordin, 2003.
[c] Andersson et al., 2009.
[d] Self-report of having been given a diagnosis by a physician.
[e] P-values refer to results of independent samples *t*-test. Ns not significant.

2.3. Stimulus material

The stimulus material consisted of acrolein (90% with 0.1-0.2 wt% hydroquinone as stabilizer, Sigma Aldrich Co., St Louis, MO, USA) and heptane (≥99%, Thermo Fisher Scientific, Waltham, MA, USA). Three different concentrations of acrolein diluted in heptane were used for the acrolein exposures, while for the control exposure, only heptane was used. The concentrations used at the three exposure times with acrolein were at or below previously reported sensory irritation thresholds (e.g., between 0.13 mg/m^3 and 1.2 mg/m^3, Weber-Tschopp et al., 1977; Gomes et al., 2001; Kuwabara et al., 2007). The lowest concentration in the chamber was 0.07 mg/m^3, the intermediate concentration was 0.16 mg/m^3, and the highest concentration was 0.36 mg/m^3. The low and high concentrations were approximately half the concentration of the Swedish occupational threshold limit for 15 min (0.7 mg/m^3) and 8 h (0.2 mg/m^3), respectively (Arbetsmiljöverket, 2011). Heptane was used as the control condition and it was present in all exposures. The concentration of heptane during the control exposure was 20.3 mg/m^3, and the measured concentration of heptane during the acrolein exposure did not differ significantly from the control exposure ($P = 0.29$).

2.4. Chemical analysis

The concentration of acrolein and heptane in the exposure chamber was monitored by direct injection into a GC-FID (gas chromatography coupled to a flame ionizing detector; HP5890) system equipped with a fused silica column HP Ultra-2; 50 m × 0.2 mm ID coated with cross-linked 5% phenylmethylsilicone; film thickness of 0.33 μm. The data were recorded, integrated, and quantified by Waters Empower software (Waters, Milford, MA, USA) using calibration curves obtained from metered amounts of acrolein and heptane (the lowest concentration of acrolein [0.07 mg/m^3] was calculated by extrapolation from the standard curve). A syringe filled with 0.1 mL of air taken from the chamber was injected into the GC-FID, which was operated in splitless mode, with a temperature starting at 35 °C and rising 2 °C/min until reaching 200 °C.

2.5. Eye irritation

The influence of time on sensory irritation detection was measured with judgements of confidence. Perceived intensity was measured using magnitude estimation. Each time the magnitude of eye irritation was rated, the subject also attached a level of confidence ranging from (1) not certain to (2) very certain. This means that if the subject did not detect eye irritation at all, the answer would be "no" and the subject added a level of confidence ranging from 1 to 2. If the subject did feel something but was not sure, the answer would be "yes", then either 1 or 2 was recorded, depending on the level of certainty. These ratings were then transformed into a scale of 1–4, where the answer "no" together with level of certainty rated as 1 or 2 became 1 or 2, respectively; the answer "yes" together with 1 or 2 became 3 or 4, respectively. The midpoint between 2 and 3 indicated the transition between "no" and "yes" (Gescheider, 1997). Confidence ratings have in earlier studies been shown to correlate well with actual detection (Cain et al., 2007).

Perceived eye irritation during the exposure was rated on the Borg CR-100 scale (Borg and Borg, 2001). The CR-100 scale is a verbally anchored ratio scale that is often used for measuring sensory perception. The scale has descriptive adjectives corresponding to specific numbers on the scale (nothing at all, 0; minimal, 2; extremely weak, 3; very weak, 5; weak, 13; moderate, 25; fairly strong, 37; strong, 50; very strong, 70; and extremely strong, 90).

Two methods were used to further assess eye irritation during exposure. First, the subjects were filmed during the exposure, and the number of eye-blinks were counted manually with a hand

Table 2
Description of the exposure conditions and range of mean sensory- and confidence rating.

