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Chemical boundaries for detection of eye irritation in humans from homologous vapors

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Running head: Chemical Boundaries for Eye Irritation
Abstract

In a series of experiments we looked at a “cut-off” effect for the detection of eye irritation from neat vapors of homologous n-alkylbenzenes and 2-ketones. Stimuli comprised pentyl, hexyl, and heptyl benzene, 2-dodecanone, and 2-tridecanone, presented to each eye at 4 and 8 L/min for 6 sec., using a three-alternative, forced-choice procedure against blanks. Detection probability corrected for chance (i.e., detectability) decreased with carbon chain length such that heptyl benzene and 2-tridecanone were virtually undetectable, irrespective of flowrate to the eye. Heating both stimuli sources to 37°C (body temperature) from 23°C (room temperature), increased vapor concentration by 5.0 and 6.9 times, respectively, for heptyl benzene and 2-tridecanone. Still, both chemicals failed to show increased detection for 13 of the 21 participants. In addition, plots of experimentally measured and calculated eye irritation thresholds as a function of carbon chain length for each series indicated that, based on the trend, the concentration of the two cut-off homologs at 37°C should have been high enough to allow detection. Taken together, the results suggest that these cut-offs rest on limitations related to the dimension of the molecules rather than on limitations related to their vapor concentration. For example, the stimulus molecule could exceed the size that allows it to fit into the receptor pocket of a receptive protein. Plots of calculated molecular dimensions across homologous alkylbenzenes, from ethyl to dodecylbenzene, and across 2-ketones, from 2-octanone to 2-octadecanone, provided additional support to the above conclusion.

Keywords: Eye irritation cut-off; Ocular chemesthesis cut-off; Trigeminal chemoreception; Homologous n-alkylbenzenes; Homologous 2-ketones; Chemosensory structure-activity.
Introduction

A key issue in understanding human chemosensory perception involves knowledge of the relevant structural and physicochemical properties of chemicals that govern potency, i.e., absolute detection, and perceptual quality. Within biochemical fields such as pharmacology and toxicology, analogous issues are often addressed as (quantitative) structure-activity relationships (QSARs or SARs). In the taste modality, where there is relative consensus on the number and types of qualitative categories, namely, sweet, bitter, salty, sour, and umami, the approach has involved the search for critical molecular features among families of tastants evoking one or another of these prototypical tastes (Bassoli et al., 2002; Bufe et al., 2005; Grigorov et al., 2003; Norwich, 2001; Sowalsky and Noble, 1998). In the olfactory modality, no such simple and convenient approach can be followed (Wise et al., 2000). A practical tactic to overcome this deficiency consists in studying olfactory responses along members of homologous chemical series, where carbon chain length becomes the “unit of chemical change” that serves as a probe to measure a concomitant olfactory outcome, for example, a detection threshold (Abraham et al., 2002). A similar tactic can be applied to develop QSARs for detection of chemically-induced somesthesis, that is, chemically stimulated feel (Cometto-Muñiz, 2001). Investigators in the chemical senses have labeled this particular kind of chemical sensitivity chemesthesia (Green and Lawless, 1991; Green et al., 1990), but it is also referred to as chemical nociception or irritation (Ferrer-Montiel et al., 2004; Hummel et al., 2003). This chemosensory modality plays an important role as a warning system against exposure to deleterious vapors, and, as such, constitutes an early indication of potentially ensuing, more severe, toxicological effects (Doty et al., 2004). From this role, the advantage of being able to predict the chemesthetic potency of
Chemesthesis in the face mucosae (ocular, nasal, and oral) is principally mediated by the trigeminal nerve (cranial nerve V). In the ocular mucosa, chemesthetic stimulation with vapors typically results in eye irritation. Studies at the molecular level have implicated a number of receptor candidates for chemesthesis, in particular, transient receptor potential (TRP) channels but also G protein-coupled receptors (GPCRs) and receptors for chemicals released due to epithelial cell damage such as K⁺, H⁺, ATP, and glutamate (Julius and Basbaum, 2001; Numazaki and Tominaga, 2004; Wood and Docherty, 1997; Zhang et al., 2005). This broad variety of reception processes agrees with the observation that vapors of volatile organic compounds (VOCs) from very diverse chemical functionality and structure can evoke eye irritation and nasal pungency (Cometto-Muñiz, 2001). Many of these VOCs are relatively non-reactive (Alarie et al., 1998) and, thus, unlikely to damage mucosal tissue, producing the release of the above mentioned mediators, simply upon a brief vapor exposure. There is evidence that typical pungent compounds other than capsaicin, and structurally very different from it, also activate ion channels directly (Bautista et al., 2005; Jordt et al., 2004; Macpherson et al., 2005). Electrophysiological recordings revealed that multiple types of trigeminal sensory neurons respond differentially to irritating VOCs, suggesting the involvement of multiple mechanisms, some known others yet to be identified, distributed across different modalities of neurons (Inoue and Bryant, 2005). Within this context, a likely possibility is that the integrated process (Tominaga et al., 1998) that governs the chemesthetic potency of most VOC vapors rests heavily on “selective” (or “transfer”) effects rather than on “specific” effects.
Support to the above hypothesis came from the successful application of a QSAR that models selective transfer processes across biological matrices (Abraham, 1993; Abraham and Weathersby, 1994) to human psychophysical thresholds for nasal pungency (Abraham et al., 1998a) and eye irritation (Abraham et al., 1998b), two typical chemesthetic sensations evoked by VOCs (Abraham et al., 2001). Recent studies have provided a detailed description of this QSAR (Cometto-Muñiz et al., 2005b), including its specific application to ocular irritation where the model has successfully managed to combine in one equation eye irritation thresholds measured in humans with modified Draize test scores measured in rabbits (Abraham et al., 2003). In addition, previous studies of ocular and nasal chemesthetic thresholds along and across homologous chemical series have suggested the existence of a “cut-off”, i.e., a point where a homolog fails to stimulate even at vapor saturation (Cometto-Muñiz, 2001). All larger homologs fail as well. The phenomenon has been observed for other biological effects of VOCs, for example, anesthesia (Franks and Lieb, 1985). The cited QSAR for chemesthesis would predict a cut-off only if the calculated threshold came out higher than the saturated vapor concentration of the homolog at room temperature. In this case, one could heat the liquid or solid VOC to increase its vapor concentration allowing it to reach chemesthetic detection. Experiments on ocular and nasal chemesthesia have applied this strategy and found that most VOCs continue to fail to reach detection (Cometto-Muñiz et al., 2005a, b). The outcome suggests that, in these cases, the cut-off is not concentration-based but rather molecular dimension- or structure-based. For example, the VOC could be too large or bulky to interact or fit into the receptor pocket of a protein. The present study sought, first, to identify the particular cut-off member along homologous alkylbenzenes and 2-ketones and, second, to probe the likely basis for the effect.
Experiment 1: Detection of eye irritation at room temperature (23°C)

