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Comparison of Two Stimulus-delivery Systems for Measurement of Nasal Pungency Thresholds

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Abstract

Using representative members of each of three homologous series of chemicals—ketones, acetates and alcohols—we measured nasal pungency thresholds in anosmics via two stimulus-delivery systems. The first system consists of the fairly commonly used 270 ml, plastic 'squeeze bottles'. The second system consists of 1900 ml, glass vessels with Teflon tubing and nose-pieces. Although bulkier and more susceptible to mechanical breakage, the glass vessels possess advantages that can allow them to provide 'environmentally realistic' chemosensory thresholds, i.e. thresholds closer in absolute values to those that might be obtained under whole-body exposures. Such advantages include a larger volume of the vapor-source to accommodate whole sniffs, and a tight nose–nose-piece connection to avoid stimulus dilution. The outcome revealed that, for every chemical, the glass vessels provided nasal pungency thresholds significantly lower than those provided by the squeeze bottles. The difference amounted, on average, to a factor of 4.6, though the relative potency of the compounds remained the same under both systems. Additionally, when tested with the highest homologues used here, namely, octyl acetate and 1-octanol, anosmics using the glass vessels had little or no difficulty achieving the criterion for threshold whereas they did have difficulty when using the squeeze bottles.

Introduction

Thresholds constitute important endpoints in the characterization of a sensory modality. They define the limits of sensitivity of the modality towards a particular stimulus. In the case of the chemical senses, thresholds show a picture of wide variability for any particular substance (Cain and Gent, 1991). Among the reasons for such variability, aside from any true individual differences, can be included the psychophysical procedure, the stimulus-delivery system and the number of repetitive measurements.

Over the past few years we have measured thresholds for odor, nasal pungency, eye irritation and nasal localization (i.e. right/left nostril), using homologous series of chemicals, where physicochemical properties change in orderly progression, with the goal of relating those properties to sensory potency (Cometto-Muñiz and Cain, 1994, 1996, 1998). Chemesthetic thresholds, mediated by the trigeminal nerve, can be satisfactorily modeled by a quantitative structure–activity relationship (QSAR) based on a solvation approach (Abraham *et al.*, 1996, 1998a,b). Olfactory thresholds, in contrast, proved more difficult to model by such a QSAR (Abraham, 1996).

Our reported nasal pungency thresholds were measured in subjects lacking olfaction, i.e. anosmics, for whom a background of odor would not interfere. Such a background of odor is invariably present, even at lower concentrations than those evoking barely perceptible pungency. The use of anosmics raises the question of whether their nasal trigeminal sensitivity is comparable to that of normosmics, i.e. subjects with normal olfaction. Some observations have indicated that normosmics can report considerable nasal pungency at, or even below, anosmics' thresholds (Cometto-Muñiz and Cain, 1990; Kendal-Reed *et al.*, 1998). A study of chemosensory event-related potentials evoked by carbon dioxide also suggested decreased nasal trigeminal sensitivity in subjects with impaired or absent olfaction (Hummel *et al.*, 1996). Nevertheless, alternative measures of trigeminal sensitivity such as eye irritation and nasal localization, measured in both anosmics and normosmics, and for compounds of varied chemical structure, failed to show differences of statistical significance between the two groups (Cain and Cometto-Muñiz, 1996; Cometto-Muñiz and Cain, 1998; Cometto-Muñiz *et al.*, 1998b). Additionally, eye

irritation thresholds in normosmics fell into register with nasal pungency thresholds in anosmics, a result obtained across a number of homologous series (Cometto-Muñiz and Cain, 1995). Also, an investigation of irritation-induced reflex changes in respiration in mice showed no effect of anosmia on nasal irritation sensitivity (Hansen *et al.*, 1994). The issue has not yet found closure but any difference in trigeminal sensitivity between anosmics and normosmics seems relatively small.

