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Chapter 4: Chemical Sensing in Humans and Machines

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<u>Abstract</u>

Chemosensory detection of airborne chemicals by humans is accomplished principally through olfaction and mucosal chemesthesis. Odors are perceived via stimulation of the olfactory nerve (CN I) whereas nasal chemesthetic sensations (i.e., prickling, irritation, stinging, burning, freshness, piquancy, etc), grouped under the term nasal pungency, are mediated by the trigeminal nerve (CN V). Airborne compounds elicit odor sensations at concentrations orders of magnitude below those producing pungency but the physicochemical basis for odor and

pungency potency of chemicals, either singly or in mixtures, is far from being understood. The sensitivity of the sense of smell often outperforms that of the most sophisticated chemico-analytical methods like gas chromatography and mass spectrometry. Still, the combined used of these techniques with human odor detection (i.e., olfactometry) has proved an invaluable tool to understand the chemosensory properties of complex mixtures such as foods, flavors, and fragrances.

1) <u>Human chemosensory perception of airborne chemicals</u>

Humans detect the presence of volatile organic compounds (VOCs) in their surroundings principally through their senses of olfaction and "chemesthesis" [1, 2]. The latter is also known as the "common chemical sense" [3, 4]. Activation of the olfactory nerve (CN I) produces odor sensations. Chapter 3 describes the biological basis of this chemosensory pathway. Activation of chemoreceptors on the trigeminal nerve (CN V) innervating the face mucosae produces chemesthetic responses (see, for example, [5]). These responses evoked in the nose include stinging, piquancy, burning, freshness, tingling, irritation, prickling, and the like. All these nasal sensations can be grouped under the term nasal pungency [6]. Chemesthetic responses to airborne VOCs can also be produced in the ocular, oral, and upper airway mucosae, where they are referred to as eye, mouth, and throat irritation. In the case of the back of the mouth and the throat, other nerves, such as the glossopharyngeal (CN VIII) and vagus (CN X), are also stimulated by airborne VOCs and contribute to perceived irritation.

In this chapter we will focus on human smell and nasal chemesthesis. We will review psychophysical studies performed on both sensory modalities addressing the possible basis for the odor and irritation potency of VOCs. We will also summarize various techniques that combine the power of the human nose with that of chemical-analytical instruments, such as gas chromatography and mass spectrometry, to quantify the chemosensory activity of volatile chemicals and to help understand better the characteristics of human chemosensory perception.

2) <u>Nasal Chemosensory Detection</u>

Odor thresholds represent an important biological characteristic of airborne chemicals. Nevertheless, compilation of such values [7-9] show an extreme variability for any particular substance, even after attempting to standardize the values reported in different sources [10]. This scatter severely limits the practical application of the information available. An important

roadblock in our understanding of smell and nasal chemesthesis is the lack of information regarding what particular characteristics of chemicals govern the potency (i.e., threshold and suprathreshold) and type (i.e., quality) of olfactory and trigeminal sensations that they evoke. The situation stands in sharp contrast with the senses of vision and hearing where we have a precise knowledge of the range of electromagnetic and vibrational energy, respectively, to which our eyes and ears are tuned. From a few known, well-defined parameters of any light and sound it is relatively straightforward to predict its visual and auditory perceptual properties. From the structural and physicochemical properties of a compound it is not easy to predict its odor or chemesthetic perceptual properties.

Attempts to correlate odor with structural and physicochemical properties of odorants have focused, typically, on one or a small number of odor qualities (see reviews in [11, 12]), probably because broader generalizations have failed to lead to a productive outcome. As has been pointed out [13], an important drawback of many structure-activity relationships in olfaction [14-19] is the difficult interpretation of the chemical features that are shown to correlate with odor activity.

Regarding chemesthesis in the upper airways, a pioneer review paper [20] described the possible chemical mechanisms of sensory irritation. This study focused principally on "reactive" chemicals, that is, substances producing chemesthetic responses principally via direct chemical reaction with mucosal tissues. A more recent review of the topic [21] also addressed the mechanism by which relatively nonreactive compounds could produce pungency. In fact, relatively nonreactive VOCs are the prime candidates for the production of adverse chemosensory symptoms in cases of indoor air pollution such as the sick building syndrome (cf. [22]).

