Exp Brain Res (1998) 118:180–188 

RESEARCH ARTICLE

**J. Enrique Cometto-Muñiz · William S. Cain**
**Michael H. Abraham**

# Nasal pungency and odor of homologous aldehydes and carboxylic acids

Received: 12 January 1997 / Accepted: 16 July 1997

**Abstract** Airborne substances can stimulate both the olfactory and the trigeminal nerve in the nose, giving rise to odor and pungent (irritant) sensations, respectively. Nose, eye, and throat irritation constitute common adverse effects in indoor environments. We measured odor and nasal pungency thresholds for homologous aliphatic aldehydes (butanal through octanal) and carboxylic acids (formic, acetic, butanoic, hexanoic, and octanoic). Nasal pungency was measured in subjects lacking olfaction (i.e., anosmics) to avoid odor biases. Similar to other homologous series, odor and pungency thresholds declined (i.e., sensory potency increased) with increasing carbon chain length. A previously derived quantitative structure-activity relationship (QSAR) based on solvation energies predicted all nasal pungency thresholds, except for acetic acid, implying that a key step in the mechanism for threshold pungency involves transfer of the inhaled substance from the vapor phase to the receptive biological phase. In contrast, acetic acid – with a pungency threshold lower than predicted – is likely to produce threshold pungency through direct chemical reaction with the mucosa. Both in the series studied here and in those studied previously, we reach a member at longer chain-lengths beyond which pungency fades. The evidence suggests a biological cutoff, presumably based upon molecular size, across the various series.

**Key words** Trigeminal nerve · Olfaction · Sensory irritation, mode of action · Chemosensory thresholds · Aldehydes and acids

J.E. Cometto-Muñiz (✉) · W.S. Cain
Chemosensory Perception Laboratory,
Department of Surgery (Otolaryngology), Mail Code 0957,
University of California, San Diego, La Jolla, CA 92093-0957, USA
Tel.: +1-619-622-5832; Fax: +1-619-458-9417,
e-mail: ecometto@ucsd.edu

M.H. Abraham
Christopher Ingold Laboratories, Department of Chemistry,
University College London, 20 Gordon Street,
London WC1H 0AJ, UK

## Introduction

Most volatile organic compounds (VOCs) can be detected via olfaction and via chemesthesis, or the common chemical sense (see Green et al. 1990), which mediates pungent sensations such as tingling, piquancy, prickling, burning, freshness, stinging, and irritation, among others. The nerves responsible for chemesthesis in the mucosal tissue of the face and head include primarily the trigeminal nerve, which innervates the ocular, nasal, and anterior oral mucosae, and the glossopharyngeal and vagus nerves, which innervate the posterior oral, pharyngeal, and tracheal mucosae.

Although sensory irritation has long figured as a concern regarding exposure to VOCs in the industrial workplace, in recent times it has figured as a concern in residential (e.g., Lindstrom et al. 1995) and commercial (e.g., Wilkins et al. 1993) environments as well. Issues of indoor air pollution have evoked considerable interest in the irritation caused by relatively benign VOCs such as vapors from solvents, adhesives, carpet backing, and the like (Cometto-Muñiz and Cain 1994a).

For a typical VOC noncorrosive to tissue, the threshold for pungency or irritation lies above the odor threshold by as little as 1 or as many as 4 orders of magnitude (Cometto-Muñiz and Cain 1994a). The concentration at which odor takes on an irritating character can prove difficult to specify. When both olfaction and chemesthesia are active together, it often proves difficult to distinguish how much of the sensation derives from the one and how much derives from the other. The level where chemesthesia just begins, however, can have significance for health. Whereas odor may annoy, irritation may become a symptom and may in some cases reach the point of a clinical sign.

There exists a growing set of data on the odor, nasal pungency, and eye irritation thresholds of VOCs in humans. Substances tested include both individual members and mixtures of homologous series of alcohols, acetates, ketones, alkylbenzenes, and other miscellaneous compounds (Cometto-Muñiz and Cain 1990, 1991, 1993,

1994b, 1995; Cometto-Muñiz et al. 1997b). The research has specifically targeted relatively benign compounds. For these, it has appeared that physicochemical properties govern potency. The study of homologous series has provided one way to explore the matter conveniently. Judicious selection of stimuli has been complemented by selection of two types of subjects: a group with no olfaction (i.e., anosmics) has provided nasal pungency thresholds unbiased by odor sensations, and another with normal olfaction (i.e., normosmics) – matched for age, gender, and smoking status to the anosmics – has provided odor thresholds. Both groups have provided eye irritation thresholds.

Given that thresholds for odor lie orders of magnitude below those for nasal pungency, normosmics can be tested for nasal pungency thresholds only under a substantial odor background. In this context, we found that different normosmics used different response criteria to judge when a strong odor becomes "barely" pungent. Unfortunately, it is not possible to control for response bias in normosmics through a forced-choice comparison with a blank, since samples can always be detected by their odor. Although this complicating factor provides rationale for the study of anosmics, it does not guarantee that thresholds from such subjects reflect the true common chemical sensitivity of the normosmic population. In comparison with a group of hyposmics and anosmics, normosmics have produced marginally larger peak-to-peak amplitudes in the early P1N1 wave of cortical evoked potentials elicited by carbon dioxide, a stimulus with predominantly trigeminal impact (Hummel et al. 1996). Nevertheless, alternative tests of human trigeminal chemosensitivity, such as thresholds for eye irritation and nasal localization (i.e., lateralization) have indicated virtual parity between normosmics and anosmics against the range of stimulation explored, up to 6 orders of magnitude (Cain and Cometto-Muñiz 1996; Cometto-Muñiz and Cain 1997; Cometto-Muñiz et al. 1997a). Furthermore, a study of irritation-induced reflex changes in respiration in mice also implied no effect of anosmia on sensitivity to nasal irritation (Hansen et al. 1994). If anosmics and normosmics exhibit any differences between them such differences would seem too small to have material relevance in studies of structure-activity relationships.