In the present study we have measured nasal pungency thresholds in anosmics via two stimulus-delivery systems: 'plastic squeeze bottles' and 'glass vessels'. Under Materials and methods we give a detailed description of each system. Thresholds for odor and nasal pungency measured with squeeze bottles correlate well with those cited in the literature but tend to lie on the high end of the range of values computed across studies of the same chemical (Cometto-Muñiz and Cain, 1993, 1998; Cometto-Muñiz *et al.*, 1998a). The design of the glass vessels aimed at producing environmentally realistic thresholds through the following improvements over the squeeze bottles: (i) avoidance of dilution of the stimulus by providing a tight nose-piece–nostril connection (whether testing just one or both nostrils); (ii) increase in the volume of stimulus vapor available to accommodate a human sniff completely (Laing, 1982); and (iii) elimination of any low-odor background inherent in the plastic squeeze bottles.

Human thresholds for nasal pungency, eye irritation and odor evoked by volatile organic compounds (VOCs) relate readily to issues of indoor air quality, one of various fields of practical application. It is widely acknowledged that among the likely causes for building-related complaints, the effect of VOCs deserves particular attention (Rothweiler and Schlatter, 1993; Hodgson *et al.*, 1994; Kostiainen, 1995). Among the symptoms evoked, sensory responses, especially irritation, not only figure prominently but also lend themselves to psychophysical measurement in humans (Cometto-Muñiz and Cain, 1992; Hudnell *et al.*, 1992; Kjærgaard *et al.*, 1992; Mølhave, 1991; Mølhave *et al.*, 1991).

Materials and methods

Stimuli

Selected members of three homologous chemical series (ketones, acetates and alcohols) were chosen. The ketones comprised 2-pentanone (99+%), 2-heptanone (98%) and 2-nonanone (99+%). The acetates comprised butyl acetate (99+%), hexyl acetate (98+%) and octyl acetate (99+%). The alcohols comprised 1-butanol (99.8%), 1-hexanol (98%) and 1-octanol (98%). Mineral oil (light, Food Chemical Codex quality) served as the solvent for all stimuli. Starting from each undiluted chemical (100% v/v, called dilution step 0), threefold dilution steps were prepared in duplicate, generating the following series (in % v/v): 33, 11, 3.7, 1.1, etc., labeled dilution step 1, 2, 3, 4, etc., respectively.

Stimuli were stored and presented from two types of vapor-delivery system. Both types classify as 'static' olfactometry (see Cain *et al.*, 1992). The first type consisted of 270 ml, high-density polyethylene (HDPE), squeezable bottles containing 30 ml of solution (Cain, 1989). Each bottle had a cap with a pop-up spout that could fit into the nostril and thereby allowed testing of each nostril separately. It has been widely used both in clinical (Cain, 1989) and basic research (Cometto-Muñiz and Cain, 1998) studies. The second type consisted of 1900 ml, glass vessels containing 200 ml of solution and equipped with two Teflon nose-pieces that fitted snugly into the subject's nostrils (see Figure 1). In addition, a third Teflon tube (plugged at the top) also penetrated the Teflon-lined cap of the vessel and ended at the level of the 200 ml liquid stimulus. After the subject connects to the nose-pieces, this third tube is unplugged, allowing the participant to sniff and draw air through it. The air causes slight turbulence at the surface of the liquid, which guarantees that an airtight connection is made between the nose and the vessel, and an aliquot of headspace is driven into the nose. This system allowed testing of each nostril separately and of both nostrils simultaneously. In the case of monorhinal testing, the subject plugged the nostril not being tested.

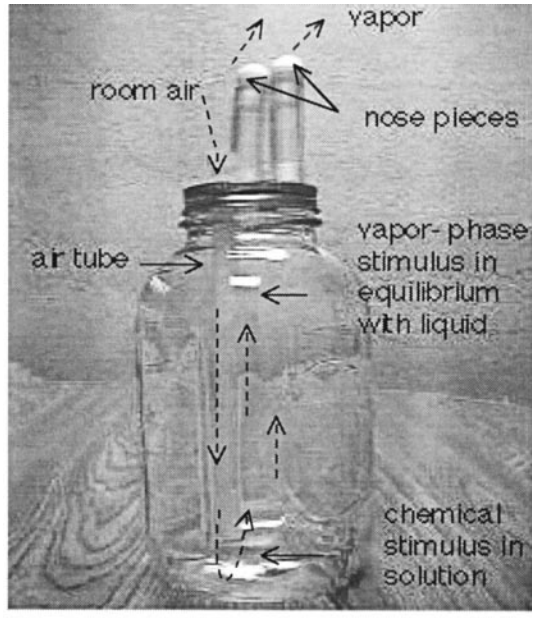
Quantification of all vapor stimuli, whether in squeeze bottles or in glass vessels, was achieved through gas chromatography (FID detector) by direct sampling via a gas sampling valve (1 ml sampling loop) or a gas-tight syringe. We used a 5890 Hewlett-Packard Gas Chromatograph equipped with a DB-1, 30 m × 0.53 mm i.d., 5.0 μm film thickness column purchased from J&W Scientific, Folsom, CA. Concentration measurements were made off-line right after preparation of the stimuli, concomitantly with testing and after all subjects had been tested, to confirm stability. Overall, the coefficient of variation for FID readings averaged 11 ± 9% (SD). All readings were referred to those of the undiluted chemical, assumed to represent saturated vapor at room temperature (23°C).