Among the various factors accounting for the large variability of measured odor thresholds, apart from true biological variability, we can mention: method of vapor-stimulus

control and/or delivery, psychophysical methodology, criterion to arrive at a threshold response, number of subjects, and number of trials per subject [23, 24]. In the case of nasal pungency thresholds, a crucial additional factor is the use of a procedure that avoids odor biases since almost all chemicals have both odor and pungency and the odor could be quite strong at the concentrations needed to produce barely perceptible nasal pungency. Additionally, in order to build a chemosensory structure-activity relationship, a chemical stimulus continuum of some sort can be very helpful.

In a long-range effort started more than 10 years ago [25], odor and nasal pungency thresholds were measured using a standardized procedure aimed to minimize many of the variability sources mentioned above and to produce a data set with robust internal consistency. Some of the procedural features employed included: 1) Delivery of vapors monorhinally (i.e., one nostril at a time) via "static" olfactometry [26] from plastic squeeze bottles [27]. 2) Short-term exposures (1-2 seconds). 3) Rigorous measurement and follow-up of presented vapor-phase concentrations by gas chromatography. 4) Use of a two-alternative, forced-choice procedure against a blank (to minimize biases), presentation of chemicals in an ascending concentration series (to minimize sensory adaptation), and use of, at least, duplicate bottles with identical concentration (to alternate sniff sampling and avoid depletion of stimulus in the headspace). 5) Use of a constant and fixed criterion for threshold (i.e., five correct choices in a row) across subjects, repetitions, chemosensory modality (i.e., odor and nasal pungency), and studies. 6) Selection of subjects with no sense of smell (called anosmics) to measure nasal pungency thresholds (thus avoiding odor biases), and of subjects with normal sense of smell (normosmics) to measure odor thresholds. Normosmics were matched to the anosmics by age, gender and smoking status, all demographic variables known to influence chemosensory perception (see review in [28]). 7) Selection of stimuli from homologous chemical series, where physicochemical properties change systematically and where carbon chain length provides a convenient "unit of change" (i.e., a continuum) against which to relate the sensory results.

a) <u>Thresholds for Odor and Nasal Pungency</u>

These systematic studies of odor and nasal pungency thresholds along homologous chemical series included testing of n-aliphatic alcohols [25], n-acetate esters [29], sec- and tert-alcohols and acetate esters [30], ketones [30], alkylbenzenes [31], and aliphatic aldehydes and carboxylic acids [6]. Figure 1 summarizes the results obtained with all these series.

Insert figure 1 about here

The outcome clearly shows how both chemosensory thresholds decline as carbon chain length increases. This means that sensory potency (both olfactory and trigeminal) increases along each homologous series. The rate at which odor thresholds decline, at least for the first few members of each series, tends to be higher than that for nasal pungency thresholds. In various instances, odor thresholds seem to reach a plateau, like for acetate esters, ketones, and alkylbenzenes. Nasal pungency thresholds, in contrast, reach a "cut-off" effect [6]: beginning with a certain homolog member, nasal pungency fails to be consistently evoked, and this effect deepens for all ensuing members. In other words, the ability of that particular homolog and of all following homologs to produce nasal pungency fades away. The cut-off effect to produce a biological response seen at some point in a chemical series is a well-known pharmacological phenomenon in the field of anesthesia [32, 33]. At least two mechanisms can account for such cut-offs [33]: a physical mechanism whereby the maximum vapor-phase concentration of the stimulus molecule (at a certain temperature and pressure) falls below the threshold, and a biological mechanism whereby the stimulus molecule lacks a crucial property to trigger transduction. For example, the molecule could be too large to fit into the binding pocket of a receptive macromolecule or to interact effectively with a target site.

b) <u>Stimulus-response (i.e., psychometric) functions for odor and nasal pungency</u>

Studies that aimed at measuring thresholds for olfaction and nasal chemesthesis with a uniform methodology, particularly in the context of testing homologous chemical series, proved to be useful tools in understanding how physicochemical properties govern sensory potency. The use of a standard testing procedure was instrumental to develop robust quantitative structure-activity relationships (QSARs) (see below). Nevertheless, measurement of a punctate chemosensory threshold according to a fixed criterion of performance has limitations [34]. A more comprehensive knowledge of the chemosensory processes involved can be gained by measurement of complete stimulus-response (called psychometric) functions (e.g., [23, 24]). These functions span the range from chance detection to virtually perfect detection and, thus, cross the boundaries between perithreshold and suprathreshold sensations. Given a certain set of testing conditions, psychometric functions depict a continuous track of how the detectability of the chemical(s) grow with increasing concentration, rendering a dynamic picture of the process.