Concentration (mg/m^3)	Duration (min)	C x T (mg/m^3–min)	Sensory rating (0–100)[a]	Confidence rating (1–4)[b]
0.36	15	0.7–5.0	0.3–13.8	1.9–3.3
0.16	45	0.8–7.2	2.3–6.9	1.7–2.7
0.07	60	0.4–4.2	0.0–11	1.5–2.7
Sham exposure	30	–	1.9–6.4	1.8–2.5

[a] Borg CR-100 scale (Borg and Borg, 2001).
[b] 1–2 indicates "no detection" and 3–4 indicates "yes detection".

tally counter at three time points during exposure: after 2 min of exposure, halfway through the exposure, and 2 min before leaving the chamber. The blink frequency was calculated as the mean over 5 min and only blinks were the majority of the eye were covered were counted (twitches were ignored). Second, self-reported tear-film BUT were measured before the exposure, immediately after, and 10 min after finishing the exposure. This method measures the length of time that the subject can keep his or her eyes open while watching a fixed point on the wall. Self-reported tear-film BUT has shown good agreement with the fluorescein method for the detection of tear-film BUT (Wyon and Wyon, 1987).

2.6. Procedure

All participants visited the laboratory four times on separate days and took part in the four exposure conditions that differed both in terms of the duration (15, 45, 60, and 30 min) and concentration of acrolein (high, intermediate, low, and control). In this way the exposure times and concentrations varied such that equivalent (or overlapping) concentration x time (C x T) products were achieved and the influence of both time and concentration could be evaluated (Table 2). The exposures were executed in a balanced design. During exposure, the level of confidence and eye irritation were rated every other minute (during the 15-min exposure period) or every 5 min (during the 30-, 45-, and 60-min exposure periods). The ratings also took place before and immediately after each exposure session, together with the BUT(s) measurements.

2.7. Statistical analysis

Statistical analysis was performed using repeated measures analysis of variance (ANOVA) and independent samples *t*-test with SPSS version 22 (IBM Corporation, Armonk, NY, USA). The α-level was set to 0.05. Where the assumption of sphericity was violated, the significance level was adjusted with the Greenhouse–Geisser correction of the degrees of freedom resulting in a stricter α-level.

Table 3
F-values for the repeated measures ANOVA. Based on judgments of confidence (detectability) and magnitude estimations (perceived intensity).

Duration (min)	Detectability		Perceived intensity	
	All	Responders only	All	Responders only
15	10.5***	9.8***	8.4***	9.3***
30	3.2*	1.9	2.6	2.7
45	4.8***	3.1**	3.0*	1.7
60	5.2***	4.0**	3.1****	2.8

**** $p = 0.06$.
*** $p < 0.001$.
** $p < 0.01$.
* $p < 0.05$.

3. Results

Acrolein exposure at or below previously reported thresholds caused increased detectability and perceived sensory irritation with time. At the beginning of the exposure, none of the concentrations seemed to elicit a reaction. The response initially manifested itself as a reduction in confidence that a sensation had occurred, and also in increased perceived intensity (Fig. 1a). During all the exposures the rated confidence increased significantly but at no time did the average rating of confidence reach "yes", although the perceived intensity increased with time of exposure (from "nothing at all" to almost "weak"); that is, subjects were uncertain about their detection of irritation following exposure (Table 3).

A two-way mixed model ANOVA showed no significant main effect of exposure condition (with or without acrolein) on the two objective measurements of eye blink frequency and BUT[s] ($P = 0.59$ and $P = 0.11$, respectively). A significant main effect of time was only identified for blink frequency during the 60 min exposure condition ($P < 0.05$). No significant interactions were identified ($P > 0.15$) (Table 4).

