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Molecular Restrictions for Human Eye Irritation by Chemical Vapors

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Abstract

Previous research showed a cut-off along homologous volatile organic compounds (VOCs) in their ability to produce acute human mucosal irritation. The present study sought to specify the particular cut-off homolog for sensory eye irritation in an acetate and n-alcohol series. A 1,900 ml glass vessel system and a three-alternative forced-choice procedure served to test nonyl, decyl, and dodecyl acetate, and 1-nonanol, 1-decanol, and 1-undecanol. Flowrate to the eye ranged from 2 to 8 L/min and time of exposure from 3 to 24 sec. Decyl acetate and 1-undecanol were the shortest homologs that failed to produce eye irritation under all conditions, producing a cut-off effect. Increasing the vapor concentration of decyl acetate and 1-undecanol by 3 and 8 times, respectively, via heating them to 37 °C made either or both VOCs detectable to only half of the 12 subjects tested, even though the higher vapor concentration was well above a predicted eye irritation threshold. When eye irritation thresholds for homologous acetates and n-alcohols were plotted as a function of the longest unfolded length of the molecule, the values for decyl acetate and 1-undecanol fell within a restricted range of 18 to 19 Å. The outcome suggests that the basis for the cut-off is biological, i.e., the molecule lacks a key size or structure to trigger transduction, rather than physical, i.e., the vapor concentration is too low to precipitate detection.

Key words: Eye Irritation; Ocular Chemesthesis; Trigeminal Nerve; Chemical Irritation Cut-off; Homologous Acetates; Homologous n-Alcohols; Chemosensory Structure-Activity.

Introduction

Human chemoreception of airborne chemicals in the face mucosae (ocular, nasal, and oral) rests principally on two chemosensory systems: olfaction and chemesthesis. Smell is mediated by the olfactory nerve (cranial nerve I) whereas chemesthesis (Green et al., 1990; Green and Lawless, 1991) is mostly mediated by the various branches of the trigeminal nerve (cranial nerve V). For this reason, the latter is also known as trigeminal chemoreception, and had been originally labeled "the common chemical sense" (Bryant and Silver, 2000). Trigeminal stimulation by chemical vapors results from activation of polymodal nociceptors present in C and A_{delta} fibers (Martin and Jessell, 1991; Silver and Finger, 1991; Belmonte et al., 2004). In the ocular mucosa, the resulting sensation is eye irritation, a topic of both basic (Belmonte *et al.*, 1997) and applied (Wolkoff *et al.*, 2003) interest.

What kind of nociceptive molecular receptors might subserve the production of eye irritation from volatile organic compounds (VOCs)? Without discarding a role for G protein-coupled receptors (GPCRs) (Dong *et al.*, 2001; Lembo *et al.*, 2002), already known to be major players in olfactory chemoreception (Firestein, 2004; Gaillard *et al.*, 2004), the most likely candidates are members of the transient receptor potential (TRP) channel family (Clapham *et al.*, 2001). Among them, vanilloid (e.g. capsaicin) and menthol receptors have received particular attention, especially since it was found that their responsiveness includes thermal as well as chemical stimuli: The vanilloid receptor is sensitive to warm/hot temperatures (Caterina et al., 1997; Caterina and Julius, 2001)

and the menthol receptor to cool/cold temperatures (McKemy *et al.*, 2002; Peier *et al.*, 2002). Furthermore, vanilloid receptors are modulated by H^+ (Szolcsányi et al., 1994; Caterina and Julius, 2001), activated by chemicals structurally distinct from vanilloids (Szallasi *et al.*, 1996), and even stimulated/potentiated by an unrelated VOC, such as ethanol (Trevisani *et al.*, 2002). In this way, the vanilloid receptor seems to integrate a multiple and diverse range of stimuli (Tominaga *et al.*, 1998). In turn, it has been shown recently that cold-sensitive TRP receptors are also activated by a variety of pungent (i.e., irritating) compounds and by bradykinin, a peptide released from tissue injury and inflammation (Bandell *et al.*, 2004). This leads us to the added possibility that some VOCs, perhaps those most reactive towards tissue, could act indirectly by damaging epithelial cells producing the release of intracellular chemical triggers of nociception such as K^+, H^+, ATP, and glutamate. In particular, ATP can activate P2X receptors (Cook and McCleskey, 2002), and it has been shown that some VOCs (e.g., benzaldehyde, toluene, acetophenone) can modulate $P2X_2$ receptor-mediated currents in trigeminal neurons, although they are not able to activate them directly (Spehr *et al.*, 2004). These results and the fact that a myriad of vapor compounds are capable of evoking eye irritation and nasal pungency in humans (see (Cometto-Muñiz, 2001) suggest that chemesthesis from VOCs rests on a system of receptors characterized by wide range of chemical tuning.

We have hypothesized that the chemesthetic potency of <u>non-reactive</u> VOCs (Alarie *et al.*, 1998), that is, VOCs unlikely to damage mucosal tissue simply upon a brief vapor exposure, would rest heavily on “selective” or “transfer” processes rather than on

chemically- and structurally-restricted "specific" interactions. In agreement with this line of thought, a solvation equation (Abraham, 1993; Abraham and Weathersby, 1994) that models transfer processes across biological matrices successfully described and predicted human psychophysical thresholds for nasal pungency (Abraham et al., 1998; Abraham et al., 2001) and eye irritation (Abraham *et al.*, 2003), obtained under standardized conditions, for four dozen or more chemically diverse VOCs. This quantitative structure-activity relationship (QSAR) is based on up to five general physicochemical parameters, or descriptors, for VOCs that do not include any specific molecular size or structural limitation. The solvation equation takes the following form (Abraham, 1993; Abraham and Weathersby, 1994):

$$SP = c + e.\mathbf{E} + s.\mathbf{S} + a.\mathbf{A} + b.\mathbf{B} + l.\mathbf{L} \quad (1)$$

where SP refers to a chemosensory threshold (Abraham *et al.*, 2001). The **descriptors** (capital letters in bold) in equation (1) **are physicochemical properties of the stimuli (i.e., VOCs)**. We use here a simplified notation, with the original nomenclature in parentheses, as follows: **E** (R_2) is an excess molar refraction, **S** (π_2^H) is the VOC dipolarity-polarizability, **A** ($\sum\alpha_2^H$) and **B** ($\sum\beta_2^H$) are respectively the VOC effective hydrogen bond acidity and basicity, and **L** (log L^{16}) is defined through L^{16}, the VOC gas-hexadecane partition coefficient at 298K. The constant c and the **coefficients** e, s, a, b, and l are found by multiple linear regression analysis. However, they are not merely fitted coefficients since they **reflect** the **complementary physicochemical properties of** the biophase that would be most receptive to the VOCs, i.e., they provide a description of the chemical environment of **the receptor(s)** (Abraham *et al.*, 2003). The most recent update of this equation combines eye irritation thresholds (EIT) measured in humans for 23

VOCs with modified Draize test scores (MMAS/P°) measured in rabbits for 68 VOCs and reads as follows (Abraham *et al.*, 2003):

$$SP = -7.892 - 0.379\mathbf{E} + 1.872\mathbf{S} + 3.776\mathbf{A} + 1.169\mathbf{B} + 0.785\mathbf{L} + 0.561\mathbf{I} \quad (2)$$

where SP represents log (1/EIT) or log (MMAS/P°), all symbols are as defined above, and **I** = 0 for the log (1/EIT) series of compounds whereas **I** = 1 for the log (MMAS/P°) series of compounds. The statistics of the equation are: N = 91 (total number of VOCs), R^2 = 0.936 (proportion of variance explained), SD = 0.433 (standard deviation), AD = 0.000 (average deviation), AAD = 0.340 (absolute average deviation), and F = 204.5 (Fischer statistic).

Studies measuring nasal pungency thresholds along and across homologous series (reviewed in (Cometto-Muñiz, 2001) have indicated the existence of a cut-off in chemesthetic potency at a certain carbon-chain length. In other words, a homolog is reached such that its saturated vapor at room temperature fails to evoke chemesthesis. All larger homologs fail as well. Cut-offs have been observed for other biological processes, for example, anesthesia (Franks and Lieb, 1985). The descriptors in equation (2) can only predict a cut-off if the chemesthetic threshold comes out higher than the saturated vapor concentration of the VOC at room temperature (≈23°C). In contrast, if the cut-off rests on a structural/size molecular limitation, it could not be accommodated by any of the above descriptors used in the solvation-based chemesthetic QSAR.

In the present study, we set out a series of experiments to specify the cut-off homolog for sensory eye irritation in a series of acetate esters and n-alcohols where

previous research had suggested such effect (Cometto-Muñiz and Cain, 1990, 1991). The response we are focusing on is an acute, transient sensory response at the interface between detection and no-detection, conveying an early warning of potentially noxious vapors (Belmonte et al., 1997) that, at these very low and brief levels of stimulation, precedes any ophthalmic clinical signs (e.g., Podlekareva et al., 2002). We also investigated whether a failure to produce eye irritation could be overcome by increasing vapor concentration via heating the liquid stimulus source. This investigation represents the first step in an effort to gather information on the identity of cut-off homologs, and will be followed by studies covering additional chemical series in order to look for size, structural, and physicochemical commonalities among cut-off compounds. The information obtained could be used to update the QSAR equation with the addition of a new descriptor, most likely based on molecular dimensions, accounting for the cut-off phenomenon. As an antecedent to this, we have shown for olfactory thresholds that our QSAR equation, apart from descriptors for "selective" effects, can indeed incorporate descriptors for more "specific" effects such as molecular size and chemical functionality when they are relevant to the psychophysical results (Abraham *et al.*, 2002).