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. Fifteen participants (9 females) took part in the experiment. Their average age (±SD) equaled 21 (±4) years and ranged from 18 to 35 years. Thirteen of them (8 females) performed in the normosmic range on a clinical olfactory test (Cain, 1989). The other two performed in the very mildly hyposmic range for either one nostril (a male) or for both (a female). All participants were non-smokers. Thirteen subjects did not use contact lenses. Of the remaining two, one (female) used contact lenses regularly and another (male) used them occasionally; neither subject wore contact lenses on testing days.

Stimuli and Equipment. Based on previous research on nasal chemesthesis (Cometto-Muñiz et al., 2005a) and on preliminary testing, we selected two members from a 2-ketone homologous series and three from an alkylbenzene series. They were (purity in parenthesis): 2-dodecanone (98+%) and 2-tridecanone (≥99.6%), and pentyl (or amyl) benzene (98%), hexyl benzene (98%) and heptyl benzene (98%), respectively. These compounds were found to approach a “cut-off” point within each series such that even vapor from the neat homolog failed to evoke obvious eye irritation. Mineral oil (light, Food Chemical Codex quality) served as blank. Chemical vapors and blanks were delivered from a 1,900 ml glass vessel system containing 200 ml of neat stimuli (50 g for the solid 2-tridecanone) or blank and ending in a bowl-shaped 40-ml eyepiece, as
described previously (Cometto-Muñiz et al., 2005b). To avoid depletion of the headspace in the vessels, we prepared each chemical in duplicate, alternating their use through the session. The headspace concentration in a vessel was measured by gas chromatography (flame ionization detector) via a calibration curve for mass specific for each chemical (Cometto-Muñiz et al., 2003). The resulting concentrations in ppm by volume (±SD) were: 19.5 (±1.1) for 2-dodecanone, 5.5 (±0.5) for 2-tridecanone, 355 (±12) for pentyl benzene, 128 (±28) for hexyl benzene, and 27.5 (±1.4) for heptyl benzene.

