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**Efficacy of volatile organic compounds in evoking nasal pungency and odor**

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Running Head: Nasal irritation and odor thresholds

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### Abstract

Sensory irritation (pungency) figures prominently among the symptoms associated with polluted indoor environments. In order to separate the pungent from the olfactory response, we measured nasal pungency thresholds in subjects lacking olfaction (anosmics) and odor thresholds in normal controls (normosmics) for a homologous series of ketones, and selected secondary and tertiary alcohols and acetates. As seen before for homologous alcohols and acetates, both types of nasal thresholds decreased with increasing carbon chain length. Pungency thresholds decreased exponentially with chain length. For all nonreactive chemicals studied so far, threshold nasal pungency is achieved at a fairly constant percentage of vapor saturation, irrespective of molecular size or chemical functional group. Such a relationship does not hold for odor thresholds. The outcome for pungency implies an important role for a physical, rather than chemical, interaction with the nasal mucosa.

Key words: Olfaction – Common Chemical Sense – Odor – Smell – Nasal pungency – Sensory irritation – Odor pollution – Anosmia – Nasal sensory thresholds

### Introduction

Symptoms of nose, eye, and throat irritation emerge persistently in studies of indoor air pollution <sup>1</sup>. Most likely they originate from the summated effect of low concentrations of volatile organic compounds (VOCs) <sup>2, 3</sup>, many of them not normally considered irritants. The ability of humans to detect and react to airborne substances rests heavily upon two sensory channels: the sense of smell, mediated by the olfactory nerve (cranial nerve I), and the common chemical sense (CCS), mediated principally by the trigeminal, glossopharyngeal and vagus nerves (cranial nerves V, IX, and X, respectively), <sup>4, 5, 6</sup>. Stimulation by chemicals of the olfactory sense gives rise to odorous sensations; stimulation of the CCS gives rise to a number of sensations best described generically as pungent, but including the sensations of burning, piquancy, freshness, stinging, irritation, prickliness, and tingling, to name a few.

Olfaction and the CCS will often both respond to the same substances. At a low concentration a VOC may have just odor, but at some higher concentration it will commonly have both an odor and a pungent quality. Olfaction and the CCS function by different rules <sup>7, 8, 9</sup>, however, and have different vulnerabilities to chemical exposures, for example, tobacco smoke and smoking <sup>10, 11</sup>. It is important to try to isolate these sensory systems functionally, particularly if one wants to concentrate selectively on pungent sensations (i.e., CCS responses) which are, in many cases, more relevant than olfactory sensations to issues of air quality.

Our interest in studying the CCS devoid of the influence of olfaction led us to measure nasal pungency thresholds in anosmics, i.e., persons lacking olfaction (see <sup>12</sup>,

<sup>13</sup>), and to compare those thresholds with odor thresholds from normosmics, i.e., persons with normal olfaction. In the first of these studies we employed the homologous series of n-aliphatic alcohols (from methanol to 1-octanol),  $\beta$ -phenyl ethyl alcohol, pyridine, and menthol <sup>14</sup>. Anosmics could detect, via pungency, all but  $\beta$ -phenyl ethyl alcohol reliably. Normosmics could detect all of the stimuli and always at a concentration below that detectable by anosmics. For the aliphatic series, both the odor and pungency thresholds declined with carbon chain length in similar, but not identical, manners. The odor threshold declined more rapidly than the pungency threshold so that the gap between them grew larger with increasing carbon chain length. We pointed out that thresholds for narcosis <sup>15</sup>, <sup>16</sup> and various toxic phenomena <sup>17</sup>, <sup>18</sup> also decline throughout homologous series in a manner very similar to the pungency threshold. For the alcohols, therefore, analogously to narcosis and toxicity, pungency seemed to rely strictly on a nonspecific physical interaction with its relevant biophase.

In a second study, we employed a homologous series of acetates, from methyl to dodecyl acetate, and measured eye irritation thresholds as well as odor and nasal pungency thresholds <sup>19</sup>. All anosmics could detect the series up to heptyl acetate. Congenital anosmics, but not head trauma anosmics, detected octyl acetate, and only one congenital anosmic could detect decyl and dodecyl acetate. Again odor and nasal pungency thresholds decreased systematically with carbon chain length. Eye irritation thresholds came close to nasal pungency thresholds. The outcome again implied that nasal pungency rests upon a nonspecific physical interaction with susceptible mucosal structures.

Our experimental approach complements studies that probe the molecular mechanisms underlying olfaction (see <sup>20</sup>) and pungency (see <sup>21</sup>). It provides human data to establish quantitative structure–activity relationships (QSARs) of upper respiratory tract

irritation, based so far only on animal data (see <sup>22</sup>). QSARs for humans could in principle serve to establish threshold limit values (TLVs) for industrial exposure to airborne chemicals. QSARs and QSPRs (quantitative structure–property relationships) for odor also require coherent threshold data such as ours <sup>23, 24</sup>.

In the present investigation we extend our study of nasal pungency and olfactory thresholds to a series of homologous ketones, and to selected secondary and tertiary alcohols and acetates. If the rule that pungency from unreactive materials depends upon a nonspecific physical interaction between stimuli and membrane holds for this series also, then we should expect the kind of threshold decline with chain-length that we have seen previously. If the rule holds also for branched alcohols and acetates, then we would expect higher thresholds for them than for their unbranched analogues.

## Materials and Methods

### Stimuli

The odorants employed comprised analytical-grade 2-propanone (or acetone), 2-pentanone, 2-heptanone, 2-nonanone, 2-propanol (or isopropyl alcohol), 1-butanol, 2-butanol (or sec-butyl alcohol), 2-methyl-2-propanol (or tert-butyl alcohol), 4-heptanol, sec-butyl acetate, and tert-butyl acetate. Deionized water served as solvent for 2-propanone and 2-propanol and mineral oil for the rest.

The strongest stimulus for each substance comprised undiluted chemical (100 % v/v), labeled dilution step 0. Successive threefold dilutions comprised dilution steps 1 through 14.

Stimuli were presented in 250 ml capacity, squeezable, polypropylene bottles, each containing 30 ml of solution. The bottle closure had a pop-up spout that allowed testing of each nostril separately<sup>25, 26, 27</sup>.

Concentration of the vapor in the headspace of each bottle was measured by a Hewlett-Packard 5890A Gas Chromatograph (photoionization detector) equipped with a gas sampling valve. For every substance, repeated chromatographic readings were taken from each bottle in the series, including the bottle containing saturated vapor at room temperature (23°C). The concentration of saturated vapor for each compound is known from handbooks or databases on physical properties. Knowledge of the concentration of saturated vapor and its associated chromatographic reading allowed conversion of the readings for the other bottles into concentration units, and a calibration curve was derived.

### Subjects

Eight subjects participated. After the nature of the procedure had been fully explained to them, they provided written informed consent for participation in the experiment in forms approved by the Yale Human Investigation Committee. Half of the subjects were anosmics (former patients from either the Connecticut Chemosensory Clinical Research Center, University of Connecticut, or Yale-New Haven Hospital) and the other half were age, gender, and smoking-status-matched normosmics. The anosmic group comprised three males (ages: 41, 50, and 65 years) and a female (60 years). The 41-year-old male and the female were congenital anosmics, the other two were head-trauma anosmics. The normosmic group also comprised three males (ages: 41, 49, and 68 years) and a female (60 years). All eight subjects were current non-smokers. Two male anosmics and their normosmic counterparts were former smokers.

## Procedure

Participants presented stimulus to themselves by placing the pop-up spout inside the designated nostril and squeezing the bottle as they sniffed. Using this procedure, subjects sought on each trial to choose the bottle that gave the stronger sensation: One was a blank (solvent) and the other a dilution step of the test stimulus. Participants rapidly learned to squeeze and sniff with equal vigor on every trial. All participants were given the same instructions. Anosmics typically commented that they could not smell. They were then told to use the any nasal impression — including tingling, prickliness, stinging, piquancy, burning, irritation and alike — to reach a decision.

In a typical session, a subject would start by comparing the intensity of the lowest concentration of a stimulus to a blank and deciding which was stronger. An incorrect choice triggered presentation of the next step (a concentration three times higher) also paired with a blank. A correct choice entailed the presentation of the same concentration (from a duplicate set) paired with a blank, until either an error was made or five correct choices in a row were made, in which case that concentration was taken as the threshold. Hence, errors triggered increments in concentration, whereas correct choices led to another presentation of the same concentration. Once the threshold was measured for that nostril, the same procedure was followed, using the same substance, with the other nostril. After that, testing began with another substance in the same manner. Both the ascending concentration approach to the threshold and the alternation of nostrils, helped to minimize the effects of adaptation, a common phenomenon in olfactory investigations (e.g., 7, 28, 29, 30).



Sessions lasted between two and three hours and were repeated until 12 thresholds (6 for each nostril) per subject were obtained for each stimulus. 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 compounds differed from subject to subject. The number of times that the right or left nostril was tested first for a certain odorant was counterbalanced for each subject.

### Data Analysis

Mean dilution step summarized each subject's 12 thresholds per substance. Since thresholds show a log normal distribution<sup>31, 32, 33</sup>, the geometric mean served to average the individual thresholds, previously converted to headspace concentration (ppm).

### Results

Figure 1 shows the thresholds for nasal pungency – obtained from the anosmic group – and those for odor – obtained from the normosmic group. As expected, normosmics outperformed anosmics in the detection of every stimulus. Nevertheless, the anosmics could detect all stimuli at high enough concentrations. The ratio of vapor phase concentration between the pungency thresholds and the odor thresholds ranged from about 10 for 2-propanone to about 1600 for tert-butyl acetate, and averaged 285 across the various stimuli.

Insert Figure 1 about here

As seen before with homologous alcohols <sup>14</sup> and acetates <sup>19</sup>, nasal pungency and odor thresholds for the homologous ketones decreased with increasing carbon chain length. The ketones showed a tendency to plateau at 2-heptanone. As Figure 2 illustrates, the pattern across substances held for all individual anosmics and normosmics.

Insert Figure 2 about here

The results from our present and previous studies reveal patterns of how changing a functional group from one carbon to another influences chemosensory potency. For the alcohol series, the odor threshold tended to increase when the OH functional group was changed from a primary carbon to a secondary carbon: 1-propanol (threshold  $\approx 14$  ppm) vs. 2-propanol (threshold  $\approx 503$  ppm), 1-butanol ( $\approx 54$  ppm) vs. 2-butanol ( $\approx 95$  ppm), 1-heptanol ( $\approx 0.1$  ppm) vs. 4-heptanol ( $\approx 8.2$  ppm); or when it was changed from a secondary carbon to a tertiary one: 2-butanol ( $\approx 95$  ppm) vs. tert-butyl alcohol ( $\approx 609$  ppm). This tendency also held true for the pungency threshold of these alcohols, although for pungency the difference between members of the pairs became progressively attenuated as the carbon chain length increased: 1-propanol ( $\approx 2,500$  ppm) vs. 2-propanol ( $\approx 18,135$  ppm), 1-butanol ( $\approx 1,100$  ppm) vs. 2-butanol ( $\approx 5,711$  ppm), 2-butanol ( $\approx 5,711$  ppm) vs. tert-butyl alcohol ( $\approx 32,779$  ppm), 1-heptanol ( $\approx 210$  ppm) vs. 4-heptanol ( $\approx 335$  ppm).

The odor thresholds for n-butyl, sec-butyl, and tert-butyl acetates resulted in relatively similar values ( $\approx 2.4$ ,  $4.7$  and  $1.3$  ppm, respectively), and so did their pungency thresholds, though sec-butyl acetate had a lower pungency threshold ( $\approx 700$  ppm) than n-butyl ( $\approx 3,600$  ppm) or tert-butyl ( $\approx 2,100$  ppm) acetate.

When plotted against log saturated vapor concentration at room temperature, log pungency thresholds conformed to a linear function roughly parallel to the saturated vapor identity line (Figure 3). On the contrary, log odor thresholds did not conform as well to a linear relationship, and showed considerably more dispersion.

Insert Figure 3 about here

### Discussion

Anosmics proved able to detect all the substances tested, albeit at much higher concentrations than normosmics. The results obtained for this set of compounds extended those obtained for the previously tested series of homologous alcohols<sup>14</sup> and acetates<sup>19</sup>. Aside from the direct relevance of these values to the knowledge of the sensitivity of the olfactory and common chemical senses towards the stimuli used, the data also allow inferences about how changes in physicochemical properties affect the ability of the chemicals to elicit threshold odor and pungency.

In order to explore common trends in nasal pungency and odor thresholds among the three homologous series studied so far (alcohols, acetates, and ketones), we plotted our entire set of thresholds against saturated vapor concentration at room temperature (Figure 4). The composite outcome for nasal pungency strongly suggests the same relationship for all series. In the logarithmic coordinates of the figure, the function for nasal pungency closely parallels the saturated vapor identity line (slope=1.00 and r=1.00) indicating that pungency from the tested substances arises, in the absence of olfaction, at an approximately constant percentage of saturated vapor ( $\approx 32\%$ ) irrespective of the functional group or carbon chain length of the stimulating molecule. The outcome

supports the notion that simple physical properties could predict the level at which nonreactive airborne chemicals can provoke nasal pungency.

Insert Figure 4 about here

The results in Figure 4 for odor lack the uniformity seen for pungency. This presumably indicates that threshold olfactory reception involves mechanisms more finely tuned to the features of odoriferous molecules. In general, our odor thresholds fall in the range of values reported or reviewed by other authors<sup>34, 35, 36, 37, 38</sup>. Nevertheless, there is a great deal of variability among the results reported in different studies, sometimes as much as four or five orders of magnitude. Factors accounting for such dispersion include odorant generation and presentation techniques (see<sup>39</sup>), psychophysical procedures employed, and chemical purity of the substances used. There is also, of course, individual variability, although this factor might not be responsible for as much variation as is generally thought, once the age of the subjects is accounted for, and repetitive measurements are averaged over a few sessions<sup>33, 40</sup>. Among demographic variables, age seems to take the highest toll on both olfaction and the CCS (see<sup>41</sup>), with smoking and gender following (see<sup>42, 43</sup>).

A feature common to the homologous series studied so far is that the gap between pungency and odor thresholds increases with carbon chain length. This diverging trend continues up to 1-octanol for the alcohols, but tends to reach a plateau at hexyl acetate for the acetates and at heptanone for the ketones. This means that, even when the sensitivity of both the CCS and olfaction increases with carbon chain length, such an increase in sensitivity is more pronounced for odor than for pungency. Hence, compounds such as methanol or acetone evoke pungency at concentrations not much above their respective olfactory thresholds (about an order of magnitude), whereas compounds like 1-

octanol or 2-heptanone only evoke pungency well above their respective olfactory thresholds (about 2.5 to 4 orders of magnitude). The difference in the size of the gap could account in part for why the short chain-length members of the series are thought of as more irritant than the longer members, even though these can irritate the nasal mucosa at substantially lower airborne concentrations than their shorter chain counterparts. It is also true, however, that, as reported by the anosmics, the pungency produced by the short chain chemicals is "sharper", with more "bite" than that produced by the longer chain chemicals. The distinction suggests that the CCS can discriminate qualitative as well as quantitative differences in sensation.

Devos et al. have recently compiled and standardized many previously measured human olfactory thresholds <sup>44</sup>. There is a high correlation ( $r=0.90$ ) between the 25 thresholds of our investigations <sup>14, 19</sup> and the averaged values from the compilation (Figure 5). From the lowest to the highest values, our thresholds cover a larger span, approximately six orders of magnitude rather than their four, and thereby offer better resolution among compounds. Averaging across studies undoubtedly accounts for much of the apparent constriction in range in the compiled data. Our thresholds, which represent the point of 100% detection, understandably often fall above those in the compilation, which presumably represent principally the points of 50% or 75% detection. Our data comprise one of the larger sets obtained with a uniform methodology, procedure, and instructions, and employing the same, intensively tested subjects. Also, our vapor phase concentrations are derived from measurements rather than from the more common practice of estimating the vapor concentration from the liquid concentration.

Insert Figure 5 about here

Our nasal pungency thresholds represent the first measured in the absence of a functional sense of smell. Previously reported nasal irritation thresholds (see review in <sup>36</sup>) as well as nasal irritation suprathreshold intensities (see <sup>7, 45</sup>) were measured in normosmic subjects. Any difference found between nasal pungency thresholds from anosmics and nasal pungency "thresholds" from normosmics, once methodological and demographic factors are accounted for, can safely be ascribed to the interfering presence of olfaction. The olfactory sense of normosmics can influence their perception of the level at which a substance first starts to irritate: a) directly, if subjects confuse an odorous sensation with pungency, or b) indirectly, if a functioning sense of smell can facilitate or inhibit the response of the CCS at threshold levels. The degree to which these possibilities occur can perhaps be explored best through objective measurements of in vivo olfactory and CCS responses in humans. In this regard, evoked potentials may eventually hold promise <sup>46</sup>. Cain and Murphy <sup>8</sup> showed a mutual, albeit asymmetrical, inhibition between suprathreshold levels of pungency and odor: irritation inhibited odor markedly while odor inhibited irritation slightly. The same pattern of inhibition appeared even when the irritant (CO<sub>2</sub>) entered one nostril and the odorant (amyl butyrate) the other, suggesting that the interaction occurs in the brain. Nevertheless, we cannot rule out a facilitative interaction between olfaction and the CCS, more so at near-threshold levels.

We have concentrated our efforts on relatively nonreactive compounds since more reactive substances will exert their effect almost certainly via damage to tissue. Nevertheless, as mentioned in the Introduction, the agents putatively responsible for symptoms such as nose, eye, and throat irritation from polluted indoor environments are principally nonreactive VOCs. Many of these VOCs are present below even their odor thresholds, which raises the question for future investigations of the possibility that subthreshold levels of a wide array of chemicals add up to evoke irritation. At

suprathreshold levels, pungent sensations from mixtures of substances showed a higher degree of addition than odorous sensations<sup>47, 48</sup>.

### Acknowledgements

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### Figure Legends

Figure 1. Nasal pungency thresholds (from an anosmic group) and odor thresholds (from a normosmic group) for 11 substances. Each point represents a geometric mean across subjects and dots indicate standard deviations.

Figure 2. Thresholds for individual anosmics and normosmics. Each point represents an average of 12 threshold determinations.

Figure 3. Pungency thresholds and odor thresholds depicted as a function of saturated vapor concentration at room temperature. The saturated vapor identity line is shown for reference.

Figure 4. The same as in Figure 3, but including previously studied homologous alcohols <sup>14</sup> and acetates <sup>19</sup>.

Figure 5. Comparison between our present and previous <sup>14, 19</sup> odor thresholds, sorted in descending order, and those compiled, standardized, and averaged by Devos et al. <sup>44</sup>.

FIGURE 1

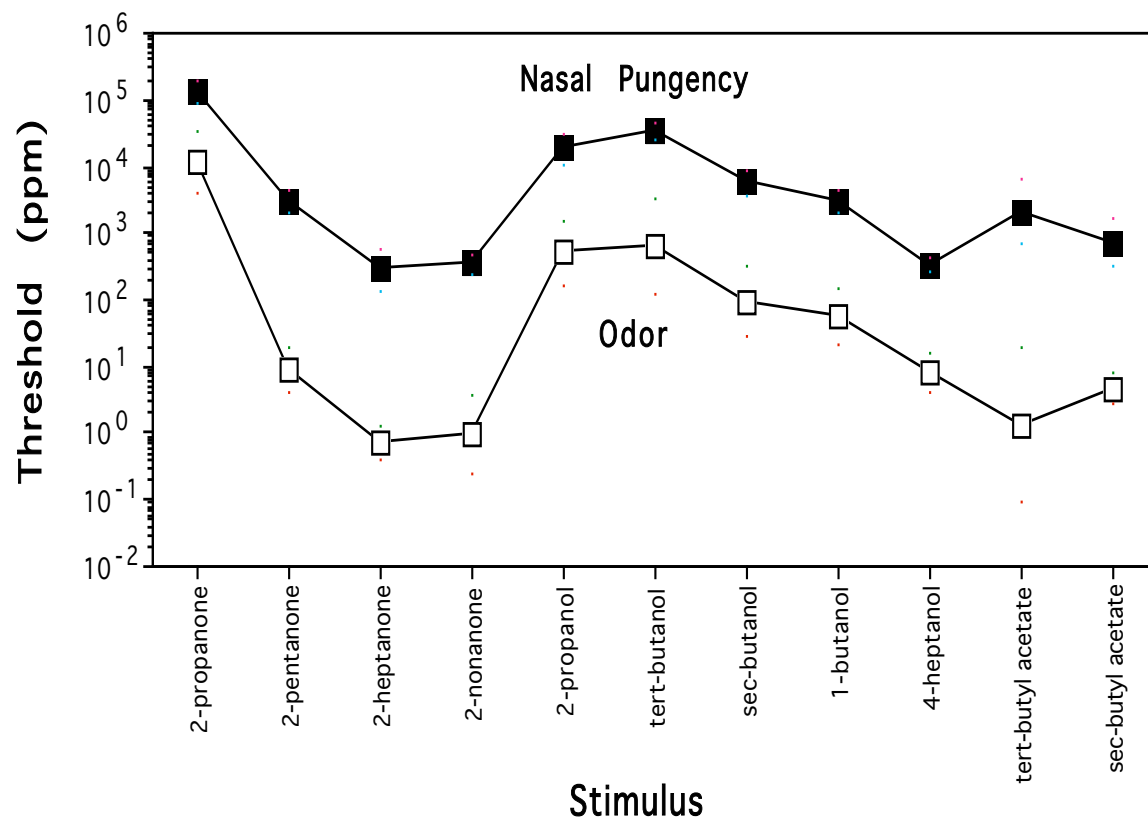




FIGURE 2

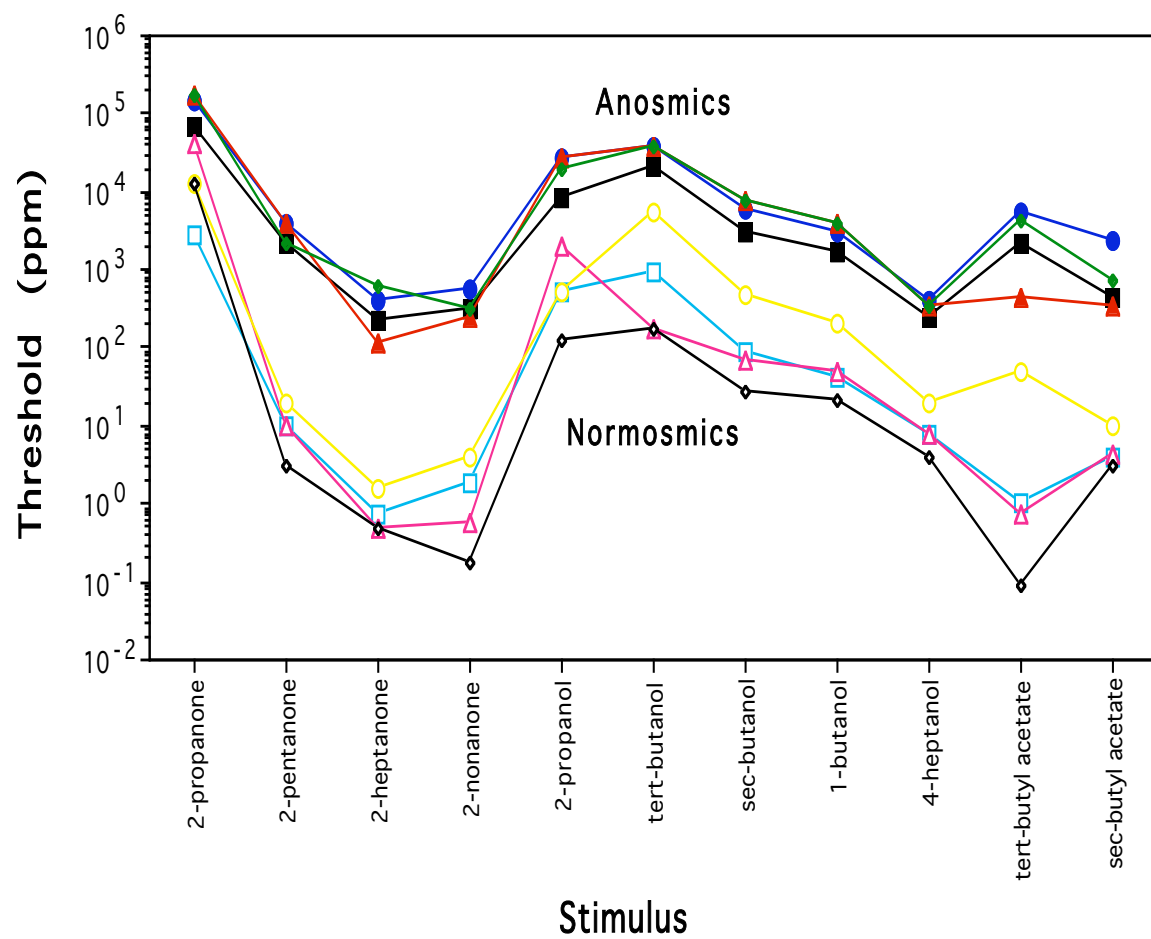


FIGURE 3

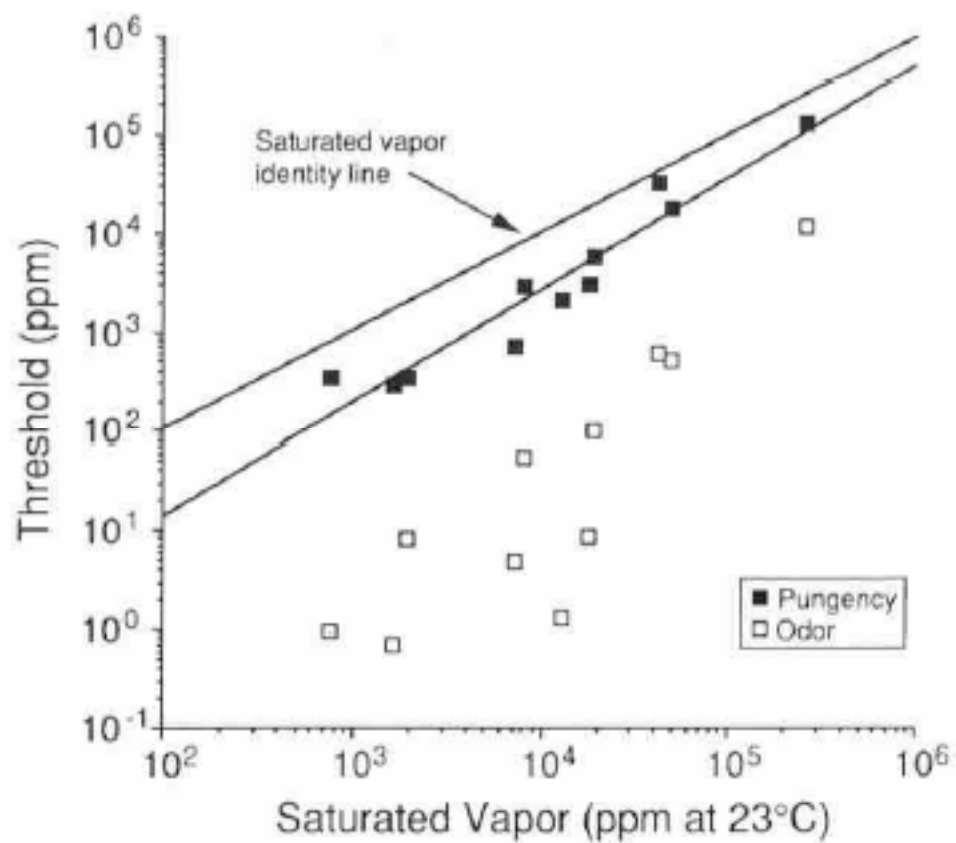


FIGURE 4

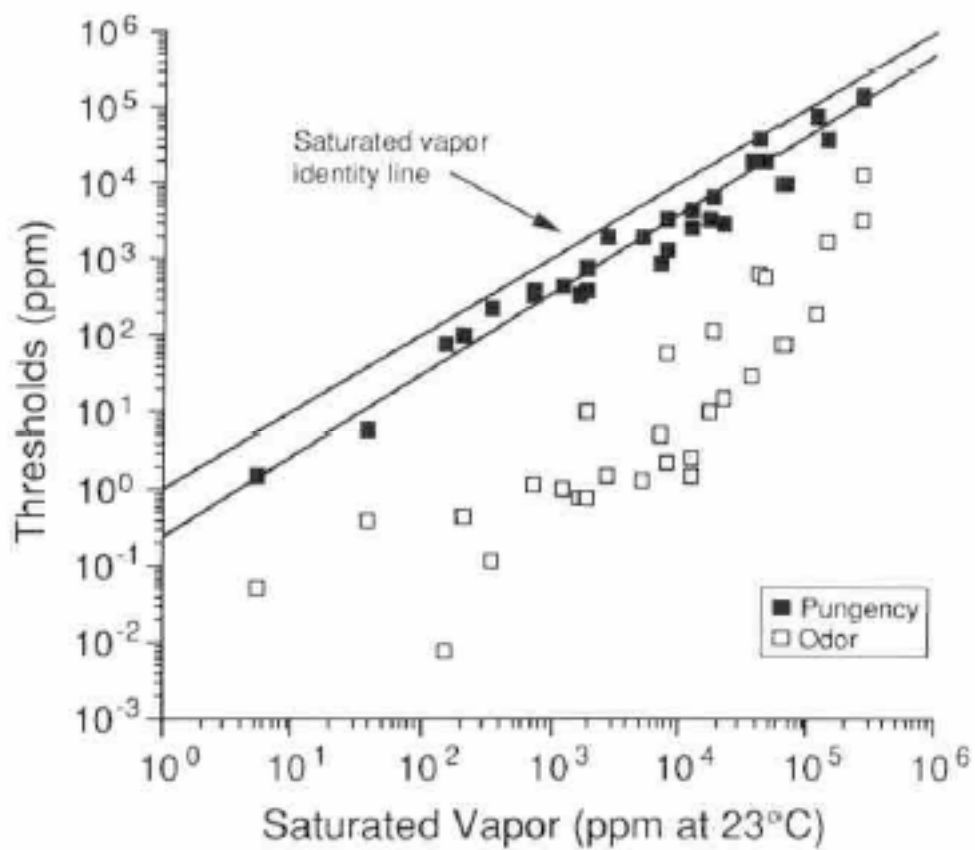
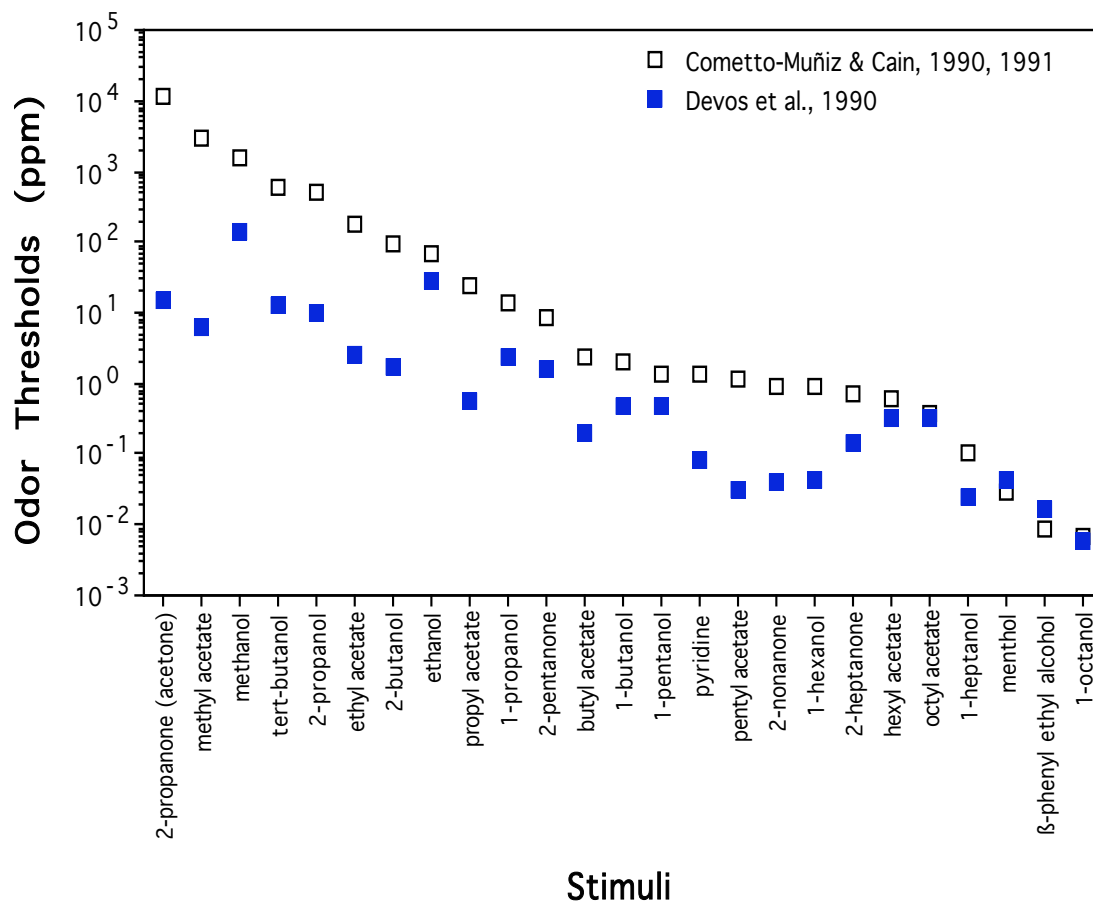


FIGURE 5



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