

SENSORY REACTIONS OF NASAL PUNGENCY AND ODOR TO VOLATILE ORGANIC COMPOUNDS: THE ALKYL BENZENES

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Research assessed the independent contribution of the trigeminal and olfactory nerves to the detection of airborne chemicals by measuring nasal detection thresholds in subjects clinically diagnosed as lacking a functional sense of smell (anosmics) and in matched normal controls (normosmics). Anosmics can provide only odor-unbiased pungency thresholds. Normosmics provided odor thresholds. Stimuli included homologous alkylbenzenes (from toluene to octylbenzene), chlorobenzene, 1-octene, and 1-octyne. As seen before with homologous alcohols, acetates, and ketones, both types of threshold declined with increasing carbon chain length. Anosmics failed to detect alkylbenzenes above propylbenzene. The strong linear correlation between pungency thresholds and saturated vapor concentration for all tested compounds, as a whole, and the constancy of pungency thresholds expressed as percent of vapor saturation, suggests that nasal pungency from these substances relies heavily on a broadly tuned physicochemical interaction with a susceptible biophase within the cell membrane. Through such a nonspecific mechanism, low, subthreshold levels of a wide variety of volatile organic compounds of low reactivity—as found in many polluted indoor spaces—could add their sensory impact to precipitate noticeable sensory irritation.

In humans an early warning of the presence of potentially toxic airborne chemicals is provided by two sensory systems: olfaction and the irritation sense—also called common chemical sense (CCS).⁽¹⁾ Receptors for the sense of smell are the bipolar olfactory neurons in the olfactory epithelium, located in the upper rear portion of the nasal cavity. Each neuron sends a single dendrite to the surface of the epithelium. This dendrite ends in an olfactory knob, giving rise to a number of cilia that protrude and are immersed in the mucus bathing the nasal cavity. These cilia are believed to be the site of olfactory transduction.^(2,3) The other end of each olfactory neuron sends an axon that joins with axons from

neighboring receptors and all together they constitute the olfactory nerve (Cranial Nerve I). After penetrating the cribriform plate, the olfactory nerve reaches the olfactory bulb, making the first synapse of the pathway in bulb structures called glomeruli.

Receptors of the CCS, on the contrary, are not specialized but are simple free nerve endings, present in all mucosae. In the face, free nerve endings from different branches of the trigeminal nerve (Cranial Nerve V) provide common chemical sensitivity to the cornea, conjunctiva, nasal mucosa, and to part of the oral mucosa. Free nerve endings lie within or below the epithelium. Thus, they are less accessible to incoming molecules than olfactory receptors. Stimulation of the CCS by chemicals gives rise to a number of sensations—different from odor and taste sensations—that can be referred to as pungent sensations. They include prickling, irritation, tingling, freshness, stinging, piquancy, and burning, among others. In the present article the term “pungency” refers to nasal common chemical sensations as a whole. As mentioned, these sensations include but are not limited to sensory irritation.

Virtually all substances that stimulate olfaction can also evoke pungency, and vice versa. How can we distinguish whether a substance is producing just an odor sensation or an odor and pungent sensation? To a certain extent humans can, on instruction, assess separately the odor and pungent components of single or mixed compounds presented as stimuli.⁽⁴⁻⁷⁾ But how can we determine the concentration of an airborne chemical needed to elicit threshold nasal pungency in the absence of olfactory cues? As a rule the odor of a substance is apparent often well before any pungency can be detected. The presence of an odorous background makes the assessment of threshold pungency a very difficult task. A subject may have considerable difficulty deciding when an odor sensation contains just detectable pungency and the results obtained may reflect his response criterion as much as or even more than his sensitivity.

Independent study of olfaction and CCS in humans has both basic and applied relevance: e.g., about half of the Threshold Limit Values (TLV®s) set by the American Con-

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ference of Governmental Industrial Hygienists (ACGIH)⁽⁸⁾ for industrial and occupational exposures to chemicals are based on sensory irritation.⁽⁹⁾ The deleterious effects of chemical exposures on olfaction and CCS have been reviewed recently.^(10,11) Even in nonindustrial environments there is growing concern about the health and toxicological effects of low-level volatile organic compounds (VOCs).^(12,13) A typical example is sick building syndrome.⁽¹⁴⁾ The symptoms of exposed subjects are generally unspecific (headache, difficulty in concentration, lassitude), with sensory irritation in nose, eyes, and throat common denominators.⁽¹⁵⁾

In a number of investigations,⁽¹⁶⁻¹⁸⁾ clinically diagnosed⁽¹⁹⁾ anosmics, i.e., subjects lacking a functional sense of smell, have been tested to gain insight into true pungency thresholds for series of homologous substances and a few miscellaneous compounds of interest. For comparison, the authors measured odor thresholds in groups of age-, gender-, and smoking status-matched normosmics. Substances employed included homologous alcohols,⁽¹⁶⁾ homologous acetates,⁽¹⁷⁾ and homologous ketones, secondary and tertiary alcohols, and acetates.⁽¹⁸⁾ Other miscellaneous compounds of interest included β -phenyl ethyl alcohol, pyridine, and menthol.⁽¹⁷⁾