Subjects

Twelve anosmics (seven men and five women) participated. They included five congenital, two head-trauma, two nasal-disease and three idiopathic anosmics, ranging in age from 22 to 74 years old, with an average (± SD) of 47 ± 15. Eleven subjects were nonsmokers and one was a previous smoker. A standardized clinical olfactory test (Cain, 1989) confirmed anosmia in each participant.

Procedure

A two-alternative forced-choice procedure with presentation of increasing concentrations served to measure all nasal pungency thresholds. The method requires participants to use the assigned nostril to select, on each trial, the stronger of two stimuli: one is always a blank (solvent) and the other a dilution step of the tested substance, starting with a



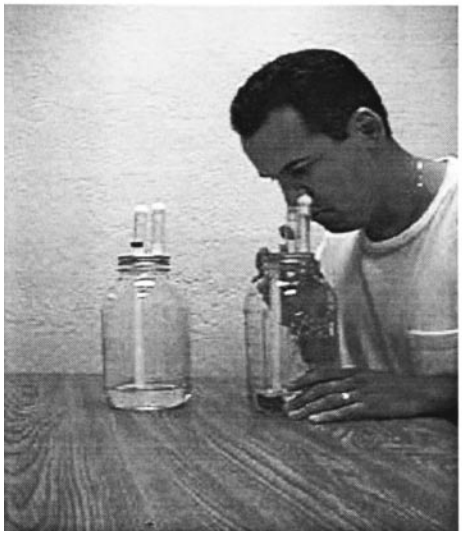


Figure 1 **(Upper)** Photo of a glass vessel. **(Lower)** Photo of subject being tested birhinally via the glass vessels.

concentration clearly below threshold. Selection of the blank triggers, on the following trial, presentation of the next step, i.e. a higher concentration, paired with a blank. Selection of the stimulus triggers, on the following trial, presentation of the same dilution step from a duplicate set, also paired with a blank. The procedure continues until the subject selects the substance over the blank five times in a row. The dilution step where this first occurs is taken as the threshold. This procedure is used to test each nostril separately and, for glass vessels, also both nostrils simultaneously.

In a typical session each subject provided, for a given chemical, all five thresholds: two using squeeze bottles—i.e. right and left nostril—and three using the glass vessels—i.e. right, left and both nostrils simultaneously. Overall, each participant completed between one and three such sessions per chemical. Due to limitations of time, not all anosmics were tested on all chemicals, but whenever they were tested on a particular compound they completed all thresholds for that compound within the same session. The order of testing for nostril(s) and delivery system (within a session) and for chemicals (across sessions) varied irregularly across subjects.

Data analysis

The geometric mean summarized measurements within and across individuals since chemosensory thresholds tend to show a log-normal distribution (Brown *et al.*, 1968; Amoore, 1986; Cain and Gent, 1991). No significant differences in thresholds were found between right and left nostril with either delivery system. Thus, measurements from right and left nostrils were averaged. In the case of glass vessels, no significant differences were found between birhinal and monorhinal thresholds, so the three thresholds (i.e. right, left and both nostrils) were averaged. Analysis of variance (ANOVA) with reported *P*-values corrected when necessary (Huynh–Feldt correction) were performed on thresholds expressed as log parts per million by volume (log ppm). The software used was SuperANOVA v.1.11 by Abacus Concepts, Inc. (Berkeley, CA).