A step in the quest to understand the particular physicochemical properties relevant to nasal pungency potency of VOCs entailed development of a solvation equation to describe the observed irritation thresholds (Abraham et al. 1996). Such an equation – a quantitative structure-activity relationship (QSAR) – should serve also to predict nasal pungency thresholds of untested VOCs.

Although developed for relatively nonreactive VOCs, such as those commonly found indoors (Saarela et al. 1993), the equation nevertheless makes predictions of potency for more reactive species. In such cases, the "predictions" refer to what we might consider baseline potency. In a previous application of a comparable QSAR to the prediction of sensory irritation potency assayed by respiratory depression in mice, Abraham et al. (1990) argued that an equation developed for nonreactive compounds should underestimate potency of sufficiently reactive compounds. The term "sufficiently reactive" has no precise a priori definition. Substances can react with tissue via various mechanisms, including breaking of disulfide bonds, chemical reaction with a nucleophilic group, oxidation, or direct acid-base reaction (Nielsen 1991). When such mechanisms exceed the leverage of mechanisms based upon solvation, then potency will exceed predictions based upon the QSAR. When they do not, the QSAR should define the boundary between odor alone and odor plus irritation according to the argument of Abraham et al. (1996).

The present work involves the saturated aldehydes and carboxylic acids, also found indoors (Wolkoff and Wilkins 1993; Brown et al. 1994). Here, we address the question of whether the solvation equation derived from measurement of thresholds for other compounds would predict the irritation of the aldehydes and acids. Some aldehydes and acids, particularly those of shorter chain length, may cause pungency via reactivity whereas most will presumably not. When and if the equation fails to describe the potency of any of these compounds, it should, by the reasoning above, give an underestimate.

## Materials and methods

### Stimuli

All chemicals met specifications in the Food Chemicals Codex (FCC) or were ACS reagent grade. The carboxylic acids tested were formic, acetic, butanoic, hexanoic, and octanoic acid. The aldehydes tested were butanal, pentanal, hexanal, heptanal, and octanal. Distilled water served as solvent for formic and acetic acids; mineral oil (light, FCC quality) served as solvent for the other compounds.

Dilution series were prepared in duplicate for each substance. Dilution series started with undiluted chemical, i.e., 100% v/v (called dilution step 0), and continued in three-fold dilution steps: 33% v/v (dilution step 1), 11% v/v (dilution step 2), 3.7% v/v (dilution step 3), etc. Stimuli were kept in 270-ml squeezable, polypropylene bottles (Amoore and Ollman 1983; Cain et al. 1988). The volume of liquid per bottle was 30 ml. The bottle cap had a pop-out spout that could fit into the nostril and thereby allowed testing of each nostril separately.

Headspace concentrations in the bottles were measured (in duplicate or more) for every dilution step and compound via a gas chromatograph (PID or FID detector) equipped with a gas sampling valve. The headspace of the bottles containing undiluted chemical was assumed to be saturated with that chemical. Vapor pressure at room temperature (≈23°C) was obtained from handbooks or databases. Knowledge of saturated vapor concentration (dilution step 0) and its associated chromatographic reading (average of two to four samplings) allowed conversion of the readings from the other dilution steps into vapor-phase concentrations. The chromatographic column used to quantify the aldehydes was a DB-1, 30 m×0.53 mm ID, 5.0 μm film thickness (J&W Scientific, Folsom, Calif.), and the column for the carboxylic acids was a HP-FFAP, 30 m×0.53 mm, 1.0 μm film thickness (Hewlett-Packard). Both columns gave linear responses over a wide range of concentrations up to their very limit of sensitivity, though odor thresholds often needed to be extrapolated – an average of three steps for the acids and two steps for the aldehydes. Nasal pungency thresholds were always quantified through actual readings.

### Subjects

The normosmic group comprised seven nonsmoking subjects: four women aged 36, 39, 51, and 67 years, and three men aged 21, 51, and 67 years. The 39-year-old woman and the 67 year-old man and woman had previously smoked.

The anosmic group comprised four subjects: one woman aged 39 years, and three men aged 21, 67, and 75 years. The 39-year-old woman smoked, and the 67-year-old man had previously smoked. According to their clinical history, the 39-year-old woman and the 21-year-old man were congenital anosmics, the 67-year-old man was a post-URI (upper respiratory infection) anosmic, and the 75-year-old man was an anosmic with a history of nasal-sinus disease.

The subjects were given a standard olfactory test to confirm normosmia or anosmia (Cain 1989).

The study protocol was approved by the Human Subjects Committee of the University of California, San Diego. All subjects gave their written informed consent in forms approved by the Committee.

### Procedure

Odor thresholds (measured in normosmics) and nasal pungency thresholds (measured in anosmics) were obtained using two-alternative, forced-choice presentation via an ascending method of limits. Subjects were instructed which nostril to use. On a trial they were given two bottles, one with and one without the stimulus, in irregular sequence. They picked up one bottle, introduced the pop-out spout in the nostril of interest, squeezed and sniffed. They repeated the procedure with the other bottle in the same nostril. Then, they had to decide which bottle had delivered the stronger sensation: odor for normosmics or pungency for anosmics. On the first trial, the blank bottle – containing just solvent – was paired with a bottle containing the highest dilution step (lowest concentration) of the series for that compound. Testing always started at some step below the anticipated threshold. If the subject chose correctly, the same step (concentration) was presented again along with a blank. If the subject chose incorrectly, the next lower dilution step (higher concentration) was presented, paired with a blank. Trials continued until five correct choices in a row were made at a dilution step. Testing in that nostril then stopped and that step was taken as the threshold. The entire procedure was then repeated with the other nostril. After that, testing continued with another substance. The order of testing of the two nostrils and of the ten chemicals was irregular among sessions for the same subject, and among subjects.