Results from these studies showed a systematic decrease of odor thresholds with increasing carbon chain length for all series and a similar—though not identical—decrease of pungency thresholds. Also, a linear relationship emerged between pungency thresholds for all stimuli, regardless of molecular size or chemical functionality, and their saturated vapor concentration. This suggested that pungency for these nonreactive substances relies heavily on an unspecific physical interaction between the molecules and a susceptible lipid biophase in the nasal mucosa. Anosmics could detect most of the chemicals, albeit at much higher concentrations than normosmics. In each homologous series, a member was reached where anosmics ceased, or at least had difficulty, detecting even vapor-saturated stimuli. This occurred with 1-octanol, octyl acetate, and to a smaller extent 2-nonanone, in the respective series. Among the miscellaneous substances tested, β -phenyl ethyl alcohol failed to evoke a reliable response from the anosmics.

In the present study the authors gathered olfactory and pungency thresholds for homologous alkylbenzenes. These compounds were previously tested for sensory irritation potency⁽²⁰⁾ using the respiratory depression animal bioassay developed by Alarie.⁽²¹⁾ Ethyl and propyl benzene are components of a 22-substance mixture—thought to represent a more-or-less typical mixture of indoor air pollutants—used in various experiments on controlled human exposures.⁽¹²⁾ Three other substances also were included: chlorobenzene, a more reactive benzene derivative; and 1-octene and 1-octyne, compounds in the chain-length range where lack of ability to evoke pungency was previously noted, but with no oxygen-containing chemical functionality.

MATERIALS AND METHODS

Stimuli

The compounds employed, all of analytical-grade purity, included methylbenzene (toluene) through octylbenzene, chlorobenzene, 1-octene, and 1-octyne. Mineral oil served as solvent.

Duplicate series of dilutions were prepared for each substance, starting with the pure compound (100% v/v), labeled dilution step 0, and progressing through 14 successive three-fold dilution steps, labeled 1 through 14.

Stimuli were delivered from 250-mL capacity, squeezable, white, high-density polyethylene bottles, each containing 30 mL of solution. The bottles had pop-up spouts that fitted into the nostril and thereby allowed testing of each nostril separately.^(22,23) The vapor concentration in the headspace of each bottle was measured by a Hewlett-Packard 5890A Gas Chromatograph (photoionization detector) equipped with a gas-sampling valve. Repeated chromatographic readings were taken from each step in a dilution series. The concentration of saturated vapor for the compounds was obtained from handbooks or databases on physical properties. Concentration of saturated vapor (headspace of undiluted liquid) and the associated chromatographic readings allowed conversion of the readings for the other bottles into concentration units (ppm by volume), and a calibration curve was derived.

Subjects

A total of eight subjects participated. After the nature of the procedure had been fully explained to them, all gave written informed consent for participation in the experiment on forms approved by the Yale Human Investigation Committee. The experimental protocol submitted to the committee stressed the brevity of the exposures and that the only expected effect was a transient sensory impression, albeit sometimes disagreeable because of its odor quality or pungency. Subjects were free to decide whether or not to participate and to withdraw at any time. None of them withdrew, and none of them reported any effect other than the immediate odor or pungency experienced.

Half of the subjects were anosmics (former patients from either the Connecticut Chemosensory Clinical Research Center, University of Connecticut, or Yale-New Haven Hospital). The other half were age-, gender-, and smoking-status matched normosmics. The anosmic group comprised two males (age 42 and 65 years) and two females (age 61 and 67 years). One male (42 years) and one female (61 years) were congenital anosmics, the other male (65 years) and female (67 years) were head-trauma anosmics. None of the anosmics had any cognitive impairment.

The normosmic group also included two males (age 45 and 68 years) and two females (60 and 71 years). Only one female anosmic (67 years) and her normosmic control (71 years) were current smokers (both smoked menthol cigarettes).

Procedure

The procedure was identical to that used in the previous investigations.⁽¹⁶⁻¹⁸⁾ Participants presented the stimulus and blanks (mineral oil) to themselves by placing the pop-up spout inside the designated nostril and squeezing the bottle as they

sniffed. They rapidly learned to squeeze and sniff with a constant strength across trials.

The method employed was a forced-choice, ascending method of limits. Briefly, the subject sought to choose, on each trial, the stronger (forced-choice) of two stimuli: one was a dilution step of the particular chemical tested, the other a blank. Unknown to the participants, testing started with the lowest concentration (dilution step 14), and worked up to the threshold (ascending method of limits). If the choice was right, the same concentration was presented again (also paired with a blank); if it was wrong, the immediately higher concentration (e.g., dilution step 13) was presented. This continued until five correct choices were made in a row, in which case that step was taken as the threshold. The whole procedure was then repeated with the other nostril. After that, testing began with another substance in identical manner. Testing each nostril separately, waiting between 30 and 60 sec between trials, and using the ascending-method approach helped to minimize the effects of the commonly encountered phenomenon of olfactory adaptation.^(4,24-27)

Daily sessions lasted between two and three hours and were repeated until 12 thresholds (6 for each nostril) per subject were obtained for each compound. This amounted to a total of 132 thresholds per subject and 48 thresholds per substance in each group (anosmic or normosmic). The order of presentation of the chemicals differed from subject to subject. The number of times that the right or left nostril was tested first for a certain compound was counterbalanced for each subject.