Results

Figure 2(upper) shows the nasal pungency thresholds obtained for the ketones using the two systems. A two-way analysis of variance (ANOVA) using the factors carbon chain length (2-pentanone, 2-heptanone, 2-nonanone) and delivery system (squeeze bottles, glass vessels) confirmed that the glass vessels produced significantly lower pungency thresholds than the squeeze bottles [$F(1,7) = 111.65$, $P = 0.0001$] and that the thresholds declined significantly with increasing carbon chain length [$F(2,14) = 34.25$, $P = 0.0001$]. The interaction chain length × delivery system was not significant. Averaging across the three ketones, thresholds obtained via glass vessels were ~5.5 times lower than those obtained via squeeze bottles.

Figure 2(middle) presents the results for the acetates. When tested with octyl acetate via the squeeze bottles, the anosmics as a group failed to reach the criterion for threshold (even at vapor saturation) in 50% of instances (no value plotted in the figure; see Table 1). In contrast, when tested with octyl acetate via glass vessels they only failed in 7% of instances (Table 1). A two-way ANOVA using the factors carbon chain length (butyl acetate, hexyl acetate) and delivery system (squeeze bottles, glass vessels) con-

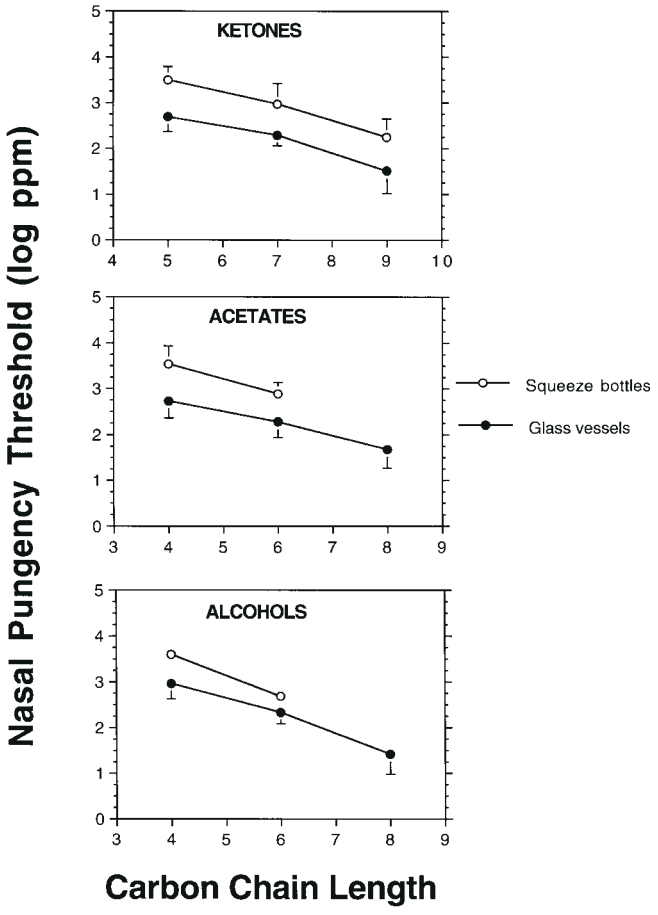


Figure 2 **(Upper)** Nasal pungency thresholds (log ppm) for homologous ketones measured via squeeze bottles (empty circles) and via glass vessels (filled circles) as a function of carbon chain length. **(Middle)** Idem for homologous acetates. No value is plotted for octyl acetate presented via squeeze bottles since, in this case, the anosmics as a group failed to reach the criterion for threshold in 50% of instances. **(Lower)** Idem for homologous alcohols. No value is plotted for 1-octanol presented via squeeze bottles since, in this case, the anosmics as a group failed to reach the criterion for threshold in 37% of instances. In all three graphs, bars, sometimes hidden by the symbol, indicate standard deviations.

firmed that the glass vessels produced significantly lower pungency thresholds than the squeeze bottles [$F(1,9) = 58.23$, $P = 0.0001$] and that the thresholds declined significantly with increasing carbon chain length [$F(1,9) = 56.20$, $P = 0.0001$]. The interaction chain length × delivery system was also significant [$F(1,9) = 6.38$, $P < 0.05$]. Averaging across butyl and hexyl acetate, thresholds obtained via glass vessels were about five times lower than those obtained via squeeze bottles.