Experiment 1: Eye irritation detectability of acetate esters

Materials and Methods

A Committee from the Human Research Protections Program of the University of California, San Diego approved the study protocol covering all experiments. All subjects gave written informed consent on forms approved by the Committee.

Subjects. Four subjects participated, three males (ages 44, 45, and 50 years old) and one female (24 years old). All were normosmics, nonsmokers, and did not use contact lenses. Normosmia (i.e., normal sense of smell) in this and following experiments was established by administration of a standardized clinical olfactory test (Cain, 1989).

Stimuli. Three homologous acetates served as stimuli: nonyl (97+%, Food Chemical Codex, FCC, quality), decyl (95+%), and dodecyl (98+%) acetate. Stimuli were stored and presented as vapors from the headspace of a specially designed 1,900-ml glass vessel-system, adapted for eye irritation testing (Cometto-Muñiz *et al.*, 2001), and containing 200 ml of the neat chemical (Figure 1). The actual vapor-phase concentration of the stimuli was measured by gas chromatography (flame ionization detector, FID) using a calibration curve for mass, specific for each chemical (Cometto-Muñiz *et al.*, 2003). The resulting concentrations in ppm by volume (±SD) were: 54 (±15), 29 (±8), and 2.6 (±0.58) for nonyl, decyl, and dodecyl acetate, respectively, at 23 °C.

Insert Figure 1 about here

Procedure. Eye irritation detectability was tested at three durations of exposure: 6, 12, and 24 sec and at three flowrates of delivery: 2, 4, and 8 L/min. Thus, there were 27

different stimuli (3 chemicals x 3 durations x 3 flowrates). To avoid depletion of the headspace in the bottles, we prepared each chemical in triplicate and we did not present any stimulus bottle a second time until the other two identical bottles with the same chemical had been presented once. We employed a three-alternative forced-choice (3AFC) procedure against blanks containing mineral oil (light, FCC quality). Subjects wore noseclips during testing to avoid odor clues. An interval of at least one minute elapsed between successive triads. Participants were instructed to end the exposure in the unlikely event that a clear irritation was felt, and that it was important not to proceed with the next stimulus until all previous sensations (if any) had completely disappeared. We stress that all stimuli presented were at the very border of detection/no detection. After testing a triad, the subject selected the stimulus that felt "different" from the other two, guessing if necessary, and rated the confidence in the decision on a scale from 1 (not confident at all) to 5 (extremely confident). The 3AFC procedure just described controls for criterion biases and inherently contains a quantification of true detection since chance level is known: 33%, or 1 in 3. Having a defined level for no-detection (or chance), the statistical analysis performed (ANOVA, see below) shows which factors significantly raise sensory detection above chance and which ones do not. The order of presentation of chemicals, durations, and flow rates was irregular. Each subject participated in 10 to 20 sessions of 1 to 2 hours each until 20 trials per combination of chemical/duration/flowrate were collected (one subject only provided 16 trials). This produced a group total of 76 trials per chemical/duration/flowrate combination.

Data analysis. Tallying the outcome of the 3AFC procedure employed renders the number of corrects trials (i.e., cases where stimulus was selected) out of the total number of trials. The ratio number of correct trials / total number of trials produces a proportion correct, p(c). We know that solely by chance we expect p(c) = 0.33 or 33%. In order to quantify detection above chance we apply the following correction formula (Macmillan and Creelman, 1991):

$$P = \{m \cdot p(c) - 1\}/(m - 1)$$

where P = detection probability (i.e., detectability) corrected for chance, p(c) = proportion correct, and m = number of choices in the forced-choice procedure (in our case, 3). So the formula reduces to:

$$P = \{3 \cdot p(c) - 1\}/2$$

Thus, this expression provides a value for P ranging from 0.0, i.e., chance detection (equivalent to p(c) = 0.33 or 33% correct in a 3AFC), to 1.0, i.e., perfect detection (equivalent to p(c) = 1.00 or 100% correct). This quantification method serves well for near threshold (i.e., barely detectable) stimuli, such as those employed here. In addition, significance of trends was established by repeated measures analysis of variance (ANOVA) (software: SuperANOVA v. 1.11, Abacus Concepts, Inc.).

Results

Figure 2a presents the outcome for the three acetates in terms of detection probability and confidence as a function of flowrate (with exposure time as the parameter), whereas Figure 2b presents it as a function of exposure time (with flowrate as

the parameter). For nonyl acetate, detectability (P) increased monotonically with increasing flowrate from 0.15 (average across exposure times) at 2 L/min to 0.55 at 8 L/min (Figure 2a, left). Confidence ratings followed closely the trend in detectability. For decyl and dodecyl acetate, detectability remained close to chance (i.e., P≈0) and was not significantly affected by flowrate (Figure 2a, middle and right). Confidence ratings rose slightly, if at all, with flowrate. None of the acetates showed a significant change in detectability or confidence with increasing exposure time (Figure 2b). The effect of flowrate exclusively on the detectability of nonyl acetate and the lack of effect of exposure time on the detectability of all three acetates is illustrated in Figure 3. The results indicated that decyl and dodecyl acetate failed to be consistently detected above chance levels. The statistical significance of these trends was confirmed in a repeated-measures ANOVA including the factors acetate (three levels), flowrate (three levels), exposure time (three levels), and their interactions. The results showed that: 1) the detectability of the three acetates was significantly different ($F(2,6) = 24.98$, $p = 0.001$); 2) flowrate was not significant but the interaction acetate x flowrate was ($F(4,12) = 6.81$, $p = 0.004$), indicating that only the detectability of nonyl acetate increased with flowrate; 3) neither time nor the interactions acetate x time or flowrate x time were significant; 4) the triple interaction acetate x flowrate x time was significant ($F(8,24) = 2.446$, $p = 0.043$), indicating that only the detectability of nonyl acetate grew monotonically with flowrate while remaining constant across exposure times.

Insert Figures 2 and 3 about here

Experiment 2: Eye irritation detectability of n-alcohols

Materials and Methods

Subjects. Nine subjects (7 females, 2 males) participated. Their average age (±SD) was 25 (±8) years. All were normosmics. One subject (female) had participated in Experiment 1. One female (21 years old) was a smoker and another female (18 years old) used contact lenses but did not wear them on testing days. The rest of the participants were nonsmokers and did not use contact lenses.

Stimuli. Three homologous n-alcohols served as stimuli: 1-nonanol (98+%, FCC), 1-decanol (98+%, FCC), and 1-undecanol (98+%). Stimuli were stored and presented via glass vessels as in Experiment 1. The actual vapor-phase concentration of the alcohols was also measured by gas chromatography (flame ionization detector, FID) using a calibration curve for mass, specific for each chemical (Cometto-Muñiz *et al.*, 2003). The resulting concentrations in ppm by volume (±SD) were: 85 (±20), 33 (±9), and 8.2 (±1.9) for nonanol, decanol, and undecanol, respectively, at 23 °C.

Procedure. Eye irritation detectability was tested at three durations of exposure: 3, 6, and 12 sec and at three flowrates of delivery: 2, 4, and 8 L/min. Thus, there were 27 different stimuli (3 chemicals x 3 durations x 3 flowrates). Since at the durations of exposure used in Experiment 1 (6, 12, 24 sec), time played a non-significant role, we decided to speed up testing sessions and to reduce the load to the participants by dropping the longest

exposure (24 sec) and replacing it by a 3 sec exposure. All other aspects of the procedure were identical to those in Experiment 1. Each subject participated in 5 to 10 sessions of 1 to 3 hours each until 20 trials per combination of chemical/duration/flowrate were collected. This produced a group total of 180 trials per chemical/duration/flowrate combination.

Data analysis. Same as in Experiment 1.

Results

Figure 4a presents the outcome for the three alcohols in terms of detection probability and confidence as a function of flowrate (with exposure time as the parameter), whereas Figure 4b presents it as a function of exposure time (with flowrate as the parameter). For nonanol and decanol, detectability (P) increased monotonically with flowrate (Figure 4a, left and center). Confidence ratings followed the trend in detectability. For undecanol, detectability remained close to chance (i.e., $P \approx 0$) and was not affected by flowrate (Figure 4a, right). Confidence ratings also remained unchanged with flowrate. The alcohols showed little change in detectability or confidence with increasing exposure time (Figure 4b). The effect of flowrate on the detectability of nonanol and decanol and the marginal effect of exposure time on the detectability of all three alcohols is illustrated in Figure 5. The results indicate that only undecanol failed to be detected above chance levels. The statistical significance of these trends was confirmed in a repeated-measures ANOVA including the factors alcohol (three levels),

flowrate (three levels), exposure time (three levels), and their interactions. The results showed that the three main factors were significant: alcohol ($F(2,8) = 62.34$, $p < 0.001$), flowrate ($F(2,16) = 17.59$, $p < 0.001$), and, even, time ($F(2,16) = 8.53$ $p = 0.003$). The only significant interaction was that of alcohol x flowrate ($F(4,32) = 6.78$, $p < 0.001$), indicating that whereas the detectability of nonanol and decanol increased with flowrate, that of undecanol did not (cf. Figure 4a).

Insert Figures 4 and 5 about here

Experiment 3: Eye irritation detectability of decyl acetate and 1-undecanol at a higher vapor pressure

Experiments 1 and 2 determined that a cut-off in the ability to evoke eye irritation in our experimental conditions is first reached at the level of the homologs decyl acetate and 1-undecanol in the acetate and n-alcohol homologous series, respectively. Since the chemicals were presented neat from the glass vessels, their vapors represented the saturated vapor concentration at room temperature (≈23°C). One way to increase such concentration is to heat the vessels to a higher temperature. As described below, we used water baths to heat the glass vessels and achieve a higher vapor concentration of decyl acetate and 1-undecanol, in order to test if, under those conditions, the homologs could then evoke eye irritation.