Data analysis

The mean dilution step summarized each subject's 12 thresholds per substance. These individual mean thresholds were first converted to headspace concentrations (ppm) and then averaged geometrically across subjects in each group (anosmic and normosmic), since such thresholds follow a log normal distribution.^(10,28,29)

RESULTS

Figure 1 depicts the odor and nasal pungency thresholds for the chemicals. Both types of thresholds declined through the homologous series. As expected, the odor thresholds lay well below the pungency thresholds. As seen before, the spread of odor threshold for each substance across subjects (indicated by the standard deviation) was much higher than the spread of pungency thresholds. Pungency thresholds were remarkably uniform across subjects and sessions. A clear difference emerged between the results for this family of compounds and previously studied families: in the alkylbenzenes, failure to precipitate nasal pungency appeared much earlier in the series. Members following propylbenzene failed to evoke pungency reliably in the anosmics.

Individual functions for the anosmic and normosmic groups appear in Figure 2. This figure provides a direct visual impression of the variability of thresholds across subjects in

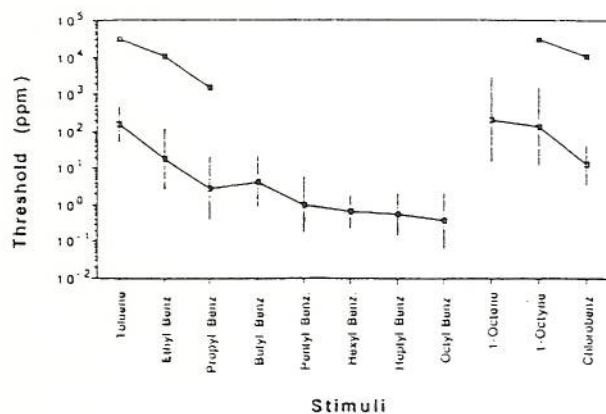


FIGURE 1. Nasal pungency thresholds (■) (\pm standard deviation) and odor thresholds (□) (\pm standard deviation) for the 11 substances tested. Each point in the pungency or odor function represents the average of 48 thresholds measured in four subjects. (The standard deviation on pungency thresholds is small enough to be covered by the symbol.)

the two groups. Figure 2 demonstrates that the profile of thresholds throughout the series is common to all subjects. The results reveal a robust general factor of sensitivity. The subject most sensitive to a particular compound tends to be the most sensitive to all the others (including the nonhomologous stimuli) and the subject least sensitive to a particular compound tends to be the least sensitive to all the others.

Figure 3 illustrates the relationship between the sensory thresholds (pungency and odor) and saturated vapor concentration at room temperature for the alkylbenzenes, chlorobenzene, 1-octene, and 1-octyne, and for the other 31 chemicals studied so far. Pungency thresholds taken as a whole exhibited a linear relationship with saturated vapor ($r = 0.97$), having a slope of 1.02. Pungency for individual homologous series con-

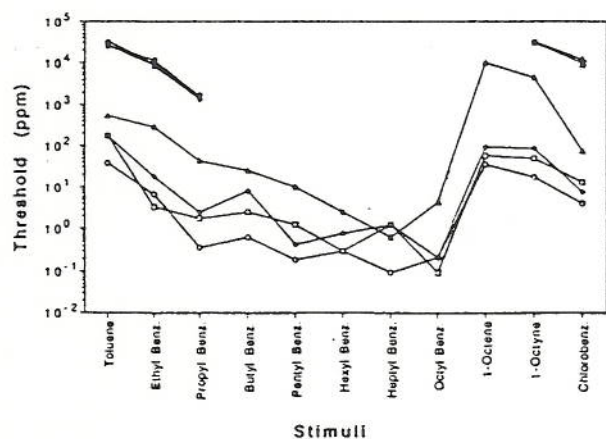


FIGURE 2. Individual nasal detection thresholds for each anosmic (●, ■, ◆, ▲) (indicating pungency thresholds) and normosmic (○, □, ◇, △) (indicating odor thresholds) participant. Each point represents the average of 12 threshold determinations.

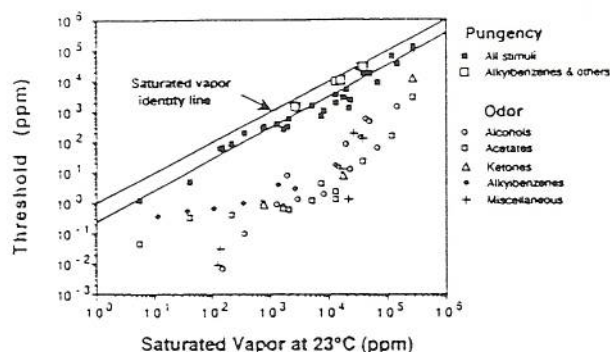


FIGURE 3. Pungency and odor thresholds for the 11 compounds studied here plus alcohols, acetates, ketones, and miscellaneous chemicals studied previously, depicted as a function of their saturated vapor concentration at room temperature. In decreasing order of saturated vapor concentrations the alkylbenzenes include toluene through octyl benzene; the alcohols include methanol, ethanol, 2-propanol, tert-butyl alcohol, 1-propanol, 2-butanol, 1-butanol, 1-pentanol, 4-heptanol, 1-hexanol, 1-heptanol, and 1-octanol; the acetates include methyl, ethyl, propyl, tert-butyl, butyl, sec-butyl, pentyl, hexyl, heptyl, octyl, decyl, and dodecyl acetate; the ketones include acetone, 2-pentanone, 2-heptanone, and 2-nonanone; the miscellaneous substances include 1-octyne, 1-octene, pyridine, chlorobenzene, menthol, and β -phenyl ethyl alcohol. The line representing pungency has a slope of 1.02 and an $r = 0.967$. The saturated vapor identity line is shown for reference.