Figure 2(lower) shows the outcome for the alcohols. When tested with 1-octanol via the squeeze bottles, the anosmics as a group failed to reach the criterion for threshold (even at vapor saturation) in 37% of instances (no value plotted in the figure; see Table 1). In contrast, when tested with 1-octanol via glass vessels all anosmics reached criterion at some concentration on every repetition. A two-way ANOVA using the factors carbon chain length (butanol, hexanol) and delivery system (squeeze bottles, glass vessels) confirmed that the glass vessels produced significantly lower pungency thresholds than the squeeze bottles [$F(1,8) = 41.32$, $P < 0.0005$] and that the thresholds declined significantly with increasing carbon chain length [$F(1,8) = 164.82$, $P = 0.0001$]. The interaction chain length × delivery system was also significant [$F(1,8) = 9.99$, $P < 0.05$]. Averaging across butanol and hexanol, thresholds obtained via glass vessels were ~3 times lower than those obtained via squeeze bottles.

The overall results clearly indicate that nasal pungency thresholds are significantly lower when obtained via the glass vessels, and that the trend of declining thresholds within each chemical series holds irrespective of the delivery system used. Two advantages of the glass vessels over the squeeze bottles include a tight nose-piece–nostril connection and a larger headspace volume. The first feature minimizes dilution of the stimulus upon sniffing by surrounding room air and the second provides enough stimulus for even large sniffs.

To probe into the role of the sniff-volume factor we measured the volume inhaled by anosmics from glass vessels, upon birhinal testing, when sampling: (i) the blank (i.e. mineral oil); (ii) a butanol stimulus slightly above the pungency threshold as measured via glass vessels (1200 ppm); and (iii) a butanol stimulus clearly below the pungency threshold as measured via glass vessels (416 ppm). Each of 11 anosmics provided, in a single session, five measurements of inhalation volume from each of the two butanol stimuli and 10 from the blank. Measurements where taken with a calibrated respirometer within a forced-choice, two-alternative experimental protocol analogous to that described above. Since each of the two butanol stimuli was always paired with a blank, the number of readings from the blank doubled those from each stimulus.

A one-way ANOVA performed on the inhalation volumes sampled from stimuli and blank showed overall significant differences [$F(2,20) = 5.45$, $P < 0.05$] (see Figure 3). Specific contrast comparisons revealed that: (i) the volumes sniffed from the blank and from the below-threshold stimulus were not significantly different. (ii) The volume sniffed from the slightly-above-threshold stimulus was significantly lower ($F = 10.53$, $P < 0.005$) than that sniffed from the below-threshold stimulus. (iii) The volume sniffed from the slightly-above-threshold stimulus was significantly lower ($F = 4.61$, $P < 0.05$) than that sniffed from the blank.

Discussion

For the three homologous series tested, nasal pungency thresholds measured via the glass vessels were significantly lower than those measured via the squeeze bottles while showing the same trend within members of each series. Furthermore, the glass vessels produced a threshold in all

Table 1 Summary, for each chemical and delivery system, of average nasal pungency threshold (NPT) in log ppm (mean ± SD), number of measurements taken, number of anosmics tested and percentage of measurements where criterion for threshold was not achieved

Stimulus	Average NPT, log ppm (mean ± SD)		Number of measurements		Number of anosmics tested		Percentage of measurements where NPT was not achieved	
	Squeeze bottle	Glass vessel	Squeeze bottle	Glass vessel	Squeeze bottle	Glass vessel	Squeeze bottle	Glass vessel
2-Pentanone	3.50 ± 0.29	2.69 ± 0.32	62	93	8	8	0	0
2-Heptanone	2.97 ± 0.45	2.29 ± 0.23	62	93	8	8	0	0
2-Nonanone	2.25 ± 0.40	1.51 ± 0.50	62	93	8	8	5	0
Butyl acetate	3.54 ± 0.40	2.74 ± 0.37	50	57	10	10	0	0
Hexyl acetate	2.89 ± 0.25	2.28 ± 0.34	52	63	10	10	2	0
Octyl acetate	1.91 ± 0.48	1.68 ± 0.41	50	57	10	10	50	7
1-Butanol	3.60 ± 0.07	2.96 ± 0.33	48	72	9	9	0	0
1-Hexanol	2.68 ± 0.07	2.32 ± 0.25	56	88	10	10	0	0
1-Octanol	1.69 ± 0.19	1.42 ± 0.44	48	71	10	10	37	0