There was a large inter-individual variability in the ratings of eye irritation among participants, as 42% did not detect acrolein at all, while 58% rated it as clearly irritating. The data were thus divided

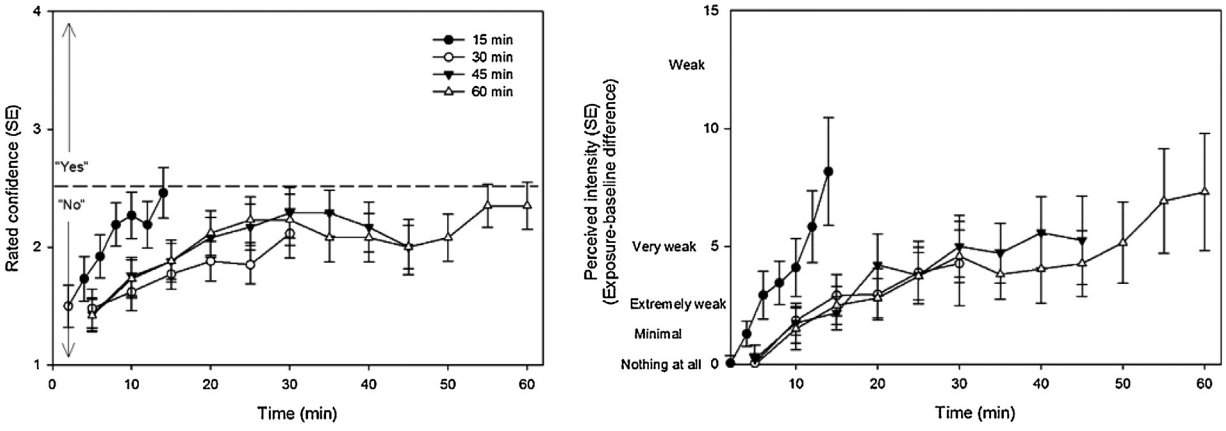

Fig. 1. Ratings of confidence (left) and magnitude (right) of eye irritation for all subjects during exposure to acrolein and heptane (15, 45 and 60 min) and heptane (30 min).

Table 4
Mean (±SD) for self-reported tear-film break-up time (BUTs) measured before and after exposure and mean (±SD) blink frequency during exposure.

Duration (min)	BUT			Blink frequency		
	Pre	post	Post10	1st	2nd	3rd
15	27.7 (25.0)	31.4 (30.0)	28.3 (24.7)	26.6 (18.3)	27.5 (18.5)	27.5 (16.0)
30	27.2 (30.0)	28.2 (29.8)	27.8 (23.7)	26.5 (17.1)	27.6 (18.4)	27.1 (19.5)
45	28.5 (24.6)	20.6 (19.1)	26.1 (28.1)	28.7 (21.8)	29.6 (20.6)	33.5 (19.3)
60	22.6 (22.3)	23.9 (22.8)	26.6 (20.5)	23.3 (15.5)	27.8 (19.0)	29.2 (18.7)

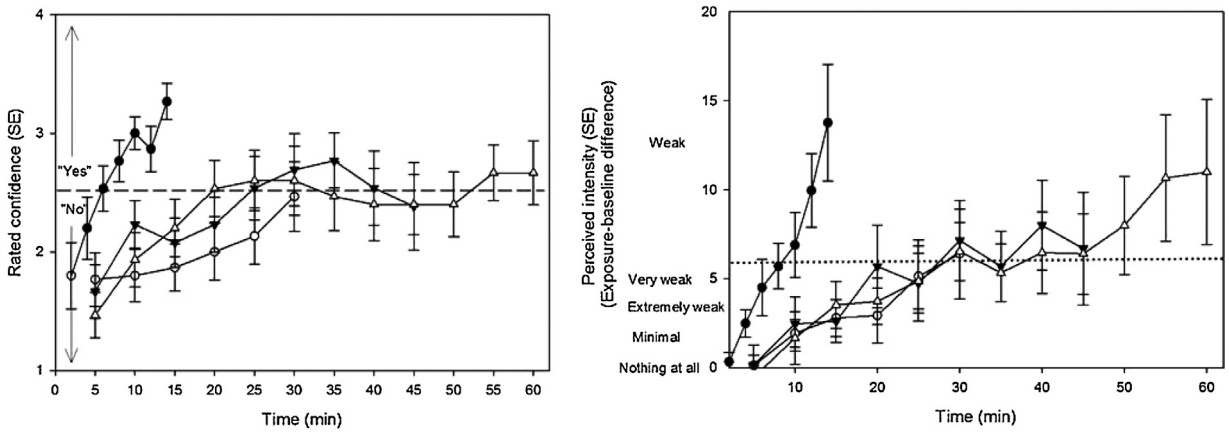

Fig. 2. Ratings of confidence (left) and magnitude (right) of eye irritation for the responders during exposure to acrolein and heptane (15, 45 and 60 min) and heptane (30 min). The dotted line represents the highest rating for heptane only performed for 60 min at the same concentration as in the present study.