formed to the general picture (slopes: 0.90 for the alcohols, 1.07 for the acetates, 1.06 for the ketones, 1.17 for the alkylbenzenes, and 0.95 for the miscellaneous substances). As a group the three alkylbenzenes and the two other chemicals used here (1-octyne and chlorobenzene) that were capable of eliciting pungency tended to lay somewhat above the average line (see Figure 3), but a linear relationship ($r = 0.999$) also held for them, and the slope was close to unity (1.15).

DISCUSSION

As seen in Figure 3, the results show that nasal pungency thresholds for all tested substances bear a strong linear relationship with their saturated vapor concentration, regardless of molecular size or chemical functional group. Even the miscellaneous substances employed conformed to that trend. Furthermore, the linear correlation had a slope close to 1.00, suggesting that when a certain uniform percentage of vapor saturation was achieved (approximately 32%) nasal pungency would occur in the anosmics, if it were to be evoked at all. Odor thresholds, on the other hand, generally failed to show the linear relationship, displaying systematic deviations; and, even if the odor threshold of an individual series approximated a linear relationship, its slope departed from unity.

Odor thresholds as a whole depicted more substance-to-substance scatter than pungency thresholds. The odor thresholds for no individual homologous series exhibited as strong a correlation with vapor saturation as did the pungency thresholds for all series and miscellaneous compounds grouped together. For odor the best correlation occurred with the ketones,

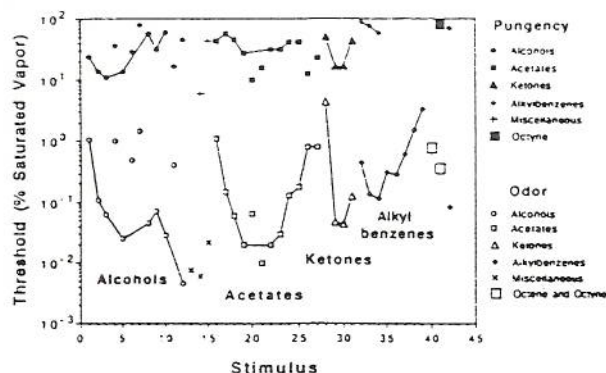


FIGURE 4. Pungency and odor thresholds for chemicals so far studied (cf. references 16-18). Members of homologous series (stimulus) are arranged according to increasing length of the main carbon chain. Alcohols (stimuli 1 to 12) comprise: methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol (sec-butanol), 2-methyl-2-propanol (tert-butanol), 1-pentanol, 1-hexanol, 1-heptanol, 4-heptanol, and 1-octanol; acetates (stimuli 16 to 27): methyl, ethyl, propyl, butyl, sec-butyl, tert-butyl, pentyl, hexyl, heptyl, octyl, decyl, and dodecyl acetates; ketones (stimuli 28 to 31): acetone, 2-pentanone, 2-heptanone, and 2-nonanone; alkylbenzenes (stimuli 32 to 39): toluene, and ethyl, propyl, butyl, pentyl, hexyl, heptyl, and octyl benzenes. For alcohols, only primary and unbranched alcohols are joined by a line; for acetates, only unbranched acetates are joined by a line. Miscellaneous compounds (stimuli 13 to 15) are, in that order, β -phenyl ethyl alcohol, pyridine, menthol. Stimuli 40 to 42 are: 1-octene, 1-octyne, and chlorobenzene.

where $r = 0.95$, and the worst occurred with the acetates, where $r = 0.87$. The slopes of the relationships for odor thresholds commonly departed from unity: the alcohols depicted a slope of 1.62; the acetates, 0.83; the ketones, 1.64; the alkylbenzenes, 0.68; and the miscellaneous chemicals, 1.44.

Figure 4 depicts pungency and odor thresholds expressed as percent of saturated vapor at 23°C for the alkylbenzenes and the other three compounds tested in the present study, as well as for all the other homologous series and compounds previously studied. Straight-chain members of homologous series are connected by lines. A striking difference between pungency and odor is the span of the threshold values across and along the series. Pungency thresholds display considerable constancy, with a range below about one order of magnitude. Odor thresholds show much more variation, about three orders of magnitude. On average (geometric mean), pungency thresholds occurred at 32% of vapor saturation (\pm SD = 16 to 62%), whereas odor thresholds occurred at 0.14% (\pm SD = 0.02 to 0.80%).

Odor thresholds for secondary and tertiary alcohols and acetates fell outside the trend of their straight chain analogues and, to some extent, so did chlorobenzene compared to the alkylbenzenes. Pungency thresholds for the same substances showed a less pronounced departure.