Materials and Methods

Subjects. Twelve subjects participated (4 males, 8 females). Their average age (±SD) was 27 (±10) years. All were normosmics and nonsmokers. One subject (female) had participated in Experiments 1 and 2, another (male) had participated in Experiment 1, and three others (1 male, 2 females) had participated in Experiment 2. One of these latter females used contact lenses but did not wear them on testing days. All other participants did not use contact lenses.

Stimuli. Four of the six chemicals specified in Experiments 1 and 2 were tested: nonyl acetate, decyl acetate, 1-decanol, and 1-undecanol. The same glass vessel system as described above was used for stimulus storage and delivery. Nonyl acetate and 1-decanol were presented at room temperature (≈23 °C). Glass vessels containing decyl acetate and undecanol, and their respective mineral oil blanks, were heated in a calibrated water bath to keep the temperature in the headspace of the vessels at 37°C. Air that fed vessels located in the water bath passed through coiled tubing immersed in the heated water in order to reach the vessel at the proper temperature within the flowrate range 4 to 8 L/min and within the time-of-flowing range 0 to 20 sec. Measurements taken with a thermocouple (Omega Instruments) confirmed that air and air+stimulus exiting the vessels within these range of conditions did so at 37.5(±0.53)°C. In this experiment, all presentations had a flowrate of 4 L/min and a duration of 6 sec. The concentration of the stimulus (±SD) in the headspace of the heated bottles, measured by gas chromatography,

showed decyl acetate at 77±8 ppm (vs. 29±8 ppm at 23°C) and undecanol at 67±4 ppm (vs. 8.2±1.9 ppm at 23°C).

Procedure. As in Experiments 1 and 2, we used a three-alternative forced-choice procedure with irregular order of presentation of: 1) stimulus and blanks within triads and 2) chemicals across triads. Stimuli were prepared in quintuplicate and no bottle was sampled a second time until all other bottles containing that chemical had been sampled once. Each subject provided 20 judgments per stimulus. This produced a group total of 240 judgments per stimulus.

Data analysis. Same as in Experiment 1.

Results

Figure 6 illustrates the results from Experiment 3. Unheated nonyl acetate and 1-decanol where re-tested here as positive controls, that is, VOCs for which, based on Experiments 1 and 2, we would expect detection to occur above chance. For nonyl acetate, the detectability value taken from Experiment 1 includes all exposure times since, for the acetates, the factor time was non-significant. Figure 6 shows that, for nonyl acetate, the detection probabilities obtained in Experiments 1 and 3 were close, both around 0.30. For 1-decanol, the detectability value taken from Experiment 2 includes only the 6 sec exposure since, for the alcohols, time was significant. Figure 6 shows that, also for 1-decanol, the detection probabilities obtained in Experiments 2 and 3 were very

close, both around 0.30. How do the detection probabilities of decyl acetate and 1-undecanol, heated to 37 °C in Experiment 3, compare with those obtained for the same chemicals at room temperature (23 °C) in Experiments 1 and 2, respectively? The detection probability of decyl acetate rose from 0.12 (Experiment 1, all durations, at 23 °C) to 0.25 (Experiment 3, 6 sec exposure, at 37 °C). A two-sample t-test indicated that this rise was not significant. The detection probability of 1-undecanol rose from 0.02 (Experiment 2, 6 sec exposure, at 23 °C) to 0.21 (Experiment 3, 6 sec exposure, at 37 °C). A two-sample t-test indicated that this rise came very close to significance ($p = 0.06$). The next experiment sought to maximize the comparability of eye irritation detectability between unheated and heated decyl acetate and 1-undecanol by testing the same group of subjects on both chemicals under both heating conditions in the same experiment.

Insert Figure 6 about here

Experiment 4: Direct comparison of the eye irritation detectability of unheated (23 °C) and heated (37 °C) decyl acetate and 1-undecanol

The low levels of detectability of decyl acetate and 1-undecanol, whether unheated or heated, and the inherent variability across subjects left doubts about the role that increasing vapor concentration played in the detectability of these stimuli. In a direct test to quantify any possible significant increase in detectability of the two chemicals with an increase in their vapor concentration, we ran an experiment where the same

group of subjects, in the same testing sessions, sought to detect decyl acetate and 1-undecanol presented unheated and heated in irregular order.

Materials and Methods

Subjects. Twelve subjects (6 males, 6 females) participated. Their average age (±SD) was 27 (±9) years. All were normosmics and did not use contact lenses. All but one male were nonsmokers. Six subjects (3 males, 3 females) had participated in Experiment 3. Of them, one subject (female) had also participated in Experiments 1 and 2, another (male) had also participated in Experiment 1, and still another (male) had also participated in Experiment 2.

Stimuli. Two chemicals (decyl acetate and 1-undecanol) were tested under two conditions, 23 °C and 37 °C. Presentation of unheated and heated stimuli was analogous to that in Experiment 3, including a flow rate of 4 L/min and a 6 sec exposure time.

Procedure. As in all previous experiments, we used a 3-alternative, forced-choice procedure and the order of stimulus and blanks within a triad and of stimuli across triads was irregular. Each stimulus (i.e., combination of chemical and temperature) was prepared in quintuplicate and no bottle was sampled a second time until all other bottles containing that stimulus had been sampled once.

Data analysis. Same as in Experiment 1.

Results

Figure 7 shows that increasing the vapor concentration of decyl acetate and 1-undecanol did produce an increase in their detectability via eye irritation. The outcome of a repeated measures ANOVA with two factors (chemical and heating condition) revealed that the increase was significant ($F(1,11) = 8.66$, $p = 0.013$ for the factor "heating condition"), but that neither the factor "chemical" nor the interaction "chemical x heating condition" were significant.

Insert Figure 7 about here

The larger variability of the data from the heated condition (see standard error bars in Figure 7) and the availability of enough data from each subject prompt a look at the individual data (Figure 8). For both decyl acetate and undecanol tested under the heated condition, the outcome revealed that about half the subjects clearly increased their detectability of the stimulus whereas the other half did not. The division did not appear to be gender-related since the two groups included both females and males. In the case of decyl acetate, the unheated stimulus failed to be detected clearly above chance by most subjects ($-0.2 \leq P \leq 0.2$) but two subjects did detect it at $P=0.4$ and $P=0.6$, respectively. Detection of the unheated decyl acetate did not guarantee increased detectability of that stimulus when heated since the second of these two subjects detected the heated chemical ($P=0.6$) no better than the unheated one. In contrast, in the case of 1-undecanol, the

unheated stimulus failed to be detected clearly above chance by any of the participants ($-0.2 \leq P \leq 0.2$). As mentioned above, when decyl acetate and 1-undecanol were heated they became detectable (to various degrees: $0.2 \leq P \leq 0.7$) for about half the subjects.

Insert Figure 8 about here

Discussion

In the process of measuring acute nasal pungency and eye irritation thresholds along and across homologous chemical series (see review in (Cometto-Muñiz, 2001), we found, in each series, that a homolog could be reached where detection by chemesthesis failed, even at vapor saturation. The failure would first be evident for one or two subjects, but it would invariably extend to additional subjects when a larger homolog was tested. It seemed, then, that a cut-off for evoking chemesthesis could be reached for each chemical series. The present study aimed at specifying the cut-off homolog in a series of acetate esters and n-alcohols and at probing the likely basis for such effect.

We investigated the possible role on eye irritation detection of two relevant variables: Flowrate of stimulus to the eye and time of exposure of the eye. Regarding flowrate, preliminary testing (not shown) revealed that, with our glass-vessel system, a blank (headspace of mineral oil) delivered at a) 2 L/min barely produced a “feel” of flow in the eye, b) 4 L/min produced a noticeable “feel”, and c) 8 L/min produced a clear “feel”. Figures 3 and 5 clearly show that an increase in flowrate enhances detectability

but only for those homologs situated before the cut-off, i.e., nonyl acetate in the acetate series, and 1-nonanol and 1-decanol in the alcohol series. For the cut-off homolog and those beyond it, increasing flowrate did not precipitate detection. Figures 2a (left graph) and 4a (left and middle graphs) suggest that, for homologs situated before the cut-off, the gain in detectability with higher flowrate slows down as flowrate reaches 8 L/min. In the case of 1-nonanol (Figure 4a, left graph), this might reflect a ceiling effect (P cannot be higher than 1), but no such ceiling effect could be argued for nonyl acetate (Figure 2a, left graph) and 1-decanol (Figure 4a, middle graph).

Time of exposure, in the acute range explored of 3 to 24 sec, had no effect or much less effect than flowrate (compare right and left graphs on Figures 3 and 5). Consistent with the results from flowrate, those homologs situated at or beyond the cut-off did not become detectable with an increase in time of exposure of the eye.

Once the cut-off homologs were defined for both series, we explored the possible basis for the effect. In other physiological processes where a cut-off effect is observed, for example anesthesia (Franks and Lieb, 1985), at least two different mechanisms have been proposed (Franks and Lieb, 1990). Under a physical mechanism, there is not enough concentration of stimulus in the vapor to reach detection. Under a biological mechanism, the stimulus lacks a key property to trigger transduction; for example, a molecule could exceed the size that allows it to interact effectively with a target site or to fit into the binding pocket of a receptive macromolecule. By heating the vessels containing the neat chemicals from room temperature (≈23 °C) to 37 °C we increased the vapor

concentration 2.7 times for decyl acetate and 8.2 times for 1-undecanol. The group results of Experiments 3 and 4 revealed that, under the new condition, both cut-off homologs now became detectable at a low (Figure 6) but significant (Figure 7) level. Inspection of the individual data for the 12 participants revealed a sharp contrast, for both decyl acetate and 1-undecanol, between the half for whom detectability clearly increased and the other half for whom detectability remained unchanged, typically close to chance level (Figure 8).