A participant produced a total of eight thresholds (four with the right and four with the left nostril) for each compound. Typically, the odor threshold for a compound represents the average of 56 (8×7) measurements and the nasal pungency threshold an average of 32 (8×4) measurements.

### Data analysis

The geometric mean served to summarize performance both within and across subjects for each chemical (Brown et al. 1968; Amoore 1986; Cain and Gent 1991).

## Results

Figure 1 shows the odor and nasal pungency thresholds. The general trend agreed with that obtained for homologous alcohols (Cometto-Muñiz and Cain 1990), acetates (Cometto-Muñiz and Cain 1991), ketones (Cometto-Muñiz and Cain 1993), and alkylbenzenes (Cometto-Muñiz and Cain 1994b), that is: (1) both types of sensory threshold tended to decline with increasing carbon chain-length, (2) nasal pungency thresholds lay one or more orders of magnitude above odor thresholds, (3) the gap between odor and pungency thresholds tended to increase with carbon chain-length, (4) variability of odor thresholds (indicated by SD) exceeded that of pungency thresholds, and (5) in the case of nasal pungency thresholds, a member was reached beyond which anosmics began to experience difficulty in detection.

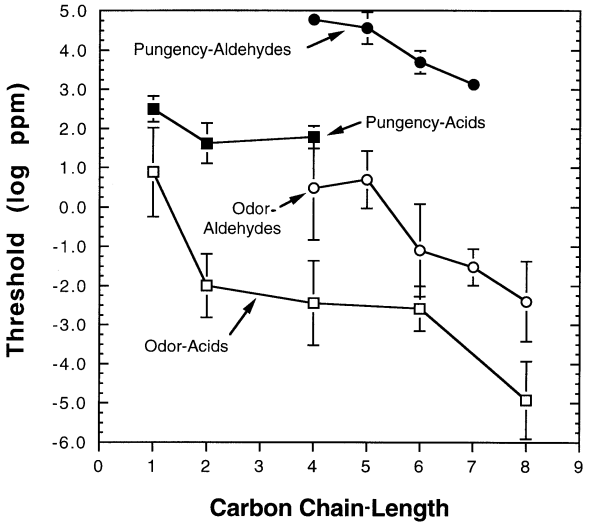


**Fig. 1** Average odor and nasal pungency thresholds for aliphatic aldehydes and carboxylic acids as a function of carbon chain-length. *Bars* indicate standard deviations

For carboxylic acids, the gap between odor and nasal pungency thresholds ranged from a low of 1.6 orders of magnitude for formic acid to a high of 5.2 orders for octanoic acid (octanoic and hexanoic acids sometimes failed to be detected by anosmics and are not plotted in Fig. 1, see below). For aliphatic aldehydes, the odor/pungency gap was much more uniform across the members of the series, amounting to about 4 orders of magnitude for butanal and pentanal, about 5 for hexanal and heptanal, and 5.6 for octanal (octanal also failed to be detected in some instances and is not plotted in Fig. 1: see below).

The range of odor thresholds between highest and lowest values across members of a series equaled 5.8 orders of magnitude for the acids (over a chain-length of eight carbon atoms) and 2.9 orders of magnitude for the aldehydes (over a chain-length of five carbon atoms). In contrast, the range of nasal pungency thresholds across members of a series equaled only about 2.2 and 1.6 orders of magnitude for the acids and aldehydes, respectively.

As observed in other series, the gain in potency with chain-length ceased rather abruptly at a certain point in the series. After that transition point, some anosmics failed to detect pungency on some or all occasions even for saturated vapor (Cometto-Muñiz and Cain 1996). After failure of detection first appeared, it increased at higher chain-lengths**.** In the case of the acids, a loss of potency first appeared with hexanoic acid, where one of the four anosmics consistently failed to detect it, and two other anosmics failed once out of eight instances. The loss grew larger for octanoic acid, where two of the anosmics con-

sistently failed to detect it, and the other two failed, respectively, on two of eight occasions and on one of eight occasions. For the aldehydes, only octanal showed signs of partial loss of ability to elicit pungency: two anosmics failed to detect it in three of eight instances. The possible meaning of these "cut-offs" in pungency for acids and aldehydes, in the context of cut-offs in pungency for other homologous series, is explored further in the Discussion.

### Prediction of nasal pungency

The solvation equation that Abraham et al. (1996) proposed for human nasal pungency contained the descriptors dipolarity/polarizability ($\pi_2^H$), overall or effective hydrogen-bond acidity ($\Sigma\alpha_2^H$), overall or effective hydrogen-bond basicity ($\Sigma\beta_2^H$), and gas-liquid partition coefficient on hexadecane at 298 K ($L^{16}$) as follows:

$$\log 1/\mathrm{NPT} = -8.562 + 2.209\pi_2^H + 3.417\Sigma\alpha_2^H + 1.535\Sigma\beta_2^H + 0.865\log L^{16}$$

$$n = 34 \quad r = 0.976 \quad SD = 0.27 \quad F = 144$$

Here, NPT is nasal pungency threshold, $n$ is the number of data points (VOCs), $r$ is the correlation coefficient, SD is the standard deviation in log 1/NPT, and $F$ is the $F$-statistic. Values of the descriptors for the stimuli in the present investigation have all been published (Abraham 1993) with one exception. The exception is formic acid, for which a set of descriptors was obtained from water-solvent partitions; the log $L^{16}$ value for formic acid was obtained from a calculated gas-water partition and a calculated water-hexadecane partition. A full account of how to obtain descriptors other than log $L^{16}$ has been recently published (Abraham and Chadha 1996).