**Procedure.** A trial consisted of a three-alternative forced-choice (3AFC) against blanks, where subjects had to decide which ocular exposure from a triad produced a different, typically stronger, sensation. The position of the stimulus within the triad and the order of testing of the five chemicals were randomized. Time of exposure was constant at 6 sec for all presentations. Flowrate to the eye was set to either 4 L/min or 8 L/min, in irregular order, alternating 20 trials at 4 L/min and 20 at 8 L/min. Thus, there were 10 different stimuli (5 chemicals X 2 flowrates). Participants wore noseclips during trials to avoid odor cues, and alternated the exposed eye (right or left) from one triad to the next. An interval of at least one minute elapsed between such successive triads testing different eyes. The vessels in a triad were presented covered by an opaque plastic sleeve to avoid visual clues. Subjects were instructed to end the exposure in the unlikely event that clear irritation was felt, and, importantly, not to proceed with the next member of a triad until all previous sensations (if any) had disappeared completely. We stress that stimuli were specifically chosen to be at the very border of chemesthetic detection/no detection and that these low and very brief levels of stimulation precede any clinical ocular signs (Podlekareva et al., 2002). In addition, participants rated their confidence on the decision made for each triad, using a scale ranging from “1” (not confident at all) to
“5” (extremely confident). Subjects participated in 4 to 6 sessions, each lasting between 2 and 3 hours, until they accumulated 20 to 26 judgments per stimulus per subject. This provided a group total of 312 to 316 group judgments per stimulus.

**Data Analysis.** Results are presented as detection probability (i.e., detectability) or as confidence of detection as a function of the stimuli. Detection probability was corrected for chance using the following formula (Macmillan and Creelman, 1991):

\[ P = \frac{m \cdot p(c) - 1}{m - 1} \]

where \( P \) = detectability corrected for chance, \( m \) = number of choices per trial (in our case, three), and \( p(c) \) = proportion correct (i.e., number of correct trials / total number of trials). Statistical significance was established by analysis of variance (ANOVA) for repeated measurements (Software: SuperANOVA v.1.11, Abacus Concepts, Inc., Berkeley, CA).

**Results**

Figure 1 summarizes the results for the group in terms of detectability and confidence of detection for each stimulus. As observed along other homologous series (Cometto-Muñiz et al., 2005b), chemesthetic detectability decreased with increasing carbon chain length, and confidence of detection followed this trend closely. Increasing the flowrate to the eye produced a slight increase in detectability but only for vapors detectable near the middle range of the scale (i.e., \( 0.35 \leq P \leq 0.65 \)), for example, hexyl benzene and 2-dodecanone, not for those that were very detectable (i.e., \( P \geq 0.75 \)), for example, pentyl benzene, or for those that were practically undetectable (i.e., \( P \leq 0.15 \)), for example, heptyl benzene and 2-tridecanone. In other words, an increased flowrate
did not modify the detectability of readily detectable stimuli and, very importantly, not that of almost undetectable stimuli. In an ANOVA for repeated measurements with the factors flow (2 levels: 4 and 8 L/min) and chemical (5 levels), the factor flow was significant ($F(1,14) = 8.376, p = 0.0118$) and so was the factor chemical ($F(4,56) = 41.096, p < 0.0001$), but not their interaction. A look at the individual data reveals that, with minor variations, all subjects showed the trend observed for the group (Figure 2).

Insert Figures 1 and 2 about here

**Experiment 2: Comparison of the eye irritation detectability of heptyl benzene and 2-tridecanone at 23 and at 37 °C**

**Materials and Methods**

**Subjects.** Twenty-one participants (11 females) took part in the experiment. Their average age ($\pm$SD) equaled 29 ($\pm10$) years and ranged from 19 to 51 years. Seventeen of them (10 females) performed in the normosmic range on a clinical olfactory test (Cain, 1989). The other four performed in the very mildly hyposmic range for either one nostril (2 males) or for both (1 female, 1 male). All participants but one (a male) were non-smokers. Seventeen subjects did not use contact lenses. Of the remaining four, one (female) used them regularly, another (male) used them occasionally, and the other two (males) were former users. No subject wore contact lenses on testing days. Three subjects (2 males, 1 female) had also participated in Experiment 1.

**Stimuli and Equipment.** Stimuli comprised heptyl benzene and 2-tridecanone as defined before. Time of exposure was 6 sec and flow rate was 4 L/min. Vapors were delivered either at 23°C (room temperature) or heated to 37°C, employing the same glass vessel
system used in Experiment 1. Heated stimuli and blanks were presented from vessels standing in a calibrated water bath covered by a perforated sheet of lab surface protector (made of cellulose fibers on top and polyethylene backing on bottom). This sheet served to hide the content of the bottles from view, and to minimize temperature loss from the water bath to the testing room. Measurements taken with a thermocouple (Omega Instruments, Stanford, CT) revealed that the air and air+stimulus exiting the vessels in our conditions of stimulation did so at 36.4 (±1.3) °C. Gas chromatography showed that the vapor concentration (ppm by volume ±SD) of heated heptyl benzene was 141 (±15) and that of heated 2-tridecanone was 38 (±3). These values are 5.0 and 6.9 times higher, respectively, than the corresponding vapor concentrations at 23°C (see Experiment 1).

Procedure. Analogous to that in Experiment 1 (i.e., 3AFC and rating of confidence) except that in Experiment 2 there were only 4 different stimuli (2 chemicals X 2 temperatures), as described above. Subjects participated in 2 to 3 sessions, each lasting between 2 and 3 hours, until 20 judgments per stimulus per subject were gathered. This provided a group total of 420 judgments per stimulus.