into one of two groups based on the confidence ratings for the 15-min exposure period. Individuals that reached "yes" in terms of confidence (>2.5) at some point during the 15-min acrolein exposure were called "responders" (n = 15), and individuals that did not reach this level of confidence were called "non-responders" (n = 11). Detectability, expressed as ratings of confidence and perceived intensity for the responders is shown in Fig. 2 and Table 3. The judgements of confidence during all three exposures containing acrolein increased significantly with time for the group of responders, whereas the sham exposure with only heptane did not. The perceived intensity as rated by the responders increased only with time for the 15 min exposure. Also, the level of confidence and perceived intensity was only during the 15 min exposure significantly higher than the sham exposure. The shortest exposure with the highest concentration also elicited significantly more eye irritation immediately after and also 10 min after the exposure was ended (Fig. 3).

To compare sensory irritation detection (for the responders only) across exposure conditions and to evaluate the influence of response bias (from heptane exposure) the detection rate for each concentration corrected for false alarms was calculated by the equation (Gescheider, 1997):

$$P_c = (P_{hit} - P_{FA}/1 - P_{FA})$$

Where P_c represents the proportion of a correct response (confidence rating ≥ 3) corrected for response bias. P_{Hit} represents the proportion of a positive response to acrolein exposure and P_{FA} a positive response to the sham exposure (the last P_{FA} for the 30 min exposure was used as false alarm rate for exposures beyond 30 min). The P_c values are presented in Fig. 4 as a function of the products of concentration and time (C x T in log units). The detection threshold was calculated by fitting a regression line by the method of least squares to the data and a value of 1 indicates certain discrimination. From the regression line a corrected detection threshold at $P_{0.5}$ was calculated for the 15 min exposure

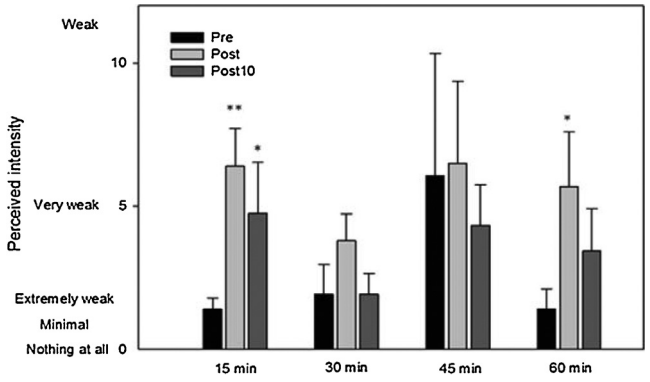

Fig. 3. Ratings of magnitude of eye irritation before, immediately after exposure and 10 min after exposure to acrolein (15, 45 and 60 min) and heptane (30 min).

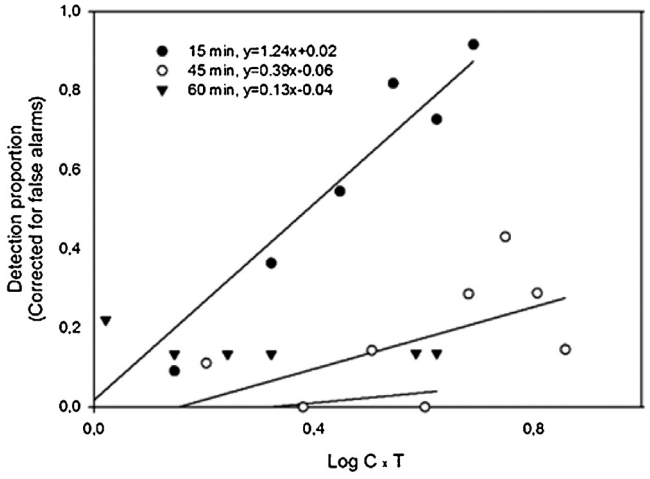

Fig. 4. Irritation detection proportion corrected for response bias as a function of the product of acrolein concentration and exposure time (C x T) in logarithmic units.