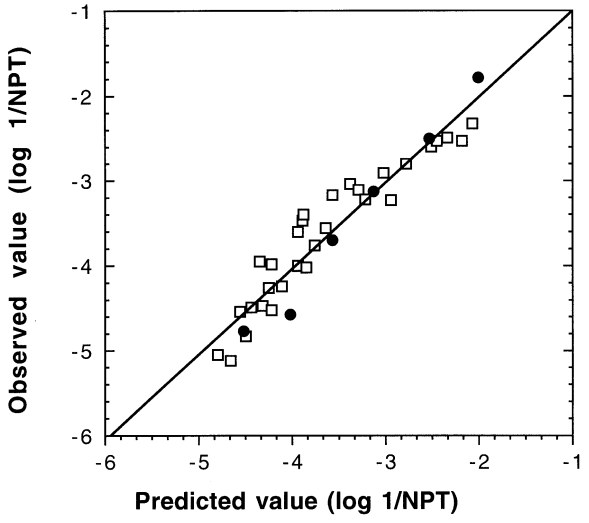


**Fig. 2** Plot of observed nasal pungency thresholds versus thresholds for aliphatic aldehydes and carboxylic acids (*filled circles*) predicted by the solvation equation (Abraham et al. 1996). Also shown are observed versus calculated values (*open squares*) for a training set of homologous alcohols, acetates, ketones, alkylbenzenes, and miscellaneous compounds used to derive the solvation equation. The *line* represents the equation for all chemicals: $y$=1.013$x$ –0.025, $r$=0.954

With the exception of acetic acid, the observed values of potency for the present stimuli, expressed as log 1/NPT, where NPT represents nasal pungency threshold in ppm, agreed closely with predictions from the solvation equation. As Fig. 2 indicates, the agreement for the new stimuli proved comparable to that for those stimuli used to derive the equation.

For acetic acid, the equation underestimated potency. Its predicted threshold of 713 ppm lay 1.2 orders of magnitude above its measured threshold of 42 ppm. (Corresponding values of log 1/NPT equaled –2.85 for predicted and –1.62 for obtained.) Abraham et al. (1996) anticipated such departures for stimuli that show chemical reactivity toward tissue. In such cases, the equation should indicate *minimum* or baseline potency for a substance with its particular physicochemical properties.

## Discussion

Three principal matters merit discussion: (1) greater relative success of the solvation equation approach for irritation than for odor, (2) existence and possible significance of "cut-off" points, and (3) perspective on the performance of the solvation equation for reactive compounds.

### Olfaction versus irritation

Although the solvation equation both described and predicted the potency of irritation successfully, it has fared much less well for potency of odorants (Abraham 1996). No other proposed relationship has done very well either. Laffort (Laffort 1968, 1969; Laffort and Dravnieks 1973; Laffort and Patte 1987) has sought through the years to predict human odor thresholds from physicochemical properties. The efforts have left enough residual variance, approximately 20%, to qualify as rough attempts. His equations, which have included terms for air-water partitioning, apolarity, hydrogen bonding, and polarizability, offer no theoretically coherent view of the interaction between stimulus and biophase that may determine potency. The equations have apparently come principally from empirical considerations, i.e., from finding associations between potency and certain parameters, with some additional assumptions about additivity and substitutability of terms.

The QSAR of Abraham et al. (Abraham 1996) has offered a coherent theoretical basis in that it describes critical features of the biophase where stimulation occurs in terms of its properties as a solvent. Insofar as transduction of sensory irritation requires the activation of a receptor, then presumably the receptor serves as the "solvent". Despite the virtue of theoretical coherence, the QSAR too leaves an unacceptable amount of unexplained variance in description of odor thresholds: about 20%. The finding

that these equations can account for more than a small amount of variance suggests some general determinants of potency from odorant to odorant (e.g., an advantage of lipophilicity), but their failure to do better suggests the existence of more specific determinants as well.

Following the analysis of Abraham et al. (1994) it is possible that the various QSARs for odor thresholds treat only that proportion of the effect that arises through transport of the odorant from the vapor phase to the receptor(s). This is why such QSARs invariably include some measure of transport, for example air-water partitioning or air-solvent partitioning. The detailed interaction of the odorants with specific receptors is largely not covered by these QSARs, which is why there is always a large residual variance.

Quite possibly, no single equation can account for potency across the entire spectrum of odorants. Since transduction of olfaction indisputably requires activation of a receptor, and since the number of such different receptors equals approximately 1000 (Ressler et al. 1994), the likelihood that a single equation could account for potency would seem remote indeed. Each receptor presumably has unique "tuning" that allows it to respond to some fraction of the vast array of thousands of possible odorants. Recent neurophysiological data on carboxylic acids and aliphatic alcohols illustrate the type of tuning that would allow discrimination of odorants not only of different functional groups but also of different chain-length within a homologous series (Sato et al. 1994).

A given olfactory receptor neuron most likely contains only one receptor type (Mori and Shepherd 1994; Axel 1995). Responses of individual neurons can accordingly yield a picture of the degree of specificity of receptor types toward odorants in a family and the degree of diversity necessary to make discriminations across attributes, such as molecular size, shape, and functional group. In measurements of calcium-ion increase in single olfactory receptor neurons of the mouse to *n*-carboxylic acids and *n*-aliphatic alcohols, one third of neurons responded to acids or alchols though not to both (Sato et al. 1994). Hence, this third of the units could discriminate functional group. When units responded to both acids and alcohols, they generally responded to those of similar, though restricted, chain-length, e.g., C4 and C5, C7 through C9. Those units, therefore, could discriminate size across functional group. Interestingly, more units responded to molecules of longer than of shorter chain-length, a finding consistent with the greater sensitivity to such stimuli as found here. Although the kind of receptor-specificity seen for olfaction in Sato et al.'s study does not preclude the possibility that a few physicochemical parameters could describe relative sensitivity, they would presumably do so across receptors and would not describe any particular biophase as a solvent in the way that may occur for sensory irritation.