Data Analysis. Same as in Experiment 1.

Results

Figure 3 summarizes the outcome of Experiment 2 and puts it in context with that of Experiment 1. The graph shows agreement between both experiments in terms of the detectability of heptyl benzene and 2-tridecanone at room temperature, 23°C. It also shows that heating the VOCs to 37°C barely increased their detectability. An analysis of
variance (ANOVA) for repeated measurements on the data from Experiment 2 including the factors chemical (two levels: heptyl benzene and 2-tridecanone) and temperature (two levels: 23 and 37 °C) revealed that neither factor nor their interaction was statistically significant (p>0.10). We conclude that, in terms of group data, the increased vapor concentration of both VOCs heated to 37°C failed to increase their detectability by eye irritation.

A more detailed level of understanding can be achieved by looking at the individual data (Figure 4). Based on the individual variability observed in Figure 4, the analysis that follows will consider that: 1) values of detectability (i.e., P) below P=0.20 imply virtually no detection, and 2) only increases in detectability of P>0.20 and falling above P=0.20 constitute relevant increases. Under these terms, and in contrast with the uniform trend for individual data seen in Figure 2, we can observe in Figure 4 two types of individual trends for each chemical: 1) for most subjects (13 out of 21 for both heptyl benzene and 2-tridecanone) the increase in concentration produced by heating to 37°C failed to either precipitate or enhance detection, and 2) for the rest of the subjects (8 out of 21 for both chemicals) the increase in concentration did precipitate detection or enhanced it. Most of the 13 individuals for whom increased concentration did not enhance detection were common for both chemicals (9 out of the 13). Many of the 8 individuals for whom concentration did enhance detection were common for both chemicals (4 out of the 8).
Figure 5 illustrates, for each chemical, a comparison in ocular chemesthetic detectability between the group unresponsive to an increase in concentration (n=13) and the group responsive to an increase in concentration (n=8). In the case of heptyl benzene, an ANOVA having one factor within subjects, i.e., temperature (which, in fact, indicates headspace concentration) and one factor between subjects, i.e., unresponsive vs. responsive subjects regarding an increase in concentration, revealed significance for temperature (i.e., concentration) \( F(1,19)=10.364, p=0.0045 \) and for the interaction temperature X group \( F(1,19)=34.358, p=0.0001 \). A comparable ANOVA for 2-tridecanone revealed significance for group \( F(1,19)=5.220, p=0.03 \) and, although temperature slightly missed significance \( (p=0.06) \), the interaction temperature X group was significant \( F(1,19)=19.506, p=0.0003 \). For both chemicals, the results support the notion that one larger group of unresponsive subjects failed to increase their ocular detectability with an increase in concentration whereas another smaller group of responsive subjects did increase it.

Discussion

The studies cited in the Introduction support the following notions: 1) chemesthesis is a receptor(s)-based process that, for most nonreactive VOCs, rests strongly on selective rather than specific interactions; 2) many and diverse receptors and neuron types are involved; 3) there is a cut-off effect in the chemesthetic ability of VOCs, and it seems to be related to molecular structure, size, and/or dimensions rather than to vapor concentration. Against this background, the present study set out to find the particular homolog reaching the cut-off point for ocular chemesthesis within a series of
alkylbenzenes and 2-ketones. The outcome of Experiment 1 identified heptyl benzene and 2-tridecanone as the cut-off homologs within their respective series. A previous investigation on detection of nasal pungency in anosmics, i.e., subjects lacking olfaction, found that they were unable to detect butyl benzene and higher alkylbenzenes, although the approach was not optimized to define a cut-off point (Cometto-Muñiz and Cain, 1994). The comparison of nasal vs. ocular cut-offs in chemesthesis from series of VOCs represents a fertile area of research to further unveil the basis for the phenomenon and warrants study on its own. The results of Experiment 2 showed that the cut-off could not be significantly overcome by an increase in vapor concentration of 5.0 times in the case of heptyl benzene or 6.9 times in that of 2-tridecanone. Considering that functions relating chemesthetic detectability of VOCs to their vapor concentration typically cover the complete range from chance to virtually perfect detection within a concentration factor of just 10 (Cain et al., 2005; Cometto-Muñiz et al., 2002), the outcome of Experiment 2 strongly suggests that the basis for the present cut-offs also rest on molecular structure or dimension, not vapor concentration.