As mentioned in the Introduction, in cellular and subcellular experimental models, heat has been shown to activate vanilloid receptors but only in the noxious range, i.e., above 43°C (Caterina *et al.*, 1997). Thus, even if vanilloid receptors are involved in the eye irritation response to VOCs of the sort tested in the present experiments, it seems unlikely that the precipitation of detection for heated VOCs responded simply to the increase in temperature of the vapor to 37°C, the normal body temperature. Still, the observation that certain factors, e.g., elevated H^+ concentrations in the case of vanilloid receptors (Tominaga *et al.*, 1998), can sensitize a nociceptor and make it responsive to otherwise ineffective levels of a stimulus warrants consideration.

What could be the reason behind the contrast between the two subgroups of subjects? One possibility is that the cut-off homolog might vary slightly among participants, perhaps due to genetic variability in the receptor(s) involved. For those subjects who failed to detect decyl acetate and/or 1-undecanol even when heated, the mechanism of a biological cut-off remains a possibility. For the other half of the subjects

that began to show an above-chance level of detection of the stimulus(i) when heated, we can say that, if there is a biological cut-off, it is not at the level of the presently tested chemical(s). Figure 9 presents values of: a) saturated vapor at 23°C, b) saturated vapor at 37°C, and c) measured (Cometto-Muñiz and Cain, 1990, 1991) or predicted (from equation (2)) eye irritation thresholds (EIT) as a function of the variable (i.e., alkyl group for acetates) carbon chain length of acetates and n-alcohols. It illustrates how the trend for EIT reaches or surpasses the value of saturated vapor concentration at 23°C, thus producing a potential physical cut-off at the levels of decyl acetate and 1-undecanol. In turn, Figure 10 also plots saturated vapor at 23°C and EIT for acetates and n-alcohols but as a function of D, the longest unfolded length of the molecule. This straightforward size parameter has proven to be a significant factor in quantifying olfactory potency in homologous series (Abraham *et al.*, 2002). The outcome shows that the cut-off appears in both series at a very restricted value of D, between 18 and 19 Å, suggesting a biological cut-off.

Insert Figures 9 and 10 about here

Additional data from more homologous series need to be gathered before the issue of the cut-off can be better understood. Among such series we can mention ketones, alkylbenzenes, carboxylic acids, aldehydes, and terpenes. As the results begin to accumulate, commonalities in both structural and physicochemical features among cut-off homologs can be searched for. The existence of a physical, a biological, or a combination of both restrictions for a vapor to be able to evoke mucosal chemesthesis is a

relevant parameter for understanding the integrated range of chemical tuning across the various receptors involved. Ultimately, this information can be captured in a new parameter on the QSAR solvation equation for ocular and nasal chemesthesis (Abraham *et al.*, 2001), further expanding its applicability and predictive ability.

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References

Abraham, MH. Scales of solute hydrogen-bonding: Their construction and application to physicochemical and biochemical processes. Chem. Soc. Rev. 1993; 22: 73-83.

Abraham, MH, Gola, JMR, Cometto-Muñiz, JE, Cain, WS. The correlation and prediction of VOC thresholds for nasal pungency, eye irritation and odour in humans. Indoor Built Environ. 2001; 10: 252-257.

Abraham, MH, Gola, JMR, Cometto-Muñiz, JE, Cain, WS. A model for odour thresholds. Chem. Senses 2002; 27: 95-104.

Abraham, MH, Hassanisadi, M, Jalali-Heravi, M, Ghafourian, T, Cain, WS, Cometto-Muñiz, JE. Draize rabbit eye test compatibility with eye irritation thresholds in humans: A quantitative structure-activity relationship analysis. Toxicol. Sci. 2003; 76: 384-391.

Abraham, MH, Kumarsingh, R, Cometto-Muñiz, JE, Cain, WS. An algorithm for nasal pungency thresholds in man. Arch. Toxicol. 1998; 72: 227-232.

Abraham, MH, Weathersby, PK. Hydrogen bonding. 30. Solubility of gases and vapors in biological liquids and tissues. J. Pharm. Sci. 1994; 83: 1450-1456.

Alarie, Y, Nielsen, GD, Abraham, MH. A theoretical approach to the Ferguson principle and its use with non-reactive and reactive airborne chemicals. Pharmacol. Toxicol. 1998; 83: 270-279.

Bandell, M, Story, GM, Hwang, SW, Viswanath, V, Eid, SR, Petrus, MJ, Earley, TJ, Patapoutian, A. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron 2004; 41: 849-57.

Belmonte, C, Acosta, MC, Gallar, J. Neural basis of sensation in intact and injured corneas. Exp. Eye Res. 2004; 78: 513-25.

Belmonte, C, Garcia-Hirschfeld, J, Gallar, J. Neurobiology of ocular pain. Prog. Retin. Eye Res. 1997; 16: 117-156.

Boublík, T, Fried, V, Hála, E. The Vapour Pressures of Pure Substances. Amsterdam: Elsevier, 1984.

Bryant, B, Silver, WL. Chemesthesis: The Common Chemical Sense. In: Finger TE, Silver WL, Restrepo D, eds. The Neurobiology of Taste and Smell. 2nd Edition. New York: Wiley-Liss, 2000, p 73-100.

Cain, WS. Testing olfaction in a clinical setting. Ear Nose Throat J. 1989; 68: 316-328.

Caterina, MJ, Julius, D. The vanilloid receptor: a molecular gateway to the pain pathway. Annu. Rev. Neurosci. 2001; 24: 487-517.

Caterina, MJ, Schumacher, MA, Tominaga, M, Rosen, TA, Levine, JD, Julius, D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 1997; 389: 816-24.

Clapham, DE, Runnels, LW, Strubing, C. The TRP ion channel family. Nat. Rev. Neurosci. 2001; 2: 387-96.

Cometto-Muñiz, JE. Physicochemical basis for odor and irritation potency of VOCs. In: Spengler JD, Samet J, McCarthy JF, eds. Indoor Air Quality Handbook. New York: McGraw-Hill, 2001, p 20.1-20.21.

Cometto-Muñiz, JE, Cain, WS. Thresholds for odor and nasal pungency. Physiol. Behav. 1990; 48: 719-725.

Cometto-Muñiz, JE, Cain, WS. Nasal pungency, odor, and eye irritation thresholds for homologous acetates. Pharmacol. Biochem. Behav. 1991; 39: 983-989.

Cometto-Muñiz, JE, Cain, WS, Abraham, MH. Quantification of chemical vapors in chemosensory research. Chem. Senses 2003; 28: 467-477.

Cometto-Muñiz, JE, Cain, WS, Abraham, MH, Gola, JMR. Ocular and nasal trigeminal detection of butyl acetate and toluene presented singly and in mixtures. Toxicol. Sci. 2001; 63: 233-244.

Cook, SP, McCleskey, EW. Cell damage excites nociceptors through release of cytosolic ATP. Pain 2002; 95: 41-7.

Dong, X, Han, S, Zylka, MJ, Simon, MI, Anderson, DJ. A diverse family of GPCRs expressed in specific subsets of nociceptive sensory neurons. Cell 2001; 106: 619-32.

Firestein, S. A code in the nose. Sci. STKE 2004; 2004: pe15.

Franks, NP, Lieb, WR. Mapping of general anesthetic target sites provides a molecular basis for cutoff effects. Nature 1985; 316: 349-351.

Franks, NP, Lieb, WR. Mechanisms of general anesthesia. Environ. Health Perspect. 1990; 87: 199-205.

Gaillard, I, Rouquier, S, Giorgi, D. Olfactory receptors. Cell. Mol. Life Sci. 2004; 61: 456-69.

Green, BG, Lawless, HT. The psychophysics of somatosensory chemoreception in the nose and mouth. In: Getchell TV, Doty RL, Bartoshuk LM, Snow Jr. JB, eds. Smell and Taste in Health and Disease. New York: Raven Press, 1991, p 235-253.

Green, BG, Mason, JR, Kare, MR. Preface. In: Green BG, Mason JR, Kare MR, eds. Chemical Senses. Vol. 2: Irritation. New York: Marcel Dekker, Inc., 1990, p v-vii.

Lembo, PM, Grazzini, E, Groblewski, T, O'Donnell, D, Roy, MO, Zhang, J, Hoffert, C, Cao, J, Schmidt, R, Pelletier, M, Labarre, M, Gosselin, M, Fortin, Y, Banville, D, Shen, SH, Strom, P, Payza, K, Dray, A, Walker, P, Ahmad, S. Proenkephalin A gene products activate a new family of sensory neuron--specific GPCRs. Nat. Neurosci. 2002; 5: 201-9.

Macmillan, NA, Creelman, CD. Detection theory: A user's guide. Cambridge: Cambridge University Press, 1991.

Martin, JH, Jessell, TM. Modality coding in the somatic sensory system. In: Kandel ER, Schwartz JH, Jessell TM, eds. Principles of Neural Science. 3rd Edition. New York: Elsevier, 1991, p 341-352.

McKemy, DD, Neuhausser, WM, Julius, D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature 2002; 416: 52-8.

Peier, AM, Moqrich, A, Hergarden, AC, Reeve, AJ, Andersson, DA, Story, GM, Earley, TJ, Dragoni, I, McIntyre, P, Bevan, S, Patapoutian, A. A TRP channel that senses cold stimuli and menthol. Cell 2002; 108: 705-15.