Figure 2 presents psychometric functions for the odor and nasal pungency evoked by 1-butanol, 2-heptanone, butyl acetate, and toluene. All functions in Figure 2 show an ogival shape with a close-to-linear section in the middle of the range. As expected from previous studies on thresholds (see review in [5]), odor detection occurred orders of magnitude below nasal pungency detection. The gap between olfactory and chemesthetic detection (at halfway between chance and perfect detection) ranged between 3.4 and 6.4 orders of magnitude. The two chemosensory modalities also differed in the slope along the linear portion of the function. Odor functions for these four chemicals have slopes between 0.35 and 0.5 [34, 35] whereas nasal pungency functions have slopes between 0.7 and 1.0, except toluene which showed an even steeper slope in the range 2.2-2.9 [34, 36].

Insert Figure 2 about here

3) Olfactory and nasal chemesthetic detection of mixtures of chemicals

In typical, everyday experiences, olfactory and chemesthetic sensations arise from exposures to mixtures of substances. Rarely are they the result of exposure to a single chemical. In addition, the study of the chemosensory detection of mixtures vis-à-vis detection of the individual components has the potential to uncover basic principles of functioning of the senses of smell and chemesthesis.

Studies on the olfactory detection of mixtures of airborne chemicals have relied, for the most part, on measurement of thresholds according to a fixed criterion of performance, and have typically expressed the results in terms of the stimulus (that is, concentration of the chemical). Their outcome suggests partial and simple stimulus agonism [37-39] with some indications of synergistic stimulus agonism as number of components increases [39-42]. To illustrate the meaning of these terms, let us take the example of a 3-component mixture whose constituents are present at sensory-equivalent concentrations (i.e., at the same multiple or submultiple of their respective individual thresholds). The terms simple, synergistic, and partial agonism indicate, respectively, that the mixture achieves threshold when each component is present at one third, less than one third, and more than one third (but less than one time) its individual threshold concentration. The term independence indicates that the mixture achieves threshold only when at least one of the components is present at its individual threshold. The term antagonism indicates that the mixture achieves threshold only when the components are present at a concentration even higher than their respective individual thresholds. A recent study looking at the olfactory (and trigeminal) detectability of binary mixtures of 1-butanol and 2-heptanone via measurement of psychometric functions lent support, as a first approximations, to an outcome of simple agonism [35].

Not surprisingly, studies on the trigeminal detection of mixtures are much fewer than those on olfaction. A comprehensive study measuring trigeminal thresholds for single chemicals and for mixtures of up to nine components revealed a trend for the degree of agonism to increase with the number of components and with the lipophilicity of such components [39]. A couple of

recent investigations measured psychometric functions to look in detail at the trigeminal detectability of binary mixtures compared to the detectability of the single components [35, 36]. The general outcome supported, once again, simple agonism with the suggestive possibility, open to further scrutiny, that, for chemesthetic responses, simple agonism might weaken to partial agonism as the detectability of the mixtures approach perfect detection [36], that is, as the mixtures leave the perithreshold region and enter into the suprathreshold region. If such weakening of agonism is confirmed and extended to olfactory responses, it would fall nicely into register with the finding of partial agonism (called hypoadditivity) very commonly reported for mixtures of odorants at the suprathreshold range (e.g., [43]) even when the analysis considers "addition" of concentration (mass) and not simply addition of sensation [44].

It has been suggested that, within each chemosensory modality, compounds with similar slopes in psychometric functions will tend to show simple agonism in mixtures whereas compounds with different slopes will tend to show a lesser degree of agonism, e.g., partial agonism [36]. At this stage, psychometric functions for additional substances tested in binary and higher order mixtures need to be measured to confirm the trend.