Are there other indicators pointing towards a cut-off based on molecular dimension? Figure 6 plots vapor concentration as a function of carbon chain length for homologous 2-ketones. Four sets of values for vapor concentration are presented. These are: 1) saturated vapor (SV) concentration at 23°C, that is, room temperature; 2) SV concentration at 37°C, that is, body temperature; 3) measured human eye irritation thresholds (EIT) (from this paper, Experiment 1, and (Cometto-Muñiz and Cain, 1995)); and 4) predicted, i.e., calculated, eye irritation thresholds from a QSAR model (Abraham et al., 2001). The figure shows that, although the model occasionally might underestimate (2-propanone) or overestimate (2-heptanone) a threshold, it is often right on target (2-pentanone, 2-nonanone, 2-dodecanone). More importantly, the equation
relating threshold concentration to carbon chain length along ketones is virtually identical, in slope and in relative position, for measured and for calculated values (Figure 6). Both equations suggest a cut-off at the level of 2-tridecanone (or even 2-dodecanone) where the threshold clearly reaches the SV concentration at 23°C. Nevertheless, the SV concentration of 2-tridecanone at 37°C is still clearly above the threshold expected from either equation, indicating that if the cut-off at 23°C were concentration-based, it should be easily overcome by heating the VOC to 37°C. This did not happen.

Insert Figure 6 about here

An analogous comparison is presented in Figure 7 for homologous alkylbenzenes, where the sources for predicted and measured EIT are as cited above. Here, the values for measured and predicted EIT diverge progressively from toluene onwards, as shown by the lines representing the corresponding equations, in the direction of calculated values increasingly overestimating the measured threshold. As a result, the equation for calculated values predicts a cut-off at the level of propyl benzene for stimuli at 23°C and at the level of octyl benzene for stimuli at 37°C. In contrast, the equation for measured values suggests a cut-off at the level of heptyl benzene (or lower) for stimuli at 23°C but no cut-off, at least until well beyond octyl benzene, for stimuli at 37°C. The present results at 23°C agree well with the trend in measured EIT, but fail to show that the cut-off is overridden when heptyl benzene is heated to 37°C as would have been expected from a concentration-based limitation. This suggests that the basis for the cut-off along alkylbenzenes is also likely to be based on molecular dimensions (or size), as for the ketones.
Despite the lack of a significant increase for the group data (n=21) in the detectability of 2-tridecanone and heptyl benzene at the higher concentration (Figure 3), the analysis of individual data (Figure 4) revealed that a sub-group of participants (n=8) did significantly increase their detection (Figure 5). A similar result was observed for the cut-off homologs decyl acetate and 1-undecanol in a study of ocular chemesthesis among acetate esters and n-alcohols (Cometto-Muñiz et al., 2005b). Given that the various chemesthetic receptors putatively involved are all likely to be proteins (Julius and Basbaum, 2001), the outcome could reflect genetic variability (Elmer et al., 1998; Mogil et al., 1999) across subjects in the precise structure or dimension of receptor sites, leading to cut-offs displaced by one or two carbon chain units. These differences across subjects have also been observed in studies of nasal chemesthesis from homologous families of esters (Cain et al., 2006). We mention that differences in tear film break-up time represent another source of variability across individuals (Nichols et al., 2002; Stodtmeister et al., 1983). An additional source of individual variability could be the thickness of the tear film. Unfortunately, investigators have not reached a consensus on either the thickness of the film or its degree of variability across subjects (King-Smith et al., 2004; Prydal et al., 1992; Radke, 2005).

An analysis similar to that performed here led to the conclusion that the cut-off previously reported for decyl acetate and 1-undecanol was unlikely to be based on a failure to reach a high enough vapor concentration (Cometto-Muñiz et al., 2005b). Instead, it was probably based on these molecules exceeding a critical size and becoming too large to be accommodated effectively by the necessary number and/or types of chemesthetic receptors. The present work incorporates heptyl benzene and 2-
tridecanone, from homologous alkylbenzene and 2-ketone series, as additional cut-off stimuli, also likely to have surpassed some critical molecular dimension to evoke ocular chemesthesis.

In order to explore the possibility of there being some critical molecular dimension, beyond which ocular chemesthesis is not evoked, we have calculated the molecular dimensions of the compounds in the two homologous series. HyperChem software (HyperChem) was used to optimize the conformation of minimum energy of a VOC in the gas phase. The geometry optimization was carried out using the semi-empirical and accurate AM1 method (Dewar et al., 1985), whereas the Polak-Ribiere method (Schwartz and Polak, 1997), a good general-purpose optimizer, was chosen as the minimization algorithm. Then we can obtain the dimensions of the box into which the minimum energy conformation of the VOC will just fit. The dimensions are X, Y and Z, corresponding to the width, depth and length of the box. We denote this box as the ‘VOC box’. It is important to note that the volume of the box, calculated as XYZ, is not the same as the volume of the particular VOC. This is why we shall refer to the box dimension just as XYZ.