Podlekareva, D, Pan, Z, Kjaergaard, S, Mølhave, L. Irritation of the human eye mucous membrane caused by airborne pollutants. Int. Arch. Occup. Environ. Health 2002; 75: 359-64.

Riddick, JA, Bunger, WB. Organic Solvents. New York: Wiley-Interscience, 1970.

Silver, WL, Finger, TE. The trigeminal system. In: Getchell TV, Doty RL, Bartoshuk LM, Snow Jr. JB, eds. Smell and Taste in Health and Disease. New York: Raven Press, 1991, p 97-108.

Spehr, J, Spehr, M, Hatt, H, Wetzel, CH. Subunit-specific P2X-receptor expression defines chemosensory properties of trigeminal neurons. Eur. J. Neurosci. 2004; 19: 2497-510.

Stephenson, RM, Malanowski, S. Handbook of the Thermodynamics of Organic Compounds. New York: Elsevier, 1987.

Szallasi, A, Jonassohn, M, Acs, G, Biro, T, Acs, P, Blumberg, PM, Sterner, O. The stimulation of capsaicin-sensitive neurones in a vanilloid receptor-mediated fashion by pungent terpenoids possessing an unsaturated 1,4-dialdehyde moiety. Br. J. Pharmacol. 1996; 119: 283-90.

Szolcsányi, J, Pórszász, R, Pethö, G. Capsaicin and pharmacology of nociceptors. In: Besson JM, Guilbaud H, Ollat H, eds. Peripheral neurons in nociception: Physio-pharmacological aspects. Paris: John Libbey Eurotext, 1994, p 109-124.

Tominaga, M, Caterina, MJ, Malmberg, AB, Rosen, TA, Gilbert, H, Skinner, K, Raumann, BE, Basbaum, AI, Julius, D. The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 1998; 21: 531-43.

Trevisani, M, Smart, D, Gunthorpe, MJ, Tognetto, M, Barbieri, M, Campi, B, Amadesi, S, Gray, J, Jerman, JC, Brough, SJ, Owen, D, Smith, GD, Randall, AD, Harrison, S, Bianchi, A, Davis, JB, Geppetti, P. Ethanol elicits and potentiates nociceptor responses via the vanilloid receptor-1. Nat. Neurosci. 2002; 5: 546-51.

Wolkoff, P, Skov, P, Franck, C, Petersen, LN. Eye irritation and environmental factors in the office environment--hypotheses, causes and a physiological model. Scand. J. Work Environ. Health 2003; 29: 411-30.

Figure legends

Figure 1. Picture of the glass vessels employed to test ocular trigeminal detection of VOCs.

Figure 2. a) Plots of eye irritation detectability (left *y*-axis) (filled symbols) and confidence (right *y*-axis) (empty symbols) for nonyl, decyl, and dodecyl acetate as a function of flowrate, with time of exposure as a parameter. b) Analogous to a) but plotted as a function of time of exposure, with flowrate as a parameter. In both sets of graphs, each point represents 76 judgments made by four subjects, and bars indicate standard errors (SE).

Figure 3. Left. Plot of eye irritation detectability for the three acetates, averaged across exposure times (6, 12, and 24 sec), as a function of flowrate. Right. Analogous to a) but averaged across flowrates (2, 4, and 8 L/min) and plotted as a function of exposure time.

Figure 4. a) Plots of eye irritation detectability (left *y*-axis) (filled symbols) and confidence (right *y*-axis) (empty symbols) for 1-nonanol, 1-decanol, and 1-undecanol as a function of flowrate, with time of exposure as a parameter. b) Analogous to a) but plotted as a function of time of exposure, with flowrate as a parameter. In both sets of graphs, each point represents 180 judgments made by nine subjects, and bars indicate standard errors (SE).

Figure 5. Left. Plot of eye irritation detectability for the three alcohols, averaged across exposure times (3, 6, and 12 sec), as a function of flowrate. Right. Analogous to a) but averaged across flowrates (2, 4, and 8 L/min) and plotted as a function of exposure time.

Figure 6. Comparison of the eye irritation detectability of nonyl acetate in Experiments 1 and 3 (both at room temperature, 23 °C), and that of decyl acetate in Experiment 1 (at 23 °C) and 3 (at 37 °C). Analogous for 1-decanol and 1-undecanol in Experiments 2 and 3. In results from Experiment 3, each point represents the average of 240 judgments made by 12 subjects. Bars indicate standard errors (SE).

Figure 7. Group data showing how the homologs decyl acetate and 1-undecanol became detectable above chance when their vapor concentration was increased by heating them from room temperature (23 °C) to 37 °C. Each point represents the average of 240 judgments made by 12 subjects. Bars indicate standard errors (SE).

Figure 8. Individual data corresponding to the group data of Figure 7 showing, for both decyl acetate and 1-undecanol, how approximately half the subjects (empty symbols or symbols with an X) increased their detectability of the stimulus when it was heated whereas the other half (filled or half-filled symbols) did not (see text). S1, S2, etc. stand for Subject 1, Subject 2, etc.

Figure 9. Values of saturated vapor concentration at 23 and 37 °C (Riddick and Bunger, 1970; Boublík et al., 1984; Stephenson and Malanowski, 1987), and of measured or

predicted EIT for homologous acetates (top) and n-alcohols (bottom) plotted as a function of variable carbon chain length. EIT for dodecyl acetate and for 1-decanol, 1-undecanol, and 1-dodecanol are predicted from equation (2); the rest are measured values (Cometto-Muñiz and Cain, 1990, 1991). The arrow marks the point at which the EIT reaches (acetates) or surpasses (alcohols) the maximum available vapor concentration at 23°C. This occurs at the level of decyl acetate and 1-undecanol, respectively, the same homologs for which we experimentally found the cut-off in eye irritation.

Figure 10. Values of saturated vapor at 23°C and of EIT from homologous acetates and n-alcohols as a function of the molecule longest distance (in Å). From top left to lower right, the acetates are: ethyl, butyl, hexyl, octyl, decyl, and dodecyl acetate; the alcohols are: ethanol, 1-butanol, 1-hexanol, 1-octanol, 1-decanol, 1-undecanol, and 1-dodecanol. The arrows mark decyl acetate and 1-undecanol, the experimentally found cut-off homologs.

FIGURE 1

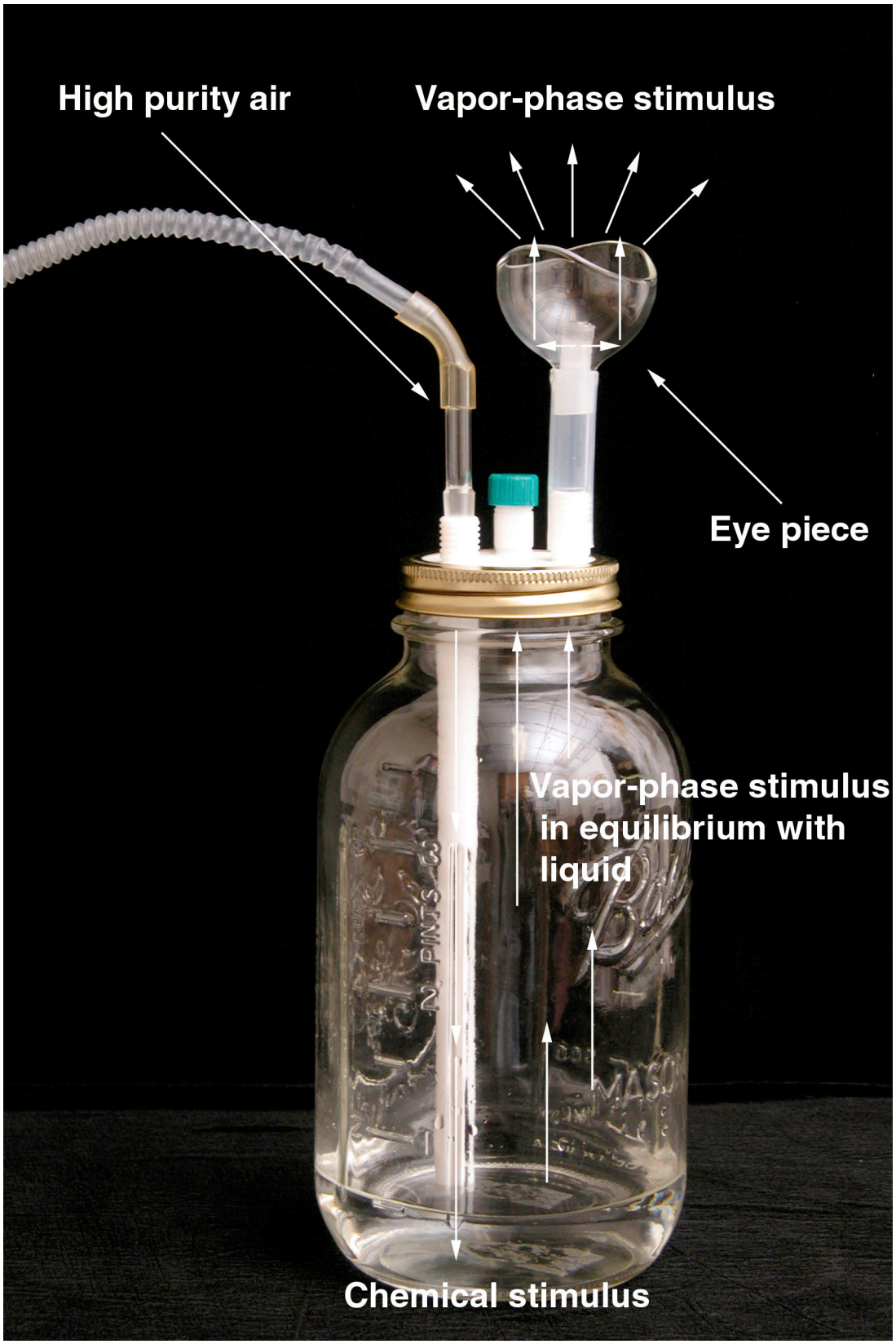

High purity air
Vapor-phase stimulus
Eye piece
Vapor-phase stimulus in equilibrium with liquid
Chemical stimulus