A picture of neural coding similar to that seen in first-order afferents – i.e., units that differentiated by size, by functional group, and by shape – emerged from studies of secondary afferent units (mitral or tufted cells) in the olfactory bulb of rabbit stimulated with carboxylic acids, aliphatic aldehydes, and aliphatic alcohols (Imamura et al. 1992; Katoh et al. 1993). A glomerulus, the point of synaptic contact between a converging set of first-order neurons and a much smaller set of second-order output neurons, approximates a functional unit. Just as the olfactory mucosa exhibited some coarse topographically-associated sensitivity, i.e., showed regions that responded preferentially to certain types of odorants, so too did the olfactory bulb. For example, Mori et al. (1992) found a cluster of mitral cells of particular sensitivity to fatty acids in the dorsomedial region of the olfactory bulb. This comports with findings that a glomerulus in any given region of the bulb receives more projections from one part of the epithelium than another, i.e., receives some point-to-point projections, though each part of the bulb also receives some input from other parts of the mucosa. Physicochemical properties could facilitate deposition of molecules preferentially toward certain peripheral regions where specific receptors could discriminate features relevant to quality perception. This adds a layer of complexity to that already expected from the "receptor as solvent." It is interesting that in our various studies of odor and irritation in homologous series, the profile of thresholds for odor always takes on an exaggerated form of that for irritation. Typically, both types of thresholds decrease with chain-length, but the odor threshold decreases more per unit increase in chain-length than does the threshold for irritation. Perhaps the olfactory mucosa can "focus" molecules into an area in such a way as to increase regional signal-to-noise ratio. Some investigators have speculated that odorant-binding proteins, which may transport molecules of odorant to the receptors, might provide the mechanism for such regional focusing (Bianchet et al. 1996).

Imamura et al. (1992) found second-order cells in the dorsomedial region of the bulb to respond differentially to straight-chain, branched-chain, and aromatic fatty acids, and to respond differentially to molecular size. Some neurons in the region responded to aliphatic aldehydes as well as to the acids, which led Imamura et al. to speculate on the existence of a specific sensitivity to the carbonyl group – a kind of odor epitope or odotope. Although insensitive to some types of odorants (e.g., alcohols and alkanes), the acid- and aldehyde-sensitive cells did show some sensitivity to other stimuli, such as ketones and esters. Toward such stimuli the neurons showed the same chain-length specificity they showed toward aldehydes and acids. In this respect, the data shared characteristics with those of Sato et al. (1994).

Exact comparisons between first-order and second-order responses to the same stimuli in isomorphic regions of the same species via the same methods of stimulation lie in the future. Nevertheless, individual units at both levels apparently exhibit a correlation between what Imamura et al. (1992) have called "tuning specificity" and the "stereochemical structure of odor molecules, with respect to (1) length and/or structure of hydrocarbon chain, (2) difference in functional group, and (3) position of the functional group within the molecule" (p. 1986). Whether the second level or higher levels of processing alter the spectrum

of sensitivity from that created at the stimulus-receptor interface has received no attention. If the spectrum remained the same, recordings from second-order neurons would afford relatively easy access to a measure of relative potency. If the spectrum does indeed remain the same, then neurophysiological and psychophysical measures of relative potency might prove straightforward to compare.

These various investigations illustrate the advisability of studies of homologous series. Often, only with such systematic changes in stimuli can the determinants of sensitivity become evident. Neither first- nor second-order processing offers evidence to support speculation that just a few physicochemical parameters might determine sensitivity. The surprisingly large number of putative olfactory receptor proteins, each with its own tuning, might confer refined qualitative discrimination.

Despite considerable progress over the last decade in understanding the second step in olfactory transduction, namely activation of second messenger systems inside the olfactory neuron (Anholt 1993), understanding of the first step, namely the primary odorant-receptor interaction (Shepherd 1994; Singer et al. 1995, 1996; Singer and Shepherd 1994), remains somewhat primitive. Understanding of the trigeminal chemoreceptive system (Green et al. 1990) lags behind that of the olfactory system. Nevertheless, the irritation sense in contrast to olfaction makes little in the way of quality discriminations (Cain 1988; Green and Lawless 1991) and may depend for its sensitivity on just one or a few receptor types (Nielsen 1991). The ability of the solvation equation to predict potency of irritants seems compatible with such a simple system.

## Possible "cut-offs"

In the present study, hexanoic acid, octanoic acid, and octanal occasionally or consistently failed to evoke pungency in one or more of the anosmics. In studies with the alcohols, acetates, and alkyl benzenes, the same occurred with 1-octanol, octyl and higher acetates, and propyl and higher alkylbenzenes. Two different mechanisms may account for such cut-offs (Franks and Lieb 1990): a physical mechanism whereby the maximum available quantity of stimulus in the vapor phase falls below the threshold, and a biological mechanism whereby the stimulus lacks a key property to trigger transduction. A molecule could, for example, exceed the size that allows it to interact effectively with a target site or to fit into a binding pocket in a receptive macromolecule.

Plots of observed and predicted nasal pungency thresholds, and saturated vapor concentrations at room temperature versus carbon chain length (N) offer a way to explore cut-off effects. Figure 3 (upper left) shows such a plot for *n*-alkyl acetates from methyl (N=1) to dodecyl acetate (N=12). As mentioned, octyl, decyl, and dodecyl acetates failed to evoke pungency in two or more of four anosmics. Since observed and predicted thresholds for the acetates fell into register reasonably well up to heptyl acetate, and since *predicted* thresholds for acetates higher than heptyl still lie below vapor saturation, the observed lack of potency of octyl and higher acetates to produce pungency (Cometto-Muñiz and Cain 1991) seems likely to stem from a biological cut-off.

The situation is less clear for the alcohols (Fig. 3, upper right). Here, observed and predicted pungency thresholds did not follow almost identical trends as for the acetates. Overall, the curved function for the observed thresholds does not rule out the possibility of a physical cut-off at the level of 1-octanol (i.e., a threshold above vapor saturation) as an explanation for its lack of pungency (Cometto-Muñiz and Cain 1990).