This procedure was carried out for the VOCs in the homologous series of ketones and alkylbenzenes. We found nothing unusual with respect to any single one of the dimensions and a ‘cut-off’ point. The dimensions increase gradually along the homologous series with no divergence from regularity at or near heptyl benzene or 2-tridecanone. The above calculations refer to the gas phase, which can be taken as almost equivalent to a non-polar non-aqueous solvent. It is well established that in non-aqueous solvents proteins adopt an extended or non-folded conformation (Soares et al., 2003) whereas in water they adopt a folded conformation (Glockshuber, 2001; Yon,
There is a lack of information on conformations of long chain homologues in solution, but many years ago Abraham (Abraham, 1984) examined the solubility of a number of homologous series in water. He showed that the aqueous solubility decreased regularly with increase of chain length along any homologous series up to a certain point, after which the solubility decrease was much less. For the n-alkanes this point was reached near dodecane, and for the n-alcohols near to 1-dodecanol (Abraham, 1984). Whether or not the origin of these effects is folding of the alkyl chain in water, we think it unlikely to be coincidental that the cut-off point for ocular chemesthesis of the n-alcohols at 1-undecanol (Cometto-Muñiz et al., 2005b) is very close to the aqueous solubility effect at 1-dodecanol.

It therefore seemed important to repeat our calculations on the homologous series, but this time for VOCs in an aqueous environment rather than in the gas phase. Calculations were therefore set up with a box containing 1264 water molecules, and the VOC placed in the center of the box. The size of this box, which determines the number of water molecules in it, was chosen based on the size of the largest VOC in the homologous series, which is 2-octadecanone, thus ensuring that even the largest VOC is surrounded by water molecules. The conformation of the VOC is then altered until it reaches the position of minimum energy. After the optimization the ‘VOC box’ was again constructed, as the smallest box enclosing the VOC solute. In this calculation, the atoms of the target VOC move during the structural optimization to their final minimum energy conformation. The surrounding water molecules do not move during the minimization, but produce a fixed potential field with which the VOC interacts. These calculations showed that for the 2-alkanones, the ‘VOC box’ XYZ value reaches a local minimum at 2-tetradecanone, and for the alkylbenzenes XYZ reaches another local minimum at octylbenzene.
The observation of local minima of XYZ at or near to the observed cut-off points in the homologous series was interesting enough to repeat the calculations, but this time allowing the water molecules to move during the optimization. Due to the large calculation time involved, we chose the molecular mechanics method MM+ (HyperChem Computational Chemistry, 2002), not as accurate as AM1, but much faster. The Polak-Ribiere method was again chosen as the minimization algorithm.

Results of the ‘VOC box’ dimensions, X, Y, and Z, for calculations in the gas phase using the AM1 procedure are in Tables 1 and 2, in units of Å and Å³. The dimensions alter quite regularly with increase in the number of carbon atoms in the VOC. The corresponding results for the VOCs in aqueous solution using the MM+ procedure are in Tables 3 and 4. It is clear by inspection of Tables 3 and 4 that the VOC dimensions do not alter regularly with the number of carbon atoms in the VOC. Plots of Z and of XYZ against carbon chain length for the two homologous series reveal that the discontinuities are only evident in plots of XYZ, not Z, against carbon chain length, Figures 8 and 9. The plot of XYZ against carbon number for the 2-ketones, Figure 8, shows a discontinuity at C9 as well as at C15, and for the alkylbenzenes the plot of XYZ against carbon number, Figure 9, shows a discontinuity at the side chain carbon number C3 and C8, corresponding to a total carbon number of C9 (propylbenzene), and C14 (octylbenzene). The discontinuities in XYZ occur at positions along the homologous series very close to the observed cut-offs in ocular chemesthesia, that is at 2-tridecanone and heptylbenzene.

These calculations refer to a completely non-biological system, but cut-off effects are indeed observed in such systems. The solubility phenomena observed by Abraham
(Abraham, 1984) is an indirect example. A direct example is the complexation of 1-alkanols with dipalmitoyl-L-α-phosphatidylcholine (DPPC) in D$_2$O/CCl$_4$ reversed micelles (Chiou et al., 1990) that showed a very sharp cut-off at 1-tetradecanol. Examples of cut-offs in the interaction of aqueous homologous series with biological systems include anesthesia (Franks and Lieb, 1985, 1990, 1994; Katz and Aharon, 2000) and effects on enzymes (Fonteh et al., 1989; Franks and Lieb, 1985; McKenzie et al., 1995). The most interesting of the latter is the cut-off effects observed by Franks and Lieb (1985) for the inhibition of activity of the firefly luciferase enzyme by aqueous alkanes and 1-alkanols. For the alkanes, a sharp but small cut-off point at hexane (C6) was observed, followed by another gradual cut-off around dodecane (C12). The 1-alkanols similarly showed a sharp small cut-off at 1-heptanol (C7) and a gradual but more severe cut-off around 1-dodecanol (C12). These twin cut-off effects are extraordinarily similar to those shown in Figures 8 and 9. Franks and Lieb suggested that the luciferase binding site was amphiphilic, and contained polar as well as non-polar parts. The binding site can accommodate two molecules of an inhibitor provided that the latter is less than C6. For homologues higher than C6, only one molecule binds, but the site cannot accommodate even a single molecule higher than C12.