condition, C x T = 2.3 (the only condition reaching above chance). This means that acrolein could be detected after 6.8 min of exposure at 0.36 mg/m^3. The mean (SD) false alarm proportion for the responders was 0.35 (0.28) and for the non-responders 0.08 (0.27) and there was a significant difference between the groups ($F = 6.00$, $P < 0.05$).

The difference between the groups of responders and non-responders was also manifested as a tendency towards differences in blink frequency and BUT(s) irrespective of exposure condition. The total number of counted eye-blinks during the four exposures seems to be higher in the group of responders compared with the non-responders (29.9 ± 18 and 16.6 ± 8.3, respectively, $P = 0.07$). Furthermore, BUT(s) measurements showed the same tendency ($P = 0.07$) with an overall difference between the groups (responders: 18.2 ± 14.5 and non-responders: 32.2 ± 18.8).

4. Discussion

This study suggests that exposure to acrolein below the threshold limit value for 15 min leads to sensory irritation in more than half of the participants. The sensory irritation was time dependent and it took about 6.8 min of exposure to acrolein at 0.36 mg/m^3 to become detectable above chance. For the prolonged exposures at lower concentrations the detection rate did not reach above chance. The level of confidence was generally low but increased significantly with time for all exposures except the sham exposure (Table 3). Exposure to the intermediate concentration (0.16 mg/m^3) indicates that the duration of exposure needs to be extended to about 90 min in order to be detected above chance (Fig. 4). This is supported by the study by Dwivedi et al. (2015), that found increased eye irritation after about 100 min of masked exposure to acrolein at 0.2 mg/m^3. The lowest concentration used in this study (0.07 mg/m^3) are according to Fig. 4 not able to generate a chemesthetic effect. Previous studies on thresholds for sensory irritation from acrolein indicate LOAEL (Lowest Observed Adverse Effect Level) in the range of 0.2–0.7 mg/m^3 which the results of this study confirms (Dwivedi et al., 2015; Kuwabara et al., 2007; Trantallidi et al., 2015; Weber-Tschopp et al., 1977). The rated perceived intensity was low but with the knowledge about the role of the TRPA1 channel in neurogenic inflammation and the time dependence identified in this study, even a low level of sensory irritation is of interest (Andrè et al., 2008; Bautista et al., 2013).

The identified time dependence on sensory irritation could be due to the nature of the chemical bonding between the TRPA1 agonist and the channel's cysteine residues, together with the effectiveness of glutathione, which acts to remove the sensory irritant (Bessac and Jordt, 2008). The design of this study with varied concentration and time generated overlapping C x T values (see Table 2) and our data show that the concentration of acrolein had a larger influence on sensory irritation than time (e.g. imperfect integration). This result is consistent with results from studies on other reactive compounds such as formaldehyde and 1-octene (Cain et al., 1986; Hempel-Jørgensen et al., 1999). On the other hand is this in contrast to another TRPA1 agonist, MITC which show near perfect integration (Cain et al., 2010). Another effect of the time dependent reaction of acrolein is that the responders in the current study not only perceived more eye irritation immediately after, but also 10 min after the 15-min exposure. This indicates a delay in the removal of acrolein (Fig. 3). Such a delay should be taken into account in the design of future studies investigating sensory irritation from TRPA1 agonists. The time dependence of sensory irritation might also have implications on the interpretation of the results from previous studies were the concentration of acrolein were continuously increased during exposure (Weber-Tschopp et al., 1977).