The odor thresholds along each of the four homologous series in Figure 4 commonly showed a U- or V-shaped function. The case of the alcohols merits special comment. For these stimuli, the U-shaped function is disrupted by the low

TABLE I. Values of nasal pungency thresholds from human anosmics (¹⁶⁻¹⁸, this paper) and of RD_{50} s from an animal (mouse) bioassay (see reviews in ^{21,22}) for 24 VOCs

Compound	Pungency (ppm)	RD_{50} (ppm)
Alcohols		
methanol	35,000	41,514
ethanol	9,000	27,314
1-propanol	2,500	12,704
2-propanol	18,135	17,693
1-butanol	1,100	4,784
1-pentanol	1,700	4,039
1-hexanol	400	239
1-heptanol	210	98.4
1-octanol	70	47.2
Acetates		
methyl acetate	112,358	829
ethyl acetate	67,272	614
propyl acetate	17,565	793
butyl acetate	3,648	730
tert-butyl acetate	2,115	15,962
pentyl acetate	1,648	1,531
hexyl acetate	635	740
Ketones		
2-propanone (acetone)	130,671	77,516
2-pentanone	2,966	5,933
2-heptanone	281	893
Alkylbenzenes		
toluene	29,574	5,300
ethyl benzene	10,100	4,060
propyl benzene	1,487	1,530
Miscellaneous		
menthol	60	45
chlorobenzene	10,553	1,054

odor threshold value for 1-heptanol and the even lower threshold for 1-octanol. The outcome stresses the high efficacy of 1-octanol as an odorant and, given its lack of chemical reactivity, suggests that its solubility properties (combined weight of polar and nonpolar zones in the molecules) closely match those of the biophase where odor reception takes place.

In the case of pungency thresholds, the constricted range obscures the presence of analogous U-shaped functions. Pungency thresholds for ketones and alcohols have a tendency to be U-shaped. Thresholds for the acetates display a striking constancy. Not much can be said about the alkylbenzenes in this regard due to their failure, early in the series, to elicit pungency in the anosmic group.

Olfactory and common chemical sensations often constitute early warnings of the presence of potentially dangerous airborne chemicals. These sensory responses have relevance to issues of air quality and health in both industrial and non-industrial environments.

The interest in this study and in previous studies⁽¹⁶⁻¹⁸⁾ has focused on assessing the independent contribution of smell and CCS to the detection of chemically related (homologous) series of substances. Data from a number of such series where physicochemical properties change uniformly and systemati-

cally should hasten the development of quantitative structure-activity relationships (QSARs) for sensory irritation in humans. So far, QSARs for irritation have relied exclusively on animal data.⁽²⁰⁾

The sensory irritation properties of most of the alkylbenzenes studied here also have been investigated with an animal bioassay that employed Swiss-Webster male mice.⁽²⁰⁾ The response parameter in this bioassay is the concentration that depresses the respiratory rate of mice by 50% (RD_{50}). As was the case with pungency thresholds, the RD_{50} in mice also decreased systematically with increasing chain length of the alkylbenzenes, except that the response could still be measured up to hexylbenzene (the highest series member tested). Interestingly, benzene failed to produce any reduction in respiratory rate in mice and, as a matter of fact, increased the rate.

Values of RD_{50} have been obtained not only for alkylbenzenes but also for many other nonreactive and reactive compounds.⁽²¹⁾ So far there are 24 compounds, mostly non-reactive VOCs, for which values of both RD_{50} from mice and nasal pungency thresholds from human anosmics exist^(16-18,21,22) (Table I). Figure 5 shows the relationship between these two sets of values. Considering all 24 substances, the correlation is only modest ($r = 0.63$), but if excluding the three lower acetates (methyl, ethyl, and propyl)—which seem outliers—the correlation reaches an $r = 0.85$. The RD_{50} values for these acetates (as well as for butyl acetate) are quite similar (Table I) and fail to follow the tendency seen in other chemical series where RD_{50} decreases with increasing carbon chain length.

RD_{50} values for ethyl acetate have repeatedly failed to bear the same relationship to TLVs as the bulk of other tested