Whether these considerations apply to ocular chemesthesis is another matter. However the present calculations strongly supports our conclusion that the observed lack of ocular chemesthesis in the higher homologues is due to a chemical effect that is connected to the size of the VOC and the receptor, and not to the low vapor pressures of the higher homologues. Furthermore, if the present results of calculations are found to be general, then they constitute a method for the prediction of the cut-off point in any homologous series. We shall explore this by extending our calculations to other homologous series.
We have previously interpreted the mathematical model we have constructed for the correlation of eye irritation thresholds (Abraham et al., 2001; Abraham et al., 2003; Abraham et al., 1998b; Cometto-Muñiz et al., 2005b) as though the receptor was in a largely non-aqueous (but quite polar) environment, which seems, at first sight, to be contrary to the present results. Franks and Lieb (Franks and Lieb, 1994), however, have argued that anesthetics act on hydrophobic protein pockets that are exposed to water. This would be the case in eye irritation, because the pre-ocular film that lies adjacent to the epithelium, the tear film, is largely composed of water (Iwata, 1973). Hence the receptor could act as somewhat polar non-aqueous medium, capable of interacting with hydrophobic parts of VOCs in a homologous series, and yet be responsive to configurational changes in the VOC brought about by the adjacent aqueous medium.
Acknowledgments

The work described in this article was supported by research grant number R01 DC 005003 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health, and, in part, by Philip Morris USA Inc. and Philip Morris International. Thanks are due to K. Sheets, E. Lu, N. Mathur, C. Valderrama, E. Kertowidjojo, and T. Chang for technical assistance.
References


differences in phenylthiocarbamide and propylthiouracil bitterness perception.


### Table 1.
Dimensions (Å and Å³) of the box enclosing the VOC for the 2-ketone series in vacuo

<table>
<thead>
<tr>
<th>VOC</th>
<th>MF</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>XYZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-octanone</td>
<td>C8H16O</td>
<td>2.71916</td>
<td>1.81467</td>
<td>10.58340</td>
<td>52.22250</td>
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<tr>
<td>2-nonanone</td>
<td>C9H18O</td>
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<td>1.81441</td>
<td>11.79974</td>
<td>58.79238</td>
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<tr>
<td>2-decanone</td>
<td>C10H20O</td>
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<td>13.07824</td>
<td>64.31862</td>
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<td>2-undecanone</td>
<td>C11H22O</td>
<td>2.71031</td>
<td>1.81547</td>
<td>14.30010</td>
<td>70.36345</td>
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<tr>
<td>2-dodecanone</td>
<td>C12H24O</td>
<td>2.71518</td>
<td>1.81485</td>
<td>15.57532</td>
<td>76.74964</td>
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<tr>
<td>2-tridecanone</td>
<td>C13H26O</td>
<td>2.70229</td>
<td>1.81460</td>
<td>16.80535</td>
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<td>2-tetradecanone</td>
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<td>1.81491</td>
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<td>2-pentadecanone</td>
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<td>1.81501</td>
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<td>2-heptadecanone</td>
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<td>2-octadecanone</td>
<td>C18H36O</td>
<td>2.76912</td>
<td>1.81491</td>
<td>23.05823</td>
<td>115.88383</td>
</tr>
</tbody>
</table>

### Table 2.
Dimensions (Å and Å³) of the box enclosing the VOC for the alkylbenzene series in vacuo

<table>
<thead>
<tr>
<th>VOC</th>
<th>MF</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>XYZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethylbenzene</td>
<td>C8H10</td>
<td>4.61663</td>
<td>2.88829</td>
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<td>propylbenzene</td>
<td>C9H12</td>
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<td>2.71558</td>
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<td>butylbenzene</td>
<td>C10H14</td>
<td>4.76730</td>
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<td>pentylbenzene</td>
<td>C11H16</td>
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<td>hexylbenzene</td>
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<td>octylbenzene</td>
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<td>3.50860</td>
<td>17.75894</td>
<td>352.84412</td>
</tr>
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</table>
Table 3.
Dimensions (Å and Å³) of the box enclosing the VOC for the 2-ketone series in water