4) Physicochemical determinants of odor and nasal pungency

As mentioned, the senses of olfaction and chemesthesis allow us to detect airborne chemicals. To gain a better understanding of how these sensory channels function it is important to know what particular features of chemicals govern their potency as odorants and irritants (including threshold and suprathreshold intensities). Regarding olfaction, a large number of such features have been suggested, including Wiswesser notation formulas [14], structural parameters directly derived from the chemical formula [45] or derived from gas chromatographic measurements [17, 19], steric and electronic descriptors [46], molecular vibration [47-49], partition coefficients (specifically, water-air and octanol-water) [50] and an electron-topological

method [51]. Some of these investigations focused on one or just a few odor qualities (e.g., musk) whereas others studied a broader spectrum.

Regarding chemesthesis, there have also been a number of chemical features reported to correlate with sensory irritation. Among them, normal boiling point [52], adjusted boiling point [53], saturated vapor pressure [54], Ostwald solubility coefficient (i.e., log L where L = concentration in solvent/concentration in gas phase) [55], and partition coefficients (specifically, water-air and octanol-water) [56]. Interestingly, all these descriptors are physicochemical parameters and do not involve the precise chemical structure of the irritant.

a) <u>The linear solvation model</u>

Many of the quantitative structure-activity relationships (QSARs) cited above for olfaction and chemesthesis are difficult to interpret either chemically or mechanistically [13]. A recently developed model has the advantage of not only providing a strong statistical fit to human psychophysical data but also conveying chemically and mechanistically meaningful information on both the stimulus (i.e., odorant or irritant) and the biophase where sensory reception initially takes place (i.e., for olfaction, the membrane covering the cilia of the olfactory receptor neuron, and, for nasal chemesthesis, the membrane of the free nerve endings of the trigeminal nerve). This model is based on a general solvation equation developed by Abraham [57, 58]:

$$\log SP = c + r \cdot R_2 + s \cdot \pi_2^H + a \cdot \Sigma\alpha_2^H + b \cdot \Sigma\beta_2^H + l \cdot \log L^{16} \tag{1}$$

where SP is the dependent variable that, in the present context, represents a sensory property defined as the reciprocal of the odor detection threshold (1/ODT) or the reciprocal of the nasal pungency threshold (1/NPT). The reciprocals are used simply because the larger the quantity, the more potent is the odorant or irritant. There are five independent variables: excess molar refraction (R_2), dipolarity/polarizability (π_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 298K (L^{16}). The L^{16} descriptor is a combination of two properties of the odorant or irritant: (i) a general measure of size and (ii) the ability of the odorant or irritant to interact with a biophase through dispersion forces. The term c and the coefficient for each of the independent variables (r, s, a, b, and l) are obtained by multiple linear regression analysis. However, these are not simply fitted coefficients. They have chemical and mechanistic meaning since they reflect the complementary properties that the biophase must show in order to be receptive to the odorant or irritant. In other words, the independent variables provide a physicochemical characterization of the stimulus (i.e., odorant or irritant) whereas the corresponding coefficients provide a characterization of the receptive biophase bound to interact with that stimulus. The r-coefficient measures the tendency of the biophase to interact with the odorant or irritant via polarizability-type interactions, mostly via π- and n-electron pairs. The s-coefficient reflects the biophase dipolarity/polarizability (since a dipolar odorant or irritant will interact with a dipolar biophase, and a polarizable odorant or irritant will interact with a polarizable biophase). The a-coefficient represents the complementary property to the odorant or irritant hydrogen-bond acidity, and, thus, is a measure of the biophase hydrogen-bond basicity (since an acidic odorant or irritant will interact with a basic biophase). Similarly, the b-coefficient is a measure of the biophase hydrogen-bond acidity (since a basic odorant or irritant will interact with an acidic biophase). Finally, the l-coefficient is a measure of the biophase lipophilicity [13].

b) Application of the solvation equation to odor and nasal pungency thresholds

The standardized procedure employed to measure the odor and nasal pungency thresholds depicted in Figure 1 provided a firm ground to develop QSARs based on the solvation model described above. Under this model, the odorant or irritant is seen as a solute that travels through a series of solvent phases (air, nasal mucus, nasal tissue) until it exerts its (sensory) action upon a receptive biophase. Thus, the model only applies to what can be called "transport" processes. These are processes where the fundamental step is either the distribution of the stimulus (i.e., odorant or irritant) between biophases or the rate of transfer of the stimulus form

one biophase to another. The model does not apply to stimuli acting through exact conformational or geometrical states since these sort of molecular changes would barely affect the above mentioned physicochemical descriptors but, when relevant, could affect potency dramatically. In addition, the model does not apply to "reactive" compounds, that is, substances that produce nasal pungency via direct chemical reaction with nasal tissue [21]. The solvation equation would underestimate the potency of such chemically reactive stimuli [59, 60].