For carboxylic acids and aliphatic aldehydes we only have data on a few members of each series. The acids (Fig. 3, lower left) depart substantially from the trend that all other stimuli show in relation to saturated vapor, making it difficult to suggest an explanation for the observed cut-off at hexanoic acid. The case of the aldehydes resembles that of the acetates in that the relationship between observed and predicted pungency thresholds suggests a biological cut-off at the level of octanal.

A solvation equation such as that of Abraham et al. (1996) predicts that the potency of a mixture should, to a first approximation, equal the linear combination of the potencies of its components. Since almost all real-world exposures to VOCs involve mixtures, their study stands high in the research agenda. Initial results with mixtures suggest that a linear combination provides a reasonable approximation, but that lipophilicity and complexity, i.e., number of components in a mixture, may influence degree of linearity (Cometto-Muñiz et al. 1997b). The possibility of multiple receptor types in trigeminal nerves may also influence degree of linearity. Further work will determine how well the solvation equation predicts nasal pungency thresholds for mixtures of substances.

## Reactive versus nonreactive stimuli

Cain and Cometto-Muñiz (1995) noted a generally good correlation between sensory irritation measured by the respiratory depression technique in mice ($RD_{50}$: concentration to depress rate of respiration by 50%) and human thresholds. In the present case, a comparison between mice and humans for three of the aldehydes yielded only weak correspondence: measurements of sensory irritation in mice for butanal, pentanal, and hexanal – among other aldehydes – by use of the respiratory depression technique showed similar irritation potency for the three aldehydes, with $RD_{50}$ values of 1100–1500 ppm (Steinhagen and Barrow 1984). Saturated aliphatic aldehydes – with the exception of the very potent formaldehyde – were less potent than cyclic aldehydes which, in turn, were less potent than unsaturated aliphatic aldehydes. In contrast to the results in mice, the human nasal pungency thresholds declined with increasing car-

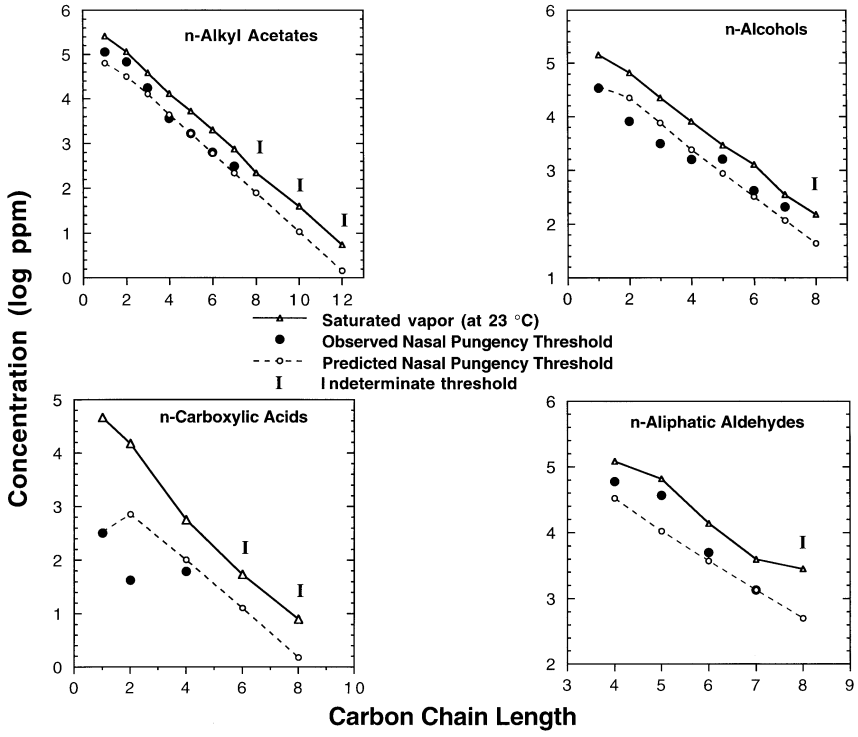


**Fig. 3** Plots of saturated vapor concentration, *observed* nasal pungency thresholds, and *predicted* nasal pungency thresholds, all as a function of carbon chain-length of homologous *n*-alkyl acetates, *n*-alcohols, carboxylic acids, and aliphatic aldehydes. The *bar symbol* refers to cases where the pungency threshold was indeterminate (i.e., one or more anosmics repeatedly failed to reach detection criterion level, even at vapor saturation)

bon chain-length, and lie above the $RD_{50}$ in mice by 1.6, 1.5, and 0.7 orders of magnitude for butanal, pentanal, and hexanal, respectively. There are various differences between the $RD_{50}$ technique and our methodology, besides the obvious species difference, but perhaps exposure time and exposed body surface are the two most relevant for sensory irritation potency. A solvation equation has also been used to describe upper respiratory tract irritation in mice (Alarie et al. 1995). The same major factors apply to both the log 1/NPT and log $1/RD_{50}$ equations, but the coefficients of $\pi$, $\alpha$, and $\beta$ are appreciably larger for the nasal pungency equation. This suggests that the receptor phase for nasal pungency is more polar, more basic, and more acidic than the phase for upper respiratory tract irritation. However, the nasal pungency phase seems unlikely to be more aqueous because a more aqueous phase would lead to a marked reduction in the coefficient "l" of the descriptor $L^{16}$. There is actually not much difference in this coefficient between the two equations (l=0.86 for nasal pungency and l=0.76 for upper respiratory tract irritation).

Manner of stimulus presentation and psychophysical method may influence absolute thresholds obtained for odor or irritation. Thresholds obtained with the polypropylene bottles used in this experiment tend to lie above those obtained by some other means (Cometto-Muñiz and Cain 1993). Nevertheless, in a recent study that employed dynamic olfactometry and a different psychophysical method from that used here, the irritation threshold for acetic acid equaled 25 ppm, which is roughly comparable to the present value of 42 ppm (Walker et al. 1989). We plan direct comparisons of sensitivity measured by different apparatus to clarify contributions of methodology.