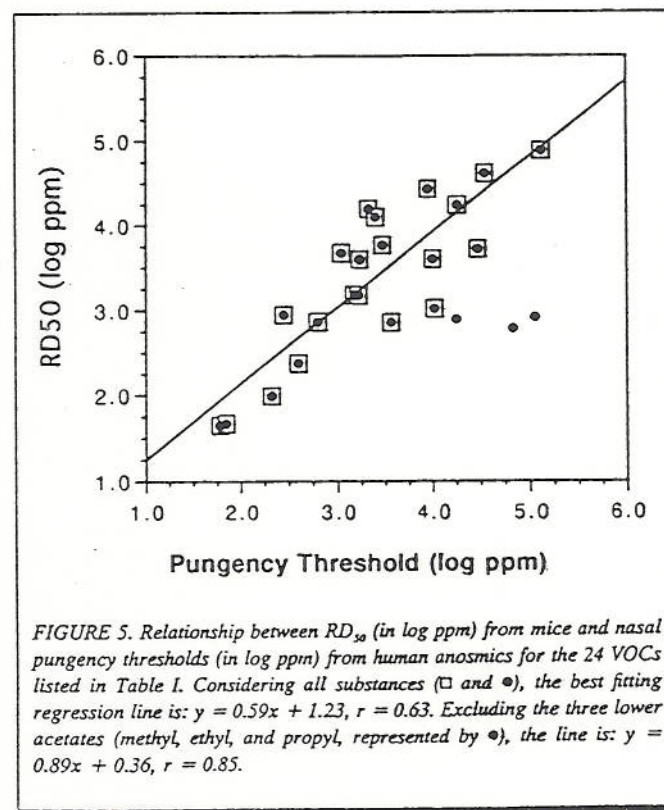


FIGURE 5. Relationship between RD_{50} (in log ppm) from mice and nasal pungency thresholds (in log ppm) from human anosmics for the 24 VOCs listed in Table I. Considering all substances (\square and \bullet), the best fitting regression line is: $y = 0.59x + 1.23$, $r = 0.63$. Excluding the three lower acetates (methyl, ethyl, and propyl, represented by \bullet), the line is: $y = 0.89x + 0.36$, $r = 0.85$.

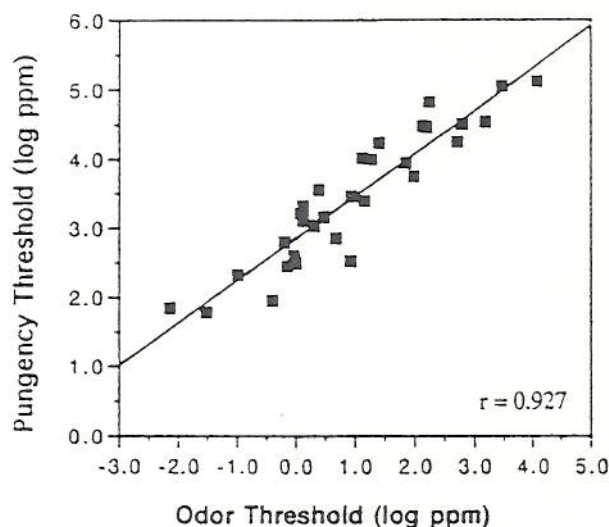


FIGURE 6. Relationship between pungency and odor thresholds in humans for 33 nonreactive VOCs comprising the alkylbenzenes, alcohols, acetates, ketones, and miscellaneous chemicals listed in the legend of Figure 3, except those not detected by anosmics (i.e., with no available pungency threshold). Best fitting regression line: $y = 0.613x + 2.864$, $r = 0.93$. Data from references 16–18.

compounds.^(33,34) Whereas the time-response function for most substances exhibits an initial depression followed by some recovery, the function for ethyl acetate shows progressive depression over time.⁽³⁴⁾ In general the initial depression is used to determine RD_{50} . For ethyl acetate the degree of depression seen at the end of the exposure was presumably used. This uncharacteristic time-course may account for the disparity between the high pungency threshold measured in a one-sniff test and the low RD_{50} . The same phenomenon may account for the similar disparity seen for methyl and propyl acetate. The time-course functions for these two compounds, unfortunately, do not appear in the literature.

The current data is consistent with the notion that the process by which nonreactive airborne molecules trigger nasal pungency rests heavily on a nonspecific physical interaction with the mucosa. A model for both physical and chemical interactions of sensory irritants with a putative receptor protein located in a lipid bilayer has already been described.^(20,32) The model proposes that a receptor protein nevertheless mediates irritation. Whether this receptor exists in the free nerve endings of the trigeminal nerve remains an issue. Presumably it exists in a lipid environment. The issue of whether the chemicals impinge directly on nasal free-nerve endings or exert their action on nonneural cells, which in turn release some endogenous substance (e.g., substance P, calcitonin gene-related peptide^(35–37)) for which highly specific receptors probably exist, is not addressed in the present approach. One systematic finding of the authors' studies is the lower variability of pungency thresholds compared to odor thresholds. This outcome is consistent with the idea that pungency for nonreactive substances is mostly determined by a nonspecific process unlike olfaction, which presumably relies on a variety of more finely

tuned receptors that may differ in their expression due to genetic polymorphism.^(38–40)

Even though both types of sensory thresholds decrease with carbon chain length, they do not decline at the same rate. The rate of decline of odor thresholds for the first three to seven members of the series is faster than that of pungency thresholds. From then on, odor tends to level off while pungency fades away. The observed effect for pungency could result from two opposing phenomena: as carbon chain lengthens, lipid solubility increases, which tends to lower thresholds; but vapor pressure decreases to a point where there are too few molecules in the air to trigger pungency. For odor the chemicals most likely interact with specific receptors for which larger ligands would have higher affinity and, thus, give lower thresholds, as implied in a recent probability model for molecular recognition in biological receptors.⁽⁴¹⁾ As Figure 6 illustrates, there is a strong relationship between odor and pungency thresholds ($r = 0.93$). This relationship shows that the lower the odor threshold for a substance, the wider the gap between its odor and pungency threshold.