FIGURE 2a

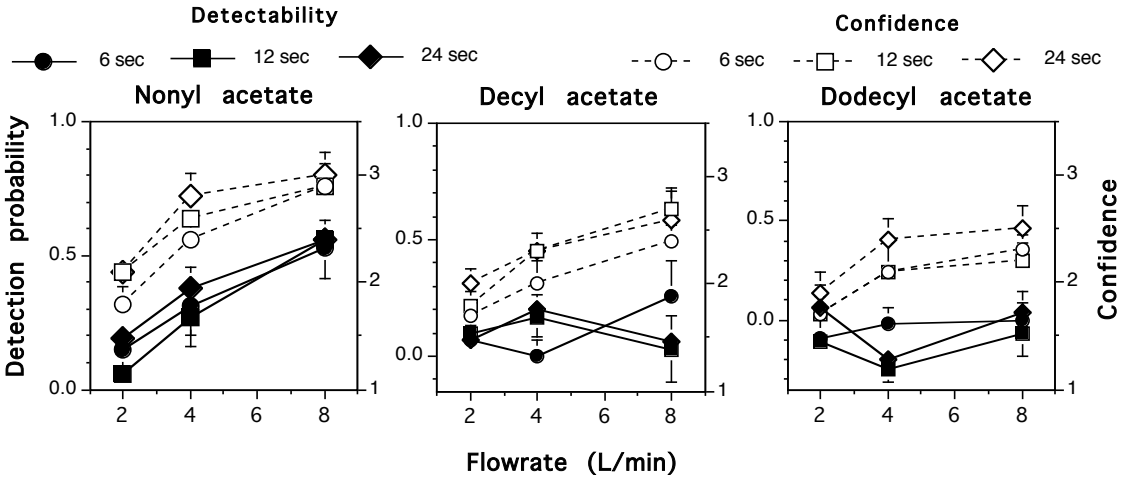


FIGURE 2b

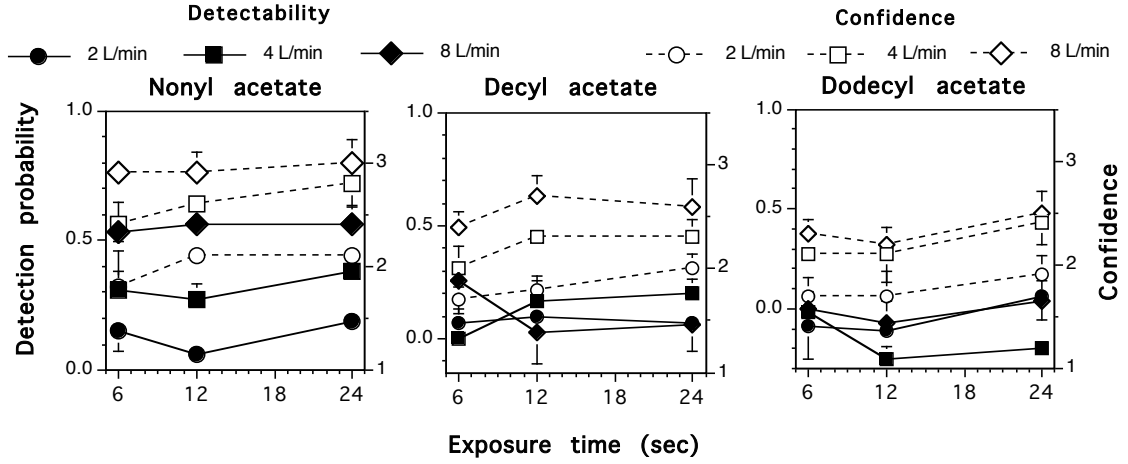


FIGURE 3

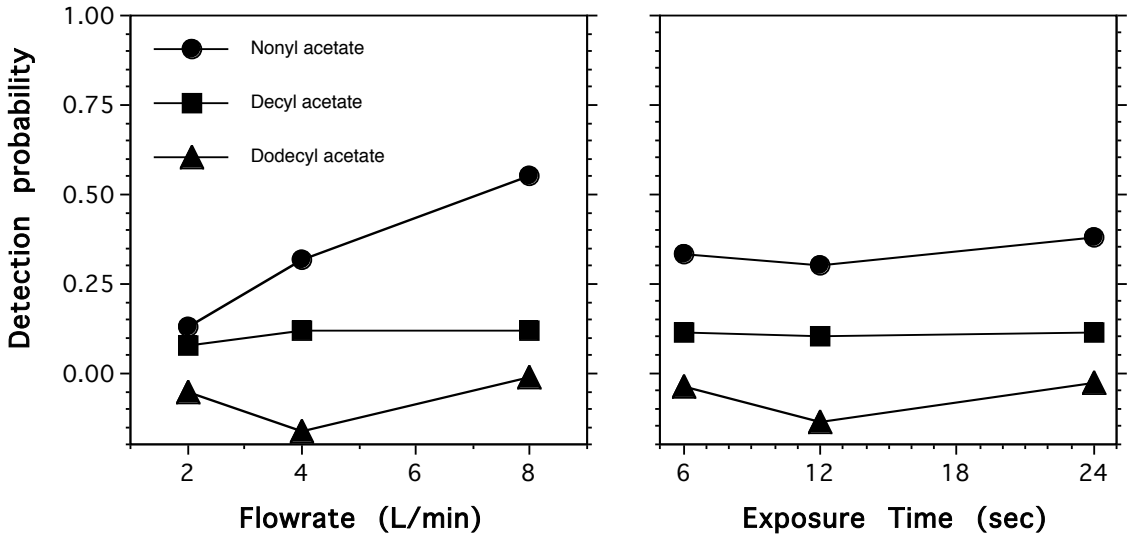

1.00
0.75
0.50
0.25
0.00
Detection probability
Nonyl acetate
Decyl acetate
Dodecyl acetate
2
4
6
8
Flowrate (L/min)
6
12
18
24
Exposure Time (sec)