<table>
<thead>
<tr>
<th>VOC</th>
<th>MF</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>XYZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-octanone</td>
<td>C8H16O</td>
<td>2.71848</td>
<td>1.99700</td>
<td>10.76183</td>
<td>58.42387</td>
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<td>2-nonanone</td>
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<td>2.37967</td>
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<td>C10H20O</td>
<td>2.72092</td>
<td>1.97314</td>
<td>13.31027</td>
<td>71.45959</td>
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<td>2-undecanone</td>
<td>C11H22O</td>
<td>2.73122</td>
<td>2.15495</td>
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<td>85.72491</td>
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<td>2-dodecanone</td>
<td>C12H24O</td>
<td>2.77172</td>
<td>1.96935</td>
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<td>C13H26O</td>
<td>2.77476</td>
<td>2.03814</td>
<td>17.12012</td>
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<td>2-tetradecanone</td>
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<td>2.11536</td>
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<td>C15H30O</td>
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<td>2-hexadecanone</td>
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<td>2-heptadecanone</td>
<td>C17H34O</td>
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<td>2.25086</td>
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<td>2-octadecanone</td>
<td>C18H36O</td>
<td>2.91829</td>
<td>2.29446</td>
<td>23.47993</td>
<td>157.21926</td>
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</tbody>
</table>

Table 4.
Dimensions (Å and Å³) of the box enclosing the VOC for the alkylbenzene series in water

<table>
<thead>
<tr>
<th>VOC</th>
<th>MF</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>XYZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethylbenzene</td>
<td>C8H10</td>
<td>4.77286</td>
<td>1.89689</td>
<td>7.34661</td>
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<td>2.63893</td>
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<td>4.86036</td>
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<td>4.85192</td>
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<td>4.85982</td>
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<td>19.74669</td>
<td>271.24688</td>
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</table>
Figure Legends

**Figure 1.** Detectability (left y-axis) and confidence of detection (right y-axis) of the group (n=15) for the five chemicals presented to the eye at two flowrates. Each point represents the average of 312 to 316 judgments provided by 15 subjects. Bars indicate standard error (SE).

**Figure 2.** Individual ocular detectability of each chemical, averaged across the two flowrates of presentation, for the 15 subjects (dotted lines, each symbol represents one subject). Single data points comprise between 40 and 54 judgments per chemical. The average data for the group is also shown (thick, continuous lines, filled diamonds). Group data points comprise between 624 and 630 judgments per chemical. The thick horizontal line highlights chance detection level.

**Figure 3.** Showing: a) agreement in the ocular detectability of heptyl benzene and 2-tridecanone between the group in Experiment 1 (n=15) and that in Experiment 2 (n=21), both tested at room temperature, 23°C, and b) failure to enhance significantly the detectability of either chemical by increasing their vapor concentration via heating them to 37°C.

**Figure 4.** Individual ocular detectability of heptyl benzene and 2-tridecanone at room temperature, 23°C, and heated (H) to 37°C, for the 21 subjects (dotted lines, each symbol represents one subject). Single data points comprise 20 judgments per stimulus. The average data for the group is also shown (thick, continuous lines, filled diamonds). Group data points comprise 420 judgments per stimulus. The thick horizontal line highlights chance detection level.
Figure 5. Comparison between a group of unresponsive (n=13) and a group of responsive (n=8) subjects in terms of their ability to precipitate or increase the ocular detectability of the VOC with an increase in concentration (achieved by increasing the temperature of the VOC). The left panel shows the results for heptyl benzene and the right panel those for 2-tridecanone. Bars indicate standard error. Data for the unresponsive groups represent the average of 260 judgments per stimulus; data for the responsive groups represent the average of 160 judgments per stimulus.

Figure 6. Relationship between various set of values for vapor concentration and carbon chain length for homologous 2-ketones. The set of values include: 1) saturated vapor (SV) concentration at 23°C; 2) SV concentration at 37°C (both sets from: extrapolated plots of log vapor pressure vs. carbon chain length and (Ambrose et al., 1975; Stephenson and Malanowski, 1987)); 3) measured human eye irritation thresholds (EIT) (from this paper, Experiment 1, and (Cometto-Muñiz and Cain, 1995)); 4) predicted, i.e., calculated, eye irritation thresholds (EIT) (from (Abraham et al., 2001)).

Figure 7. Analogous to Figure 6 but for homologous alkylbenzenes. Carbon chain length refers to the side chain attached to the benzene ring. Values of SV concentration at both temperatures from (Wilhoit and Zwolinski, 1971); the rest as in Figure 6.

Figure 8. A plot of the VOC box dimension, XYZ, against the number of carbon atoms for the 2-alkanone series in water.

Figure 9. A plot of the VOC box dimension, XYZ, against the number of carbon atoms in the alkyl side chain for the alkylbenzene series in water.
FIGURE 7

Alkylbenzenes

SV@37°C
SV@23°C
EIT-Predicted
y = -0.411x + 4.903   r = 1.000
EIT-Measured
y = -0.455x + 4.819   r = 0.999

Vapor concentration (log ppm)

Carbon chain length

Alkylbenzenes