If nasal pungency from all these chemicals relies principally on a broadly tuned physicochemical interaction with susceptible mucosal structures, it is likely that the effects of a wide variety of VOCs will exhibit a considerable degree of additivity. This means that the impact of dozens of such substances, each of them well below threshold, could add up to produce noticeable pungency. Previous studies showed that for suprathreshold binary mixtures of the pungent odorants ammonia and formaldehyde in the range where pungency was the salient feature, additivity of the sensory response was complete. In the range where odor was the salient feature, additivity was incomplete.^(6,7)

CCS shows substantial temporal integration or summation, by which pungent sensations grow with time, not only over seconds,^(4,5) but also over 20 or more minutes of constant stimulation.^(42,43) The relationship between time and concentration in the first 4 to 5 sec is close to perfect reciprocity (i.e., if inhalation time is doubled, concentration can be halved and perceived pungency would remain constant). On the other hand, the olfactory sense adapts quickly, and odor sensations soon fade to a low intensity level.^(25,44,45)

Addition of nasal pungency from many chemically different sources and build-up of sensory irritation with time could help to explain the appearance of adverse health effects in some indoor environments.⁽¹⁴⁾ Particularly relevant to both basic (e.g., QSARs) and applied (e.g., sick building syndrome) aspects of sensory irritation at this stage is the study of the level at which normosmics and anosmics respond to mixtures of pungent substances as compared to the individual components, a topic that the authors are beginning to address.

REFERENCES

- Green, B.G., J.R. Mason, and M.R. Kare, eds.: *Chemical Senses. Vol. 2: Irritation*. New York: Marcel Dekker, 1990.
- Chen, Z. and D. Lancet: Membrane proteins unique to vertebrate olfactory cilia: candidates for sensory receptor molecules. *Proc. Natl. Acad. Sci. (USA)* 81:1859–1863 (1984).