FIGURE 4a

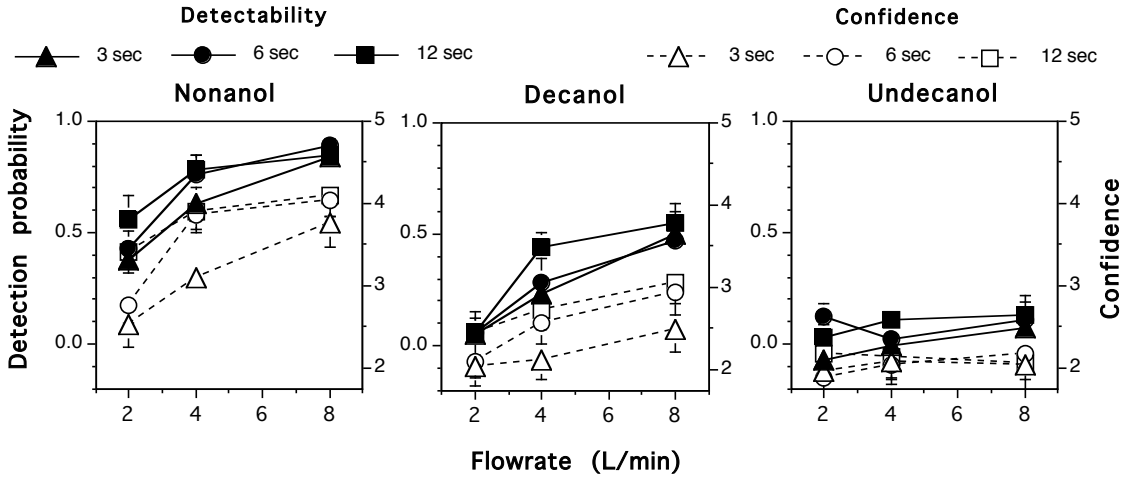


FIGURE 4b

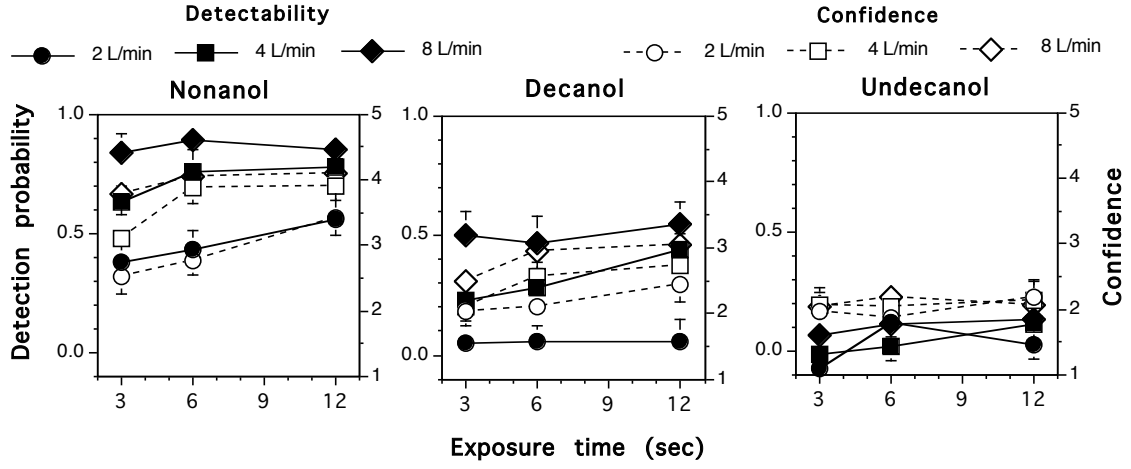


FIGURE 5

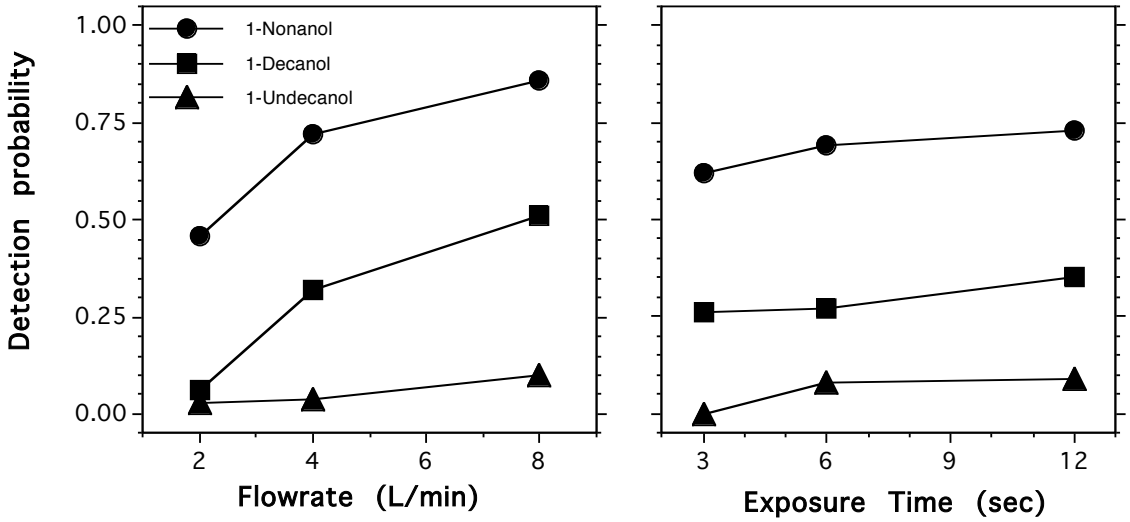

1-Nonanol
1-Decanol
1-Undecanol
Detection probability
1.00
0.75
0.50
0.25
0.00
2
4
6
8
Flowrate (L/min)
3
6
9
12
Exposure Time (sec)

FIGURE 6

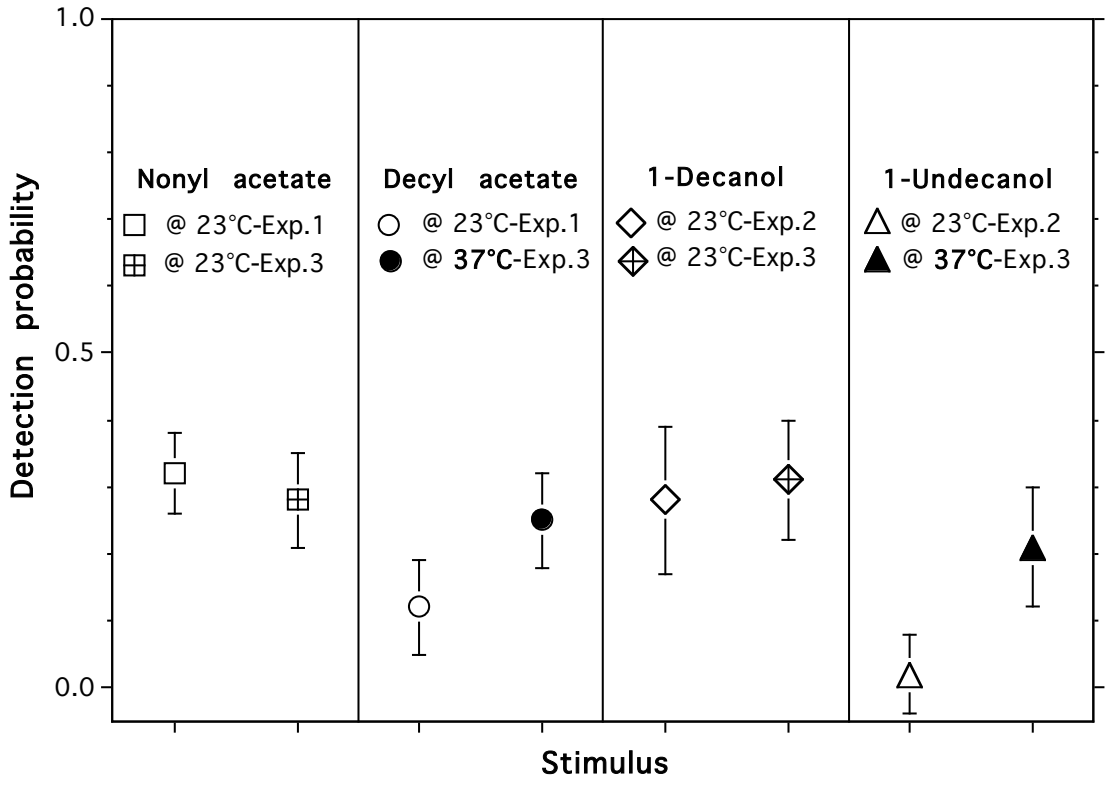

1.0
0.5
0.0
Detection probability
Nonyl acetate
@ 23°C-Exp.1
@ 23°C-Exp.3
Decyl acetate
@ 23°C-Exp.1
@ 37°C-Exp.3
1-Decanol
@ 23°C-Exp.2
@ 23°C-Exp.3
1-Undecanol
@ 23°C-Exp.2
@ 37°C-Exp.3
Stimulus

FIGURE 7

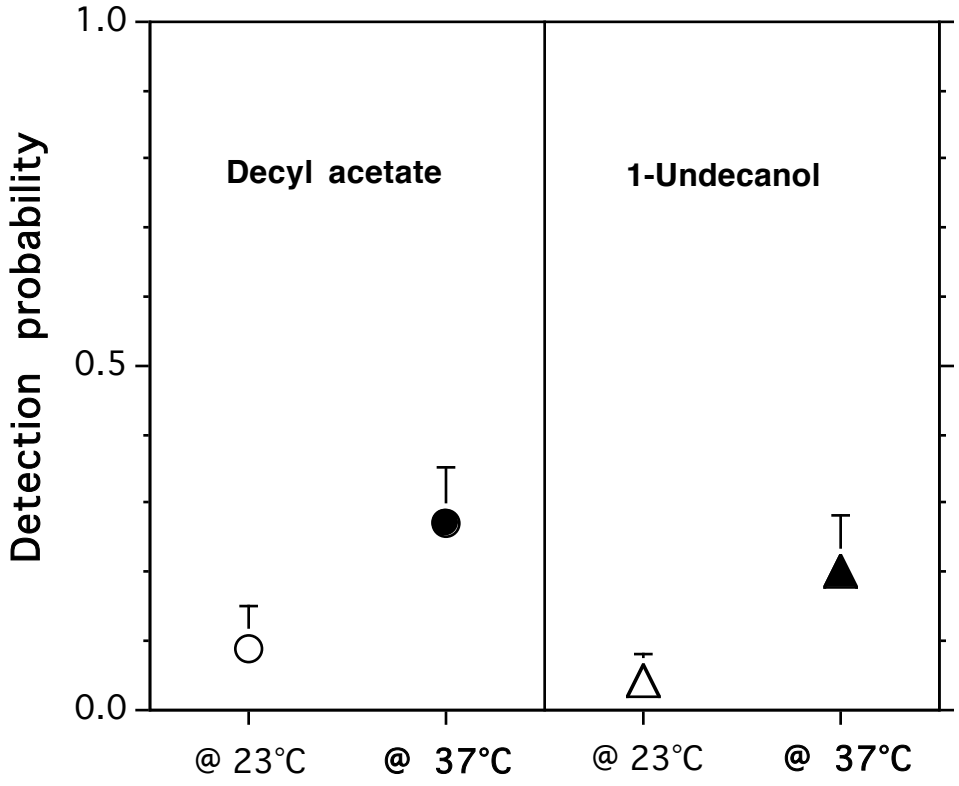

1.0
0.5
0.0
Detection probability
Decyl acetate
1-Undecanol
@ 23°C
@ 37°C
@ 23°C
@ 37°C