The current study identified large inter-individual differences in the response to acrolein and this has also been found in former studies (Dwivedi et al., 2015; Weber-Tschopp et al., 1977). Earlier studies have discussed the possibility that individual differences related to the TRPA1 channel could be dependent on the chemical atmosphere surrounding the channel. The chemical status in these cases is thus in reference to the amount of glutathione present together with endogenous compounds involved in oxidative stress or inflammation, which have been shown to activate the TRPA1 channel (Bessac and Jordt, 2008). Sensitivity towards acrolein exposure as a general trait is supported by the overall difference in blink frequency and BUT(s) between responders and non-responders.

Non-sensory factors such as stress or chemical sensitivity may explain some of the inter-individual differences found in perceived sensory irritation (Smeets and Dalton, 2005). The increased false alarm rate seen among the responders in this study suggests that the responders are more worried than the non-responders about potential health effects and therefore use other strategies when detecting possible health hazards. This awareness implies that sensitive individuals probably makes more false alarms since the consequence of making a miss is believed to be negative for the individual. However, the responders in this study, did not report more stress, affective reactions, or behavioral disruptions by odorous/pungent compounds when compared to the non-responders (Table 1). According to normative data the levels on PSQ and CSS reported by the participants in this study are within the range of a normal population (Bergdahl and Bergdahl, 2002; Nordin, 2004). Gender is another non-sensory factor that are associated with individual differences in reported sensory irritation. Women rate irritation as being stronger, and report more false alarms, when compared to men (Claeson and Nordin, 2011; Lundström et al., 2005; Shusterman et al., 2003). In the present study, too few men participated to be able to compare men and women; thus, a difference in the reported eye irritation due to acrolein exposure, as related to gender, cannot be excluded.

It has been suggested that inflammation of the upper airways affect perceived sensory irritation (Shusterman et al., 2003). There is also some empirical support for neurogenic inflammation in chemical sensitivity and building related intolerance (Dantoft et al., 2013; De Luca et al., 2010; Sahlberg et al., 2010; Zhang et al., 2010) and inflammation have been suggested to influence the sensitivity of the TRPA1 channel (Bessac and Jordt, 2008). It would therefore be interesting to include sensitive individuals in future studies that might alter the level of detectability and perceived intensity due to acrolein exposure identified in this study.

There are some limitations with the present study. The absence of a control exposure that endured for 60 min is a limitation because it is possible that the low RH produced the sensory irritation during the 45- and 60-min exposures. The significant effect of time for these two exposures must therefore be interpreted with caution. Although it can be noted that, in another study performed in our laboratory (whole-body exposure, during 60 min), using the same concentration of heptane at the same RH, no more sensory irritation for heptane than during the 30-min exposure in this study was identified (data shown in Fig. 1b). A low RH may exacerbate the effects of sensory irritants (Wolkoff et al., 2006). The RH used in this study was almost the same as the current outside humidity at the time of exposure and such RHs are common in the northern parts of the world. It might therefore be important to use RHs reflecting actual indoor air exposures for risk assessment. There was also a slight drift with time, although not significant (and not reaching above 2.5 in confidence rating), during the 30-min exposure to heptane only. Such an effect of time on reporting, even during exposures to clean air, is a common occurrence in human exposure studies (Hudnell et al., 1992); a minor change with time should thus be expected regardless of exposure. Further, exposure to heptane

at concentrations as high as 41 mg/m^3 has been shown in earlier studies to have the same effect on subjects as clean air (Cain et al., 2007).

In conclusion, exposure to acrolein resulted in both increased detectability and sensory irritation over time. The study confirm previous studies suggested LOAEL of about 0.34 mg/m^3 for adverse eye irritation due to acrolein exposure. The sensory irritation was still significant 10 min after exposure. However, acrolein was only detected by 58% of the subjects and the result suggests a pre-existing trait of sensitivity towards acrolein exposure in the responders. Based on the activation mechanism of the TRPA1 receptor, it is plausible that irritation following exposure to certain VOCs might depend on the compound, the time of exposure, and also on the individual. Occupational exposure limits are usually set to avoid sensory irritation in the majority of people (about 80%–95%) (Paustenbach and Gaffney, 2006). These results therefore have implications for both risk assessment and currently acceptable levels of compounds, such as acrolein, in the future.

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