(or almost all) repetitions, even for those chemicals (e.g. 1-octanol and octyl acetate) for which the squeeze bottles had often failed to do so both here and in past studies (Cometto-Muñiz and Cain, 1990, 1991).

Figure 4 illustrates that pungency thresholds reported here and measured via squeeze bottles agree well with those reported for the same chemicals in the past using an identical system. In turn, Figure 5 shows that pungency thresholds reported here and measured via glass vessels fall systematically below those reported earlier using squeeze bottles by an average factor of ~4 across the three homologous series.

We conclude that use of the newly developed glass vessels produces, in absolute values, significantly lower nasal pungency thresholds than those produced by squeeze bottles. Nevertheless, both systems seem to provide a similar picture of relative nasal pungency thresholds within members of homologous series (and, perhaps, even across series), confirming previous observations of a gradual decrease in thresholds with increasing carbon chain length (Cometto-Muñiz and Cain, 1996). Preliminary experiments in our laboratory with whole-body exposures in environmental chambers indicate that the glass vessels provide a closer estimate to thresholds obtained in such a realistic setting.

The enhanced performance of the glass vessels, as hypothesized in the introduction, rests on a combination of factors that includes a tighter seal between nostril and nose-piece (minimizing stimulus dilution) and a larger volume of the vapor-source from which the subjects sniff (accommodating larger stimulus inhalations). The results gathered using butanol showed that, on average, anosmics inhaled ~1200 ml from the glass vessels, on each trial, from stimuli that are below their (pungency) detection level (i.e. either blanks or below-threshold concentrations). In comparison, anosmics inhaled close to 1000 ml—an amount slightly but significantly smaller than the previous one—when sampling from stimuli above their detection level. These preliminary data indicate that anosmics draw from the glass vessels at least 1000 ml of stimulus when tested with a concentration near their detection threshold for nasal pungency. The maximum volume of vapor-phase stimulus that the squeeze bottles can deliver is only 240 ml:

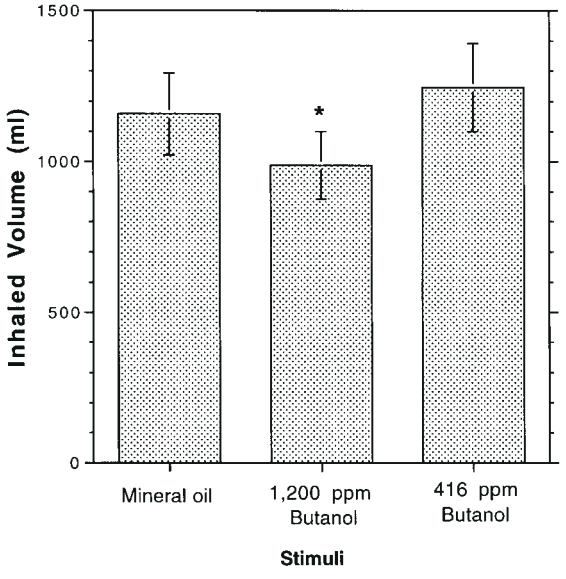


Figure 3 Average volume inhaled birhinally from glass vessels by 11 anosmics when sampling from a blank (i.e. mineral oil), from a slightly-above-threshold butanol concentration (1200 ppm), and from a clearly-below-threshold butanol concentration (416 ppm). Stimulus sampling was performed in the context of a two-alternative, forced-choice procedure where one of the alternatives was always the blank. Bars indicate standard errors. *Significantly different from the blank ($P < 0.05$) and from the below-threshold butanol concentration ($P < 0.005$).