The present data provided a very useful test of Abraham et al.'s solvation equation since they cover much of the range of potencies previously measured and add data at a higher potency. The success of the equation suggests that a key step in the evocation of nasal pungency involves transfer of the stimulus from the vapor phase to the relevant biological phase. If such transfer alone accounted for potency, it would not require postulation of an irritant receptor, though one or more receptor types for nonreactive irritants may indeed exist. To wit, recent work with the stereoisomers R- and S-nicotine indicates that trigeminal sensitivity might show stereospecific activation for certain compounds, an outcome that the solvation equation would not anticipate (Thürauf et al. 1996).

**Acknowledgements** The work described in this article was supported by research grants numbers RO1 DC 00284–12 and R29 DC 02741–01 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health, and by the Center for Indoor Air Research. Thanks are due to Mr. René Loya for his excellent technical assistance.

## References

Abraham MH (1993) Scales of solute hydrogen-bonding: their construction and application to physicochemical and biochemical processes. Chem Soc Rev 22: 73–83

Abraham MH (1996) The potency of gases and vapors: QSARs – anesthesia, sensory irritation, and odor. In: Gammage RB, Berven BA (eds) Indoor air and human health, 2nd edn. CRC Lewis, Boca Raton, pp 67–91

Abraham MH, Chadha HS (1996) Applications of a solvation equation to drug transport properties. In: Pliska V, Testa B, van der Waterbeemd H (eds) Lipophilicity in drug action and toxicology. VCH, Weinheim, pp 311–337

Abraham MH, Whiting GS, Alarie Y, Morris JJ, Taylor PJ, Doherty RM, Taft RW, Nielsen GD (1990) Hydrogen bonding 12. A new QSAR for upper respiratory tract irritation by airborne chemicals in mice. Quant Struct-Act Relat 9: 6–10

Abraham MH, Nielsen GD, Alarie Y (1994) The Ferguson principle and an analysis of biological activity of gases and vapors. J Pharm Sci 83: 680–688

Abraham MH, Andonian-Haftvan J, Cometto-Muñiz JE, Cain WS (1996) An analysis of nasal irritation thresholds using a new solvation equation. Fundam Appl Toxicol 31: 71–76

Alarie Y, Nielsen GD, Andonian-Haftvan J, Abraham MH (1995) Physicochemical properties of nonreactive volatile organic chemicals to estimate $RD_{50}$: alternatives to animal studies. Toxicol Appl Pharmacol 134: 92–99

Amoore JE (1986) Effects of chemical exposure on olfaction in humans. In: Barrow CS (ed) Toxicology of the nasal passages. Hemisphere Publishing, Washington, DC, pp 155–190

Amoore JE, Ollman BG (1983) Practical test kits for quantitatively evaluating the sense of smell. Rhinology 21: 49–54

Anholt RRH (1993) Molecular neurobiology of olfaction. Crit Rev Neurobiol 7: 1–22

Axel R (1995) The molecular biology of smell (abstract). FASEB J 9: A1323

Bianchet MA, Bains G, Pelosi P, Pevsner J, Snyder SH, Monaco HL, Amzel LM (1996) The three-dimensional structure of bovine odorant binding protein and its mechanism of odor recognition. Nature Struct Biol 3: 934–939

Brown KS, MacLean CM, Robinette RR (1968) The distribution of the sensitivity to chemical odors in man. Hum Biol 40: 456–472

Brown SK, Sim MR, Abramson MJ, Gray CN (1994) Concentrations of volatile organic compounds in indoor air: a review. Indoor Air 4: 124–134

Cain WS (1988) Olfaction. In: Atkinson RC, Herrnstein RJ, Lindzey G, Luce RD (eds) Stevens' handbook of experimental psychology. Wiley, New York, pp 409–459

Cain WS (1989) Testing olfaction in a clinical setting. Ear Nose Throat J 68: 316–328

Cain WS, Cometto-Muñiz JE (1995) Irritation and odor as indicators of indoor pollution. Occup Med: State of the Art Rev 10: 133–145

Cain WS, Cometto-Muñiz JE (1996) Sensory irritation potency of VOCs measured through nasal localization thresholds. In: Yoshizawa S, Kimura K, Ikeda K, Tanabe S, Iwata T (eds) Indoor Air '96. Proceedings of the seventh international conference on indoor air quality and climate. Indoor Air '96, Nagoya, Japan, pp 167–172

Cain WS, Gent JF (1991) Olfactory sensitivity: reliability, generality, and association with aging. J Exp Psychol: Hum Percept Perform 17: 382–391

Cain WS, Gent JF, Goodspeed RB, Leonard G (1988) Evaluation of olfactory dysfunction in the Connecticut Chemosensory Clinical Research Center. Laryngoscope 98: 83–88

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

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

Cometto-Muñiz JE, Cain WS (1993) Efficacy of volatile organic compounds in evoking nasal pungency and odor. Arch Environ Health 48: 309–314

Cometto-Muñiz JE, Cain WS (1994a) Perception of odor and nasal pungency from homologous series of volatile organic compounds. Indoor Air 4: 140–145

Cometto-Muñiz JE, Cain WS (1994b) Sensory reactions of nasal pungency and odor to volatile organic compounds: the alkylbenzenes. Am Ind Hyg Assoc J 55: 811–817

Cometto-Muñiz JE, Cain WS (1995) Relative sensitivity of the ocular trigeminal, nasal trigeminal, and olfactory systems to airborne chemicals. Chem Senses 20: 191–198

Cometto-Muñiz JE, Cain WS (1996) Physicochemical determinants and functional properties of the senses of irritation and smell. In: Gammage RB, Berven BA (eds) Indoor air and human health, 2nd edn. CRC Lewis, Boca Raton, pp 53–65

Cometto-Muñiz JE, Cain WS (1997) Trigeminal and olfactory sensitivity: comparison of modalities and methods of measurement. Int Arch Occup Environ Health (in press)

Cometto-Muñiz JE, Cain WS, Abraham MH, Kumarsingh K (1997a) Sensory properties of selected terpenes: thresholds for odor, nasal pungency, nasal localization, and eye irritation. International symposium on olfaction and taste XII and AChemS XIX, San Diego, California, 7–12 July 1997 (abstract)

Cometto-Muñiz JE, Cain WS, Hudnell HK (1997b) Agonistic sensory effects of airborne chemicals in mixtures: odor, nasal pungency, and eye irritation. Percept Psychophys 59: 665–674

Franks NP, Lieb WR (1990) Mechanisms of general anesthesia. Environ Health Perspect 87: 199–205

Green BG, Lawless HT (1991) The psychophysics of somatosensory chemoreception in the nose and mouth. In: Getchell TV, Doty RL, Bartoshuk LM, Snow JB Jr (eds) Smell and taste in health and disease. Raven Press, New York, pp 235–253

Green BG, Mason JR, Kare MR (eds) (1990) Chemical senses, vol 2, Irritation. Marcel Dekker, New York

Hansen LF, Hammer M, Petersen SH, Nielsen GD (1994) Effects of intranasal $ZnSO_4$ irrigation on olfactory and trigeminal cues. Physiol Behav 55: 699–704

Hummel T, Barz S, Lötsch J, Roscher S, Kettenmann B, Kobal G (1996) Loss of olfactory function leads to a decrease of trigeminal sensitivity. Chem Senses 21: 75–79

Imamura K, Mataga N, Mori K (1992) Coding of odor molecules by mitral/tufted cells in rabbit olfactory bulb. I. Aliphatic compounds. J Neurophysiol 68: 1986–2002

Katoh K, Koshimoto H, Tani A, Mori K (1993) Coding of odor molecules by mitral/tufted cells in rabbit olfactory bulb. II. Aromatic compounds. J Neurophysiol 70: 2161–2175

Laffort P (1968) Some new data on the physico-chemical determinants of the relative effectiveness of odorants. In: Tanyolaç NN (ed) Theories of odors and odor measurement. Technivision, Maidenhead, pp 247–268

Laffort P (1969) A linear relationship between olfactory effectiveness and identified molecular characteristics, extended to fifty pure substances. In: Pfaffmann C (ed) Olfaction and taste III. Proceedings of the third international symposium on olfaction and taste. Rockefeller University Press, New York, pp 150–157

Laffort P, Dravnieks A (1973) An approach to a physico-chemical model of olfactory stimulation in vertebrates by single compounds. J Theor Biol 38: 335–345

Laffort P, Patte F (1987) Solubility factors established by gas-liquid chromatography: a balance sheet. J Chromatogr 406: 51–74

Lindstrom AB, Proffit D, Fortune CR (1995) Effects of modified residential construction on indoor air quality. Indoor Air 5: 258–269

Mori K, Shepherd G (1994) Emerging principles of molecular signal processing by mitral/tufted cells in the olfactory bulb. Semin Cell Biol 5: 65–74

Mori K, Mataga N, Imamura K (1992) Differential specificities of single mitral cells in rabbit olfactory bulb for a homologous series of fatty acid odor molecules. J Neurophysiol 67: 786–789

Nielsen GD (1991) Mechanisms of activation of the sensory irritant receptor by airborne chemicals. CRC Crit Rev Toxicol 21: 183–208

Ressler KJ, Sullivan SL, Buck LB (1994) A molecular dissection of spatial patterning in the olfactory system. Curr Opin Neurobiol 4: 588–596

Saarela K, Kalliokoski P, Seppänen O (eds) (1993) Indoor air '93, vol 2, Chemicals in indoor air, material emissions. Proceedings of the sixth international conference on indoor air quality and climate. Indoor Air '93, Helsinki

Sato T, Hirono J, Tonoike M, Takebayashi M (1994) Tuning specificities to aliphatic odorants in mouse olfactory receptor neurons and their local distribution. J Neurophysiol 72: 2980–2989

Shepherd GM (1994) Discrimination of molecular signals by the olfactory receptor neuron. Neuron 13: 771–790

Singer MS, Shepherd GM (1994) Molecular modeling of ligand-receptor interactions in the OR5 olfactory receptor. Neuroreport 5: 1297–1300

Singer MS, Oliveira L, Vriend G, Shepherd GM (1995) Potential ligand-binding residues in rat olfactory receptors identified by correlated mutation analysis. Receptors Channels 3: 89–95

Singer MS, Weisinger-Lewin Y, Lancet D, Shepherd GM (1996) Positive selection moments identify potential functional residues in human olfactory receptors. Receptors Channels 4: 141–147

Steinhagen WH, Barrow CS (1984) Sensory irritation structure-activity study of inhaled aldehydes in B6C3F1 and Swiss-Webster mice. Toxicol Appl Pharmacol 72: 495–503

Thürauf N, Kaegler M, Dietz R, Kobal G (1996) Trigeminal and olfactory activation by R- and S-nicotine in humans (abstract). Chem Senses 21: 680

Walker JC, Warren DW, Sidman JD, Jennings RA, Reynolds JH IV (1989) Psychophysical and respiratory responses of anosmic humans to odorants (abstract). In: Døving KB (ed) Proceedings of the tenth international symposium on olfaction and taste. GCS, Oslo, Norway, p 375

Wilkins CK, Wolkoff P, Gyntelberg F, Skov P, Valbjørn O (1993) Characterization of office dust by VOCs and TVOC release: identification of potential irritant VOCs by partial least squares analysis. Indoor Air 3: 283–290

Wolkoff P, Wilkins CK (1993) Desorbed VOC from household floor dust: comparison of headspace with desorbed dust, method for TVOC release determination. In: Saarela K, Kalliokoski P, Seppänen O (eds) Indoor air '93, vol 2, Chemicals in indoor air, material emissions. Proceedings of the sixth international conference on indoor air quality and climate. Indoor Air '93, Helsinki, pp 287–292