3. Rhein, L.D. and R.H. Cagan: Role of cilia in olfactory recognition. In *Biochemistry of Taste and Olfaction*, ed. by R.H. Cagan and M.R. Kare. New York: Academic Press, 1981. pp. 47-68.
4. Cain, W.S.: Olfaction and the common chemical sense: some psychophysical contrasts. *Sens. Processes* 1:57-67 (1976).
5. Cometto-Muñiz, J.E. and W.S. Cain: Temporal integration of pungency. *Chem. Senses* 8:315-327 (1984).
6. Cometto-Muñiz, J.E., M.R. García-Medina, and A.M. Calviño: Perception of pungent odorants alone and in binary mixtures. *Chem. Senses* 14:163-173 (1989).
7. Cometto-Muñiz, J.E. and S.M. Hernández: Odorous and pungent attributes of mixed and unmixed odorants. *Percept. Psychophys.* 47:391-399 (1990).
8. American Conference of Governmental Industrial Hygienists: 1991-1992 Threshold Limit Values for Chemical Substances and Physical Agents, and Biological Exposure Indices. Cincinnati: ACGIH, 1991.
9. Alarie, Y.: Dose-response analysis in animal studies: prediction of human responses. *Environ. Health Perspect.* 42:9-13 (1981).
10. Amoore, J.E.: Effects of chemical exposure on olfaction in humans. In *Toxicology of the Nasal Passages*, ed. by C. S. Barrow. Washington: Hemisphere Publishing Corporation, 1986. pp. 155-190.
11. Cometto-Muñiz, J.E., and W.S. Cain: Influence of airborne contaminants on olfaction and the common chemical sense. In *Smell and Taste in Health and Disease*, ed. by T. V. Getchell, R. L. Doty, L. M. Bartoshuk, and J. B. Snow Jr. New York: Raven Press, 1991. pp. 765-785.
12. Kjærgaard, S.K., L. Molhave, and O.F. Pedersen: Human reactions to a mixture of indoor air volatile organic compounds. *Atmos. Environ.* 25A:1417-1426 (1991).
13. Molhave, L.: Volatile organic compounds, indoor air quality and health. *Indoor Air* 4:357-376 (1991).
14. Tucker, W.G., B.P. Leaderer, L. Molhave, and W.S. Cain, eds.: *Sources of Indoor Air Contaminants. Characterizing Emissions and Health Impacts*. Vol. 641. New York: New York Academy of Sciences, 1992.
15. Cometto-Muñiz, J.E. and W.S. Cain: Sensory irritation. Relation to indoor air pollution. *Ann. N.Y. Acad. Sci.* 641:137-151 (1992).
16. Cometto-Muñiz, J.E. and W.S. Cain: Thresholds for odor and nasal pungency. *Physiol. Behav.* 48:719-725 (1990).
17. Cometto-Muñiz, J.E. and W.S. Cain: Nasal pungency, odor, and eye irritation thresholds for homologous acetates. *Pharmacol. Biochem. Behav.* 39:983-989 (1991).
18. Cometto-Muñiz, J.E. and W.S. Cain: Efficacy of volatile organic compounds in evoking nasal pungency and odor. *Arch. Environ. Health* 48:309-314 (1993).
19. Cain, W.S., J.F. Gent, R.B. Goodspeed, and G. Leonard: Evaluation of olfactory dysfunction in the Connecticut Chemosensory Clinical Research Center. *Laryngoscope* 98:83-88 (1988).
20. Nielsen, G.D. and Y. Alarie: Sensory irritation, pulmonary irritation, and respiratory stimulation by airborne benzene and alkylbenzenes: Prediction of safe industrial exposure levels and correlation with their thermodynamic properties. *Toxicol. Appl. Pharmacol.* 65:459-477 (1982).
21. Alarie, Y.: Irritating properties of airborne materials to the upper respiratory tract. *Arch. Environ. Health* 13:433-449 (1966).
22. Amoore, J.E. and B.G. Ollman: Practical test kits for quantitatively evaluating the sense of smell. *Rhinology* 21:49-54 (1983).
23. Cain, W.S.: Testing olfaction in a clinical setting. *Ear Nose Throat J.* 68:316-328 (1989).
24. Cain, W.S.: Odor intensity after self-adaptation and cross-adaptation. *Percept. Psychophys.* 7:271-275 (1970).
25. Cain, W.S.: Perception of odor intensity and the time course of olfactory adaptation. *ASHRAE Trans.* 80:53-75 (1974).
26. Cometto-Muñiz, J.E. and W.S. Cain: Olfactory adaptation. In *Handbook of Clinical Olfaction and Gustation*, ed. by R.L. Doty. New York: Marcel Dekker, Inc., 1994. [In press]
27. Stevens, J.C., W.S. Cain, F.T. Schiet, and M.W. Oatley: Olfactory adaptation and recovery in old age. *Perception* 18:265-276 (1989).
28. Brown, K.S., C.M. MacLean, and R.R. Robinette: The distribution of the sensitivity to chemical odors in man. *Hum. Biol.* 40:456-472 (1968).
29. Cain, W.S. and J.F. Gent: Olfactory sensitivity: Reliability, generality, and association with aging. *J. Exp. Psychol.: Hum. Percept. & Perform.* 17:382-391 (1991).
30. Abraham, M.H., G.S. Whiting, Y. Alarie, J.J. Morris, P.J. Taylor, R.M. Doherty, R.W. Taft, and G.D. Nielsen: Hydrogen bonding 12. A new QSAR for upper respiratory tract irritation by airborne chemicals in mice. *Quant. Struct.-Act. Relat.* 9: 6-10 (1990).
31. Bos, P.M.J., A. Zwart, P.G.J. Reuzel, and P.C. Bragt: Evaluation of the sensory irritation test for the assessment of occupational health risk. *CRC Crit. Rev. Toxicol.* 21:423-450 (1992).
32. Nielsen, G.D.: Mechanisms of activation of the sensory irritant receptor by airborne chemicals. *CRC Crit. Rev. Toxicol.* 21:183-208 (1991).
33. De Ceaurriz, J.C., J.C. Micillino, P. Bonnet, and J.P. Guenier: Sensory irritation caused by various industrial airborne chemicals. *Toxicol. Letters* 9:137-143 (1981).
34. Kane, L.E., B.S. Dombroske, and Y. Alarie: Evaluation of sensory irritation from some common industrial solvents. *Am. Ind. Hyg. Assoc. J.* 41:451-455 (1980).
35. Stjärne, P., L. Lundblad, J.M. Lundberg, and A. Ånggård: Capsaicin and nicotine-sensitive afferent neurones and nasal secretion in healthy human volunteers and in patients with vasomotor rhinitis. *Br. J. Pharmacol.* 96:693-701 (1989).
36. Geppetti, P., B.M. Fusco, S. Marabini, C.A. Maggi, M. Fanciullacci, and F. Sicuteri: Secretion, pain and sneezing induced by the application of capsaicin to the nasal mucosa in man. *Br. J. Pharmacol.* 93:509-514 (1988).
37. Silver, W.L., J.R. Mason, D.A. Marshall, and J.A. Maruniak: Rat trigeminal, olfactory and taste responses after capsaicin desensitization. *Brain Res.* 333:45-54 (1985).
38. Buck, L. and R. Axel: A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. *Cell* 65:175-187 (1991).
39. Firestein, S.: A noseful of odor receptors. *Trends Neurosci.* 14: 270-272 (1991).
40. Korsching, S.: Sniffing out odorant receptors. *Trends Biochem. Sci.* 16:277-278 (1991).
41. Lancet, D., E. Sadovsky, and E. Seidemann: Probability model for molecular recognition in biological receptor repertoires: Significance to the olfactory system. *Proc. Natl. Acad. Sci. (USA)* 90:3715-3719 (1993).
42. Cain, W.S., L.C. See, and T. Tosun: Irritation and odor from formaldehyde: chamber studies. In *IAQ '86. Managing Indoor Air for Health and Energy Conservation*. Atlanta, GA: American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., 1986. pp. 126-137.
43. Hudnell, H.K., D.A. Otto, D.E. House, and L. Molhave: Exposure of humans to a volatile organic mixture. II. Sensory. *Arch. Environ. Health* 47:31-38 (1992).
44. Berglund, B., U. Berglund, and T. Lindvall: Olfactory self- and cross-adaptation: Effects of time of adaptation on perceived odor intensity. *Sens. Processes* 2:191-197 (1978).
45. Pryor, G.T., G. Steinmetz, and H. Stone: Changes in absolute detection threshold and in subjective intensity of suprathreshold stimuli during olfactory adaptation and recovery. *Percept. Psychophys.* 8:331-335 (1970).