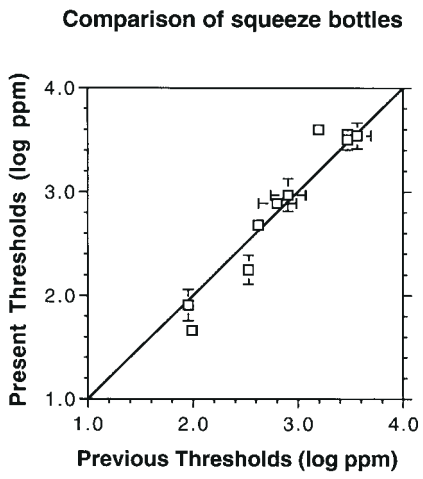


Figure 4 How nasal pungency thresholds for a common set of chemicals from the present study and from previous studies (Cometto- Muñiz and Cain, 1990, 1991), all measured via the squeeze bottles, fall into register. (We include tentative values for 1-octanol and octyl acetate, squares at the extreme lower left, for which anosmics tested via squeeze bottles often fail to reach threshold criterion; see text and Table 1.) The continuous line represents the line of identity. Bars, sometimes hidden by the symbol, indicate standard errors.

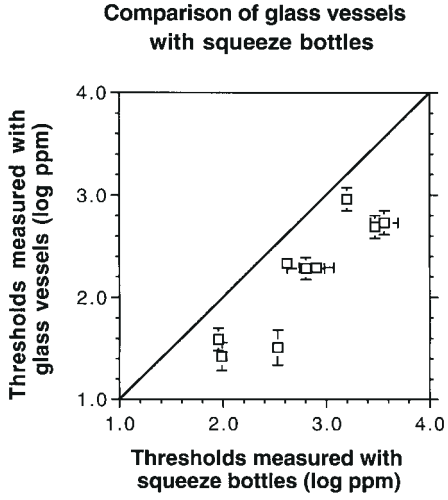


Figure 5 How nasal pungency thresholds for a common set of chemicals from the present study, measured using the glass vessels, fall systematically below those from previous studies (Cometto-Muñiz and Cain, 1990, 1991) measured using squeeze bottles. (We include tentative values for 1-octanol and octyl acetate, squares at the extreme lower left, for which anosmics tested via squeeze bottles often fail to reach threshold criterion; see text and Table 1.) The continuous line represents the line of identity. Bars, sometimes hidden by the symbol, indicate standard errors.

270 ml (total volume) minus 30 ml (liquid volume). This could account for the difference factor of ~4 (on average) observed between nasal pungency thresholds measured with the two systems. Nevertheless, anosmics do not squeeze the bottles so tight as to expel the headspace of the bottle completely. An estimate of 100–150 ml expelled would be more realistic. In summary, at this stage, we can say that the volume of the available vapor stimulus from the squeeze bottles does not reach the average demand of the anosmics and, thus, higher concentrations need to be delivered from these bottles to reach the pungency threshold.

In future studies, we plan to extend the comparison between the two stimulus-delivery systems to the olfactory modality, again using at least three different homologous series. We expect odor thresholds obtained via glass vessels to be significantly lower than those obtained via squeeze bottles. The difference might be even larger than that seen for nasal pungency thresholds given that, in the case of odor, a third factor added to the two mentioned above could enhance further the performance of the glass vessels: the absence of the plastic odor background inherent in the squeeze bottles.

Our ultimate goal is to measure thresholds for nasal pungency, eye irritation and odor for a wide variety of VOCs, from various homologous series, that have direct relevance to environmentally realistic situations such as indoor air exposures. For this purpose we have just finished building environmental chambers in our laboratory. Results from whole-body exposures represent a gold standard for many practical applications. Even so, work with the chambers cannot proceed at the speed, convenience and versatility of traditional olfactometry, represented here by the glass vessels. Still, the high number of VOCs present indoors makes it unpractical to test most of them for chemosensory potency, even via the fast and versatile glass vessels. It is here where studies of QSARs (Abraham *et al.*, 1996, 1998a,b) play a crucial role. They constitute an efficient and powerful tool capable of generalizing results obtained with a few carefully selected substances. The present finding of a general difference between pungency thresholds obtained via squeeze bottles and those obtained via glass vessels does not interfere with the previously derived QSAR, except to alter the constant.

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