FIGURE 8

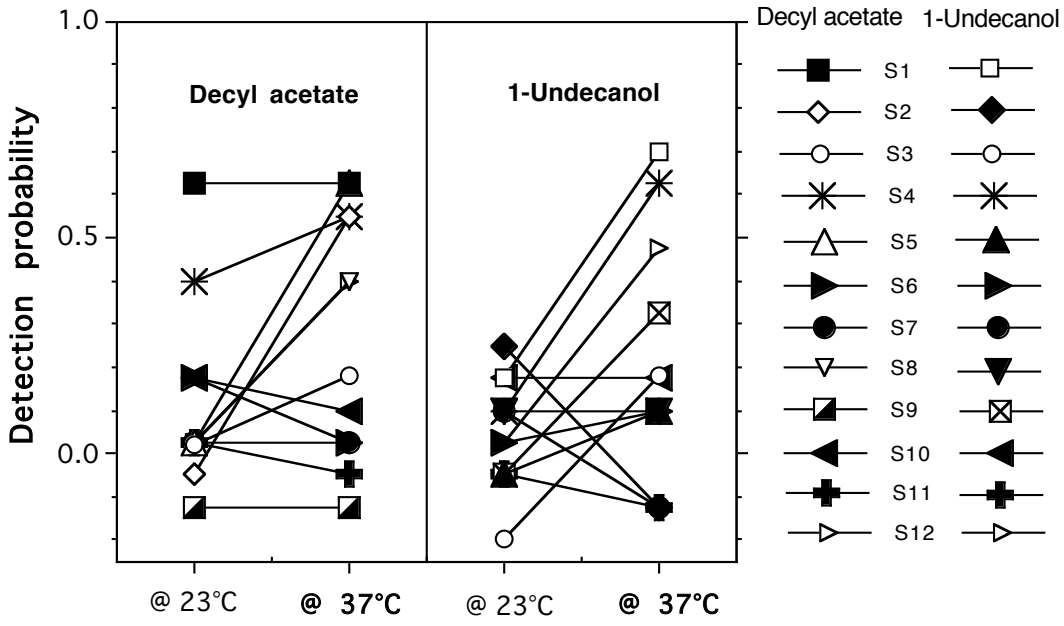
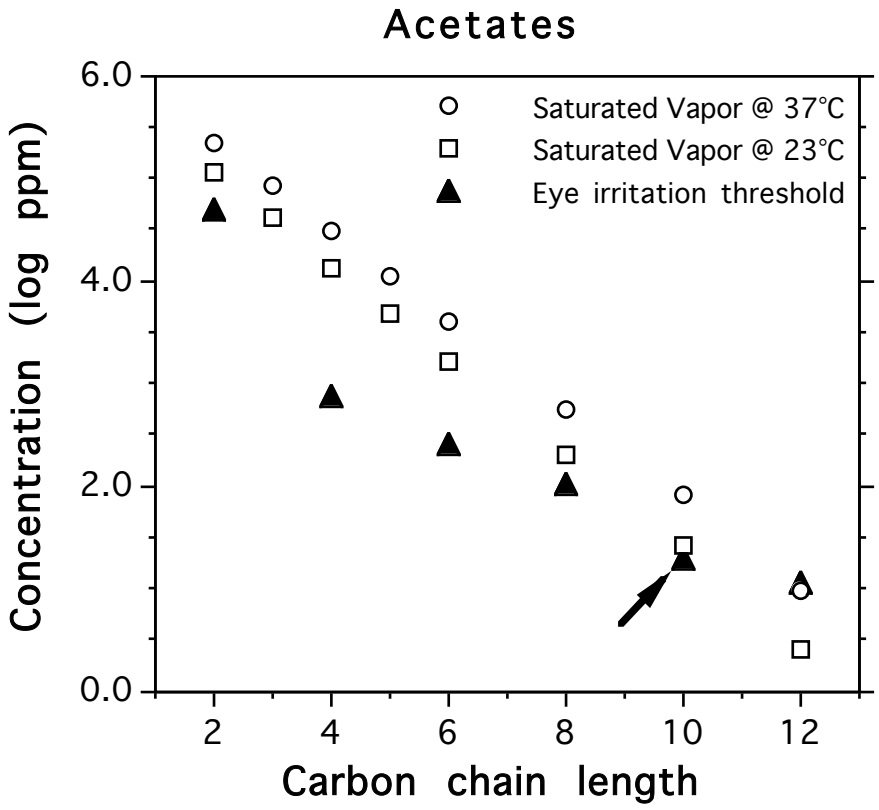

1.0
0.5
0.0
Detection probability
Decyl acetate
1-Undecanol
@ 23°C
@ 37°C
@ 23°C
@ 37°C
Decyl acetate
1-Undecanol
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
S11
S12

FIGURE 9


Acetates
Saturated Vapor @ 37°C
Saturated Vapor @ 23°C
Eye irritation threshold
Concentration (log ppm)
6.0
4.0
2.0
0.0
2
4
6
8
10
12
Carbon chain length

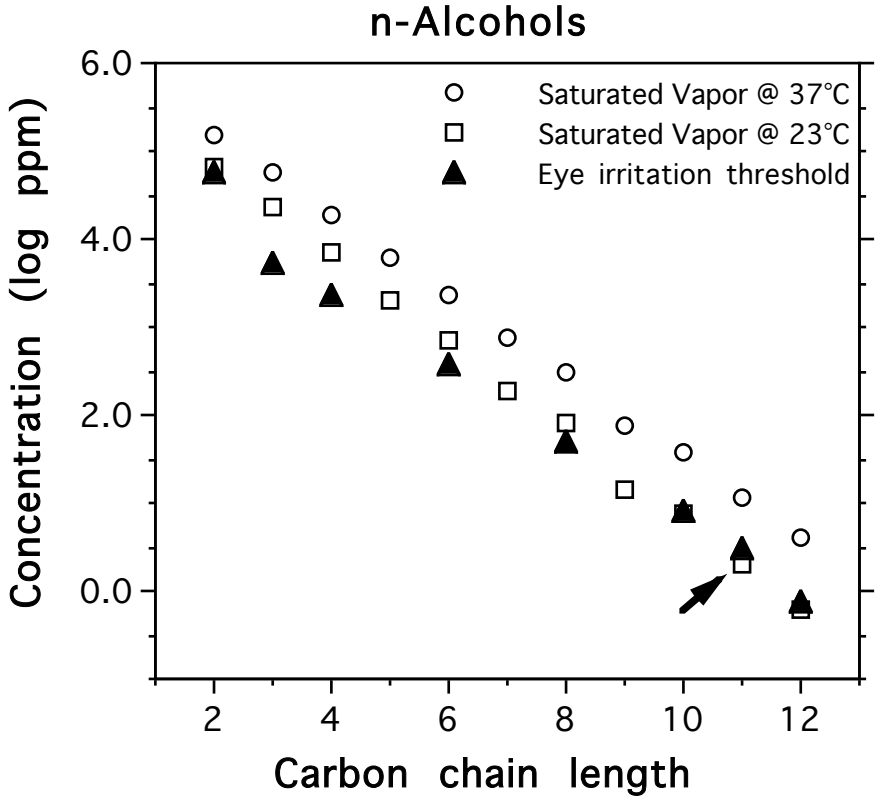
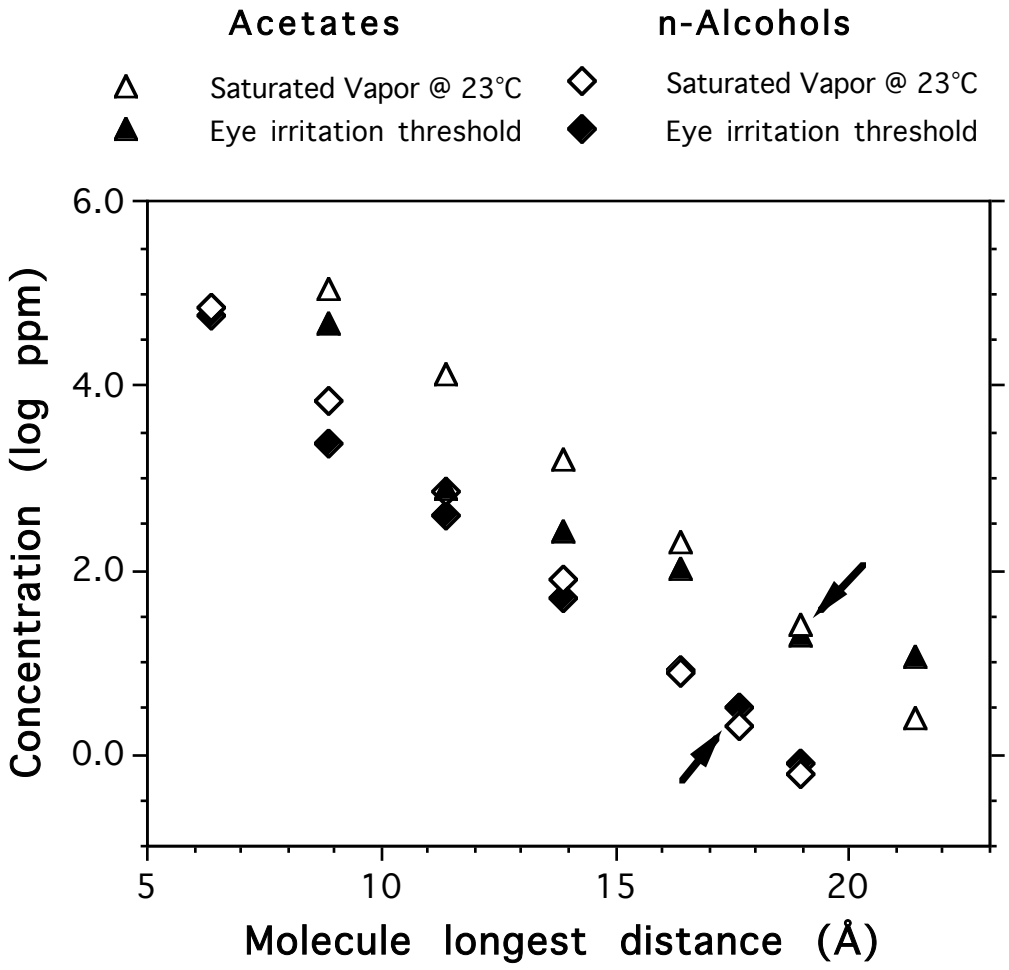

n-Alcohols
Saturated Vapor @ 37°C
Saturated Vapor @ 23°C
Eye irritation threshold
Concentration (log ppm)
6.0
4.0
2.0
0.0
2
4
6
8
10
12
Carbon chain length

FIGURE 10


Acetates
n-Alcohols
Saturated Vapor @ 23°C
Eye irritation threshold
Saturated Vapor @ 23°C
Eye irritation threshold
6.0
4.0
2.0
0.0
Concentration (log ppm)
5
10
15
20
Molecule longest distance (Å)

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