The original equation for odor thresholds [13] was recently improved [61] with the addition of two additional terms: 1) A parabolic term $(D-D^2)$ where D is the maximum length of the odorant molecule obtained by computer-assisted molecular modeling and geometry optimization. 2) An indicator variable, H, chosen as 2.0 for carboxylic acids and aldehydes and zero for all other odorants. (As discussed in [61], the need to introduce H arises because carboxylic acids and aldehydes are more potent than predicted). The odor equation looks as follows:

$$\log (1/ODT) = -7.445 + 0.304\ R_2 + 1.652\ \pi_2^H + 2.104\ \Sigma\alpha_2^H + 1.500\ \Sigma\beta_2^H + 0.822\ \log L^{16} +$$

$$+ 0.369\ D - 0.016\ D^2 + 1.000\ H$$

$$(2)$$

with n=60, r^2=0.84, SD=0.601, where n is the number of odorants included, r is the correlation coefficient, and SD is the standard deviation in the dependent variable. All symbols are as described for equation (1).

The solvation equation model has performed even better for the description and prediction of nasal pungency thresholds [6, 62-65] than for odor thresholds. Its success indicates that transport processes indeed constitute a key step in the production of nasal pungency by nonreactive airborne chemicals. The latest version of the nasal pungency equation looks as follows:

$$\log (1/NPT) = -8.519 + 2.154\ \pi_2^H + 3.522\ \Sigma\alpha_2^H + 1.397\ \Sigma\beta_2^H + 0.860\ \log L^{16}\quad (3)$$

with n = 43, r^2 = 0.955, SD = 0.27, where all letters and symbols are as defined above. In this case, the term $r.R_2$ from the general equation (1) did not achieve significance and was omitted.

It must be pointed out that equation (3) does not account for the observed cut-off effect on nasal pungency that we have mentioned under item "2) a) Thresholds for odor and nasal pungency". Future research should aim at optimizing the range of applicability of equation (3) by including a "size" factor capable of accounting for such molecular cut-offs in chemesthesis. This line of work is likely to gather critical knowledge not only on the molecular boundaries of airborne pungent stimuli but also on those of the putative nasal chemesthetic receptor as well.

5) <u>Human chemical sensing: Olfactometry</u>

All studies exploring how humans detect and perceive airborne chemicals need to devise a strategy to generate and deliver the compounds (i.e., stimuli) at predetermined concentrations (i.e., levels). Generation, delivery, and control of chemical stimuli entail more complexity than the equivalent processes for physical stimuli such as lights and sounds. In addition, there are practically no well-established, accepted, and widely used commercial devices to perform such tasks. In many cases, a one-of-a-kind olfactometer is built with much effort and time for one or a few studies, only to be left in disuse, replaced, or substantially modified for other studies. As a rule, no steps are taken in order to understand how results obtained with the "old" device compare with those obtained with the "new" one.

In this section we will discuss three broad olfactometric techniques that, with variations, have been and are still being used in the study of human chemosensory perception [26].

a) <u>Static olfactometry</u>

In general, olfactometric techniques can be classified into "static" or "dynamic" depending on whether the vapor stimulus is drawn from an enclosed container where the liquid and vapor phases of the tested chemical are in equilibrium, or the vapor flows continually in a carrier-gas stream, typically odorless air or nitrogen. Important aspects in the static approach include the type of container, the way in which the vapor is drawn to the nose, and the type of connection between the headspace of the container and the nose of the subject.

Containers in static olfactometry are typically glass or (almost) odorless plastic. As a rule, a series of dilutions of the substance(s) of interest are prepared in individual vessels using an odorless solvent. Choice of the solvent is not straightforward. Distilled and deionized water could serve in some cases but some chemicals are unstable in water. For example, esters tend to hydrolyze producing the alcohol and the carboxylic acid. Also, most odorants have little or extremely low water solubility. Alternative solvents are lipophilic substances where odorants are more stable and soluble. These include, for example, mineral oil and propylene glycol. Nevertheless, these are not always completely odorless and might present a low odor background. Many of the olfactory and nasal chemesthetic studies mentioned above resorted to the use of "squeeze bottles" [66] (Figure 3, left). Their caps have pop-up spouts that fit into one or the other nostril allowing monorhinic testing. This, added to their easy availability and simplicity of use has made them useful not only in research but also in the clinic [27]. A recent study has shown that a newly developed glass vessel system possess advantages over the plastic squeeze bottles, producing nasal pungency thresholds systematically lower by an average factor of 4.6 compared to those obtained via squeeze bottles [67] (Figure 3, right). This investigation tested three members each of homologous alcohols, acetates, and ketones.

Insert Figure 3 about here

Subjects can sample the vapors in the headspace of the container actively by sniffing or they can receive them passively, for example, when the experimenter activates a valve that

sends a fixed volume of headspace into the participant's nostrils. The second method [68] makes stimulation independent of the sniffing pattern of the subjects but it can cause progressive drying of the nasal mucosa, leading to irritation with repetitive stimulation, and can also lead to confusion between air pressure and odor sensations [69]. In addition, more recent studies have shown that natural sniffing maximizes olfactory performance in humans [70].

The type of connection between the vapor container and the subject's nostrils determines the effective concentration reaching the nose. The squeeze bottles, with their pop-up spouts that fit inside one nostril, represented an improvement over other containers that are simply open and placed under the subject's nose, but still left room for dilution of the stimulus from surrounding air. The above mentioned glass vessels include Teflon made nosepieces that fit snugly into both nostrils of the subject, maximizing the efficiency of the stimulus delivery [67].

It is important to stress that in all these techniques of static olfactometry, the actual stimulus is the vapor above the solution in the container. In principle, the vapor concentration is proportional to the liquid concentration, but such proportionality varies with odorants, solvents, and, sometimes, among concentrations of the same odorant-solvent pair. For these reasons, actual measurement of the vapor-phase concentration in each container, and periodic follow-ups to ensure stability, become the only safeguard against incorrect assumptions. Unfortunately, all too often olfactory investigations do not include such vapor measurements. Gas chromatography provides a relatively simple way to measure and calibrate vapor concentrations for use in static olfactometry.

b) Dynamic olfactometry

Under the principles of dynamic olfactometry, the chemical stimulus flows continuously in a carrier gas stream of either purified air or nitrogen. The various concentrations of the substance(s) tested are typically achieved by mixing in different proportions the carrier gas line

with the odorant line. A number of elements including tubing, capillaries, flowmeters, mass flow controllers, valves, saturating and mixing vessels, deodorizing and air conditioning (i.e., temperature and humidity) devices constitute the necessary equipment for the generation and control of odorants. As in the case of static olfactometry, the interface between the exit of the stimulus and the nose is an important feature regarding possible unwanted dilution of the targeted concentration. The complete assembly is referred to as an "olfactometer".

In a very in-detail analysis of various olfactometers and of the many principles guiding their design, Dravnieks [71] has described devices used in both animal and human studies. Dravnieks himself proposed a Binary Dilution Olfactometer [71] (Figure 4). This instrument combines portability and stability of concentrations with ease of use and maintenance. Its simplicity arises from the fact that it uses saturated vapor as the source of undiluted stimulus and employs a series of capillaries of various widths and lengths to achieve 7 fixed increasing dilutions of the odorant, all presented at a final flow rate of 160 ml/min. One of the suggested applications of this device was to use it with 1-butanol in order to allow to express the odor intensity of any source in terms of an odor equivalent concentration of 1-butanol (in ppm by volume) [72]. The technique became an ASTM (American Society for Testing and Materials) recommended procedure [73]. Dravnieks also developed a Dynamic Forced-Choice Triangle Olfactometer for measurement of thresholds [74, 75]. Both types of olfactometers found an important application in the measurement of environmental odors (e.g., [76]).

Insert Figure 4 about here

Chemical stimulation of the olfactory and trigeminal chemosensory systems in the nose gives rise to both peripheral electrical potentials [77, 78] and central evoked potentials [79]. In order to study such electrophysiological events, an olfactometer was needed that 1) delivered the stimulus without altering the mechanical or thermal conditions at the stimulated mucosa, and 2) produced a sharp, square-wave type, stimulus onset and offset. Such an instrument was

pioneered by Kobal and collaborators [77, 79]. Their instrument achieved these goals by embedding pulses of odorant or irritant in a constantly flowing air stream under controlled temperature (36.5°C) and humidity (80% RH).

An interesting development in the area of dynamic olfactometry emerged from the description of an olfactometer that also served to measure respiratory parameters [80-83] (Figure 5). The instrument evolved through the years and in its latest version presents the odorants and irritants to subjects through a mask covering nose and mouth (with a good seal, monitored by pressure) in a room-temperature warmed (≈25°C) and humidified (≈35% RH) airflow. Concentration of the stimulus on the line feeding the mask is continuously monitored by a photo-ionization detector (PID).

Insert Figure 5 about here

c) Environmental chambers

Use of whole-body environmental chambers to explore human chemosensory responses provides a close approximation to a "natural" setting. In static and dynamic olfactometry, two crucial issues to control include the actual concentration of the stimulus (typically measured via detectors used in gas chromatography such as PID or FID, flame ionization detector) and the nosepiece/nose interface. A loose interface between the nostrils and the stimulus exit, whether under a static approach (e.g., squeeze bottles) or a dynamic approach (e.g., Dravnieks olfactometer) probably results in a dilution of the effective stimulus. Perhaps different sniffing styles among subjects also contribute to variability. Investigation of the "typical" characteristics of human sniffing provide some interesting values: the "average" human sniff draws a volume of 200 ml, lasts a minimum of 0.4 sec and reaches an instantaneous flow rate of 30 l/min [70, 84, 85]. These studies also concluded that: 1) individuals vary in their sniffing techniques but are

consistent with their patterns across odorants and tasks, 2) most of the odor information is obtained in the first sniff, and 3) natural sniffing provides optimum odor perception.

Many of the above mentioned characteristics cannot be easily achieved by static or dynamic olfactometry, thus the appeal of using environmental chambers. Nevertheless, in a room-size exposure chamber, build-up, control, and rapid change of stimulus concentration become complex and problematic as the large surfaces in the chamber (including the bodies and clothing of subjects) adsorb and desorb airborne chemicals. For these reasons, even when whole-body exposures constitute the gold standard, the pace of testing under this approach is much slower. This highlights the importance of understanding the rules of interconvertibility among sensory results obtained with the different approaches and, given the enormous number of odorants and irritants, the need to develop robust quantitative structure-activity relationships for prediction of chemosensory responses. Examples of these relationships have been provided above under item "4) Physicochemical determinants of odor and nasal pungency".

Chamber studies have been particularly useful when applied to the understanding of issues of indoor air quality and associated topics. Since exposures in chambers can last for hours, they possess a clear advantage over other strategies when studying the effect of time of stimulation on chemosensory perception. Studies performed in environmental chambers have explored, among others, sensory responses to environmental tobacco smoke [76, 86-89], body odor [90], volatile organic compounds [91-96], fragrance materials in air fresheners [97], and formaldehyde (a substance off-gassing from certain home-insulation materials) [98].

6) Instruments for chemical sensing: Gas chromatography-Olfactometry

Gas chromatography (GC), one of the most widely used techniques in analytical chemistry, was first formalized in 1952 [99]. As described in a couple of recent reviews [100, 101], shortly thereafter researchers interested in odors and aromas took advantage of this powerful

separation technique to identify the principal odorants of specific products (e.g., foods, beverages, fragrances, perfumes) [102]. This particular application of GC is now known as gas chromatography-olfactometry (GC-O). In brief, the method uses GC to separate the individual components of a mixture (e.g., a food product) which, then, are presented, as they elute, to a subject (called sniffer) for sensory detection and/or characterization.

Soon researchers found that direct sniffing from the GC effluent (at the exit of a non-destructive detector) had important drawbacks. Among them, the hot and dry gases dried the nasal mucosa, producing serious discomfort, and the odorous background emitted by hot plastic components interfered with the detection of the eluting odorants [100]. This prompted the design of substantial improvements in the system that eventually led to present day GC-O. An important step along the way was the addition of humidified air to the GC effluent, resulting in the delivery of a pulsed wave of odorant, similar to that eluting from the GC, but minimizing nasal dehydration and discomfort for the human sniffer [103]. Further improvements included a venturi system that eliminated background odors, was able to handle narrow-bore GC columns with minimum loss of resolution, and provided additional comfort to the subject [104].

As the techniques of GC and GC-MS (GC-mass spectroscopy) became widespread and more sophisticated, it was possible to separate and chemically identify the dozens or hundreds of individual substances present in food, flavor, and fragrance products. It has been argued [105] that this knowledge created the illusion that the flavor chemistry of these products was well understood. These powerful analytical techniques by themselves have no way of identifying and weighting which compounds are contributing significantly to flavor, and to what extent. Thus, the crucial importance of the GC-O approach that incorporates human sensory detection. In fact, there are indications that the performance of GC-O rivals and can, even, outperform the most sensitive and selective chemico-analytical methods like GC-MS-MS, particularly towards the most powerful odorants [106]. In addition, GC-O requires comparatively little sample preparation and no need for synthesis of labeled compounds. The usefulness of GC-O continues to grow and

expand as it combines with the latest analytical tools such as solid phase microextraction (SPME) [107, 108].

Many GC-O systems are designed to split the GC effluent, sending part to a chemical detector and part to the sniffing port. Typically, humans are more sensitive than most chemical detectors so it is common that less than 10% of the effluent is directed to the sniffing port while more than 90% is directed to the detectors [109]. However, the use of non-destructive detectors (e.g., thermal conductivity detector, TCD) allows to send all the GC effluent to the sniffing port maximizing sensitivity [101].

We have discussed issues that deal with the optimization of GC effluents for chemosensory evaluation by human subjects. There are also issues that deal with the overall strategy for presenting the stimulus (typically a complex mixture of odorants and non-odorants) to the subjects and, very importantly, the procedure used to gather and quantify sensory information from the subjects [109]. The application field were many of these methods were developed and investigated relates to food and flavor research. At present, there are at least three techniques commonly used in the study of the sensory properties of the chemical components of foods and flavors by GC-O. These are "charm analysis", aroma extract dilution analysis (AEDA), and "osme" (from the Greek word meaning smell). We will briefly describe each of these methods.

a) <u>Charm Analysis</u>

This dilution technique was introduced in the middle 80's [105]. On each run, the subject is exposed to the GC effluents from one of a series of increasing dilutions of the particular stimulus investigated (typically, a complex mixture of chemicals). The participant strikes a key from a computer keyboard each time an odor begins to be detected and, again, when the odor is no longer detectable. During this interval, the subject is also required to report, for example with another key stroke, the quality of the perceived odor. The procedure renders a record of the time

on the GC run where the odor occurred, its duration, and its quality. As the authors point out, a crucial part of the method calls for the use of chromatographic standards (e.g., n-paraffins) to transform the retention times at which odors appear into retention indexes, thus associating the sensory response with a reproducible chemical property. A run as just described is made for each of the successive serial dilutions until no odor is detected.

The responses are summarized as the "charm" value "c" that is a simple function of the dilution factor "d" and the number of coincident responses "n". The term "coincident responses" refers to the number of times that an odor is detected across successive dilutions for a particular retention index. In this way, the relationship is expressed as: $c = d^{n-1}$. A charm response chromatogram is defined as a plot of c vs. retention index. Figure 6 illustrates how the charm plot is obtained. Results obtained by charm analysis compare well with those obtained by using traditional psychophysical procedures such as line-length (a visual analogue scale) and finger-span [110].

Insert Figure 6 about here

Charm analysis has been applied to study, among other products, apples [111], grapes [112, 113], orange juices [114] and the off-flavors form plastic packaging of food products [115].

b) <u>Aroma Extract Dilution Analysis</u> (AEDA)

AEDA is another dilution technique [116]. As in charm analysis, an extract from the product of interest is diluted in series and each dilution is analyzed by GC-O. In AEDA, results are expressed as flavor dilution (FD) factors. This factor is simply the ratio of the concentration of the odorant in the initial extract to its concentration at the highest dilution at which an odor is detected by GC-O [117, 118].

AEDA chromatograms plot the flavor dilution factor vs. retention index. Graphs obtained by charm analysis and by AEDA of the same flavor product are very similar [101] only that charm analysis produces areas for each relevant retention index (see Figure 3) whereas AEDA produces heights, that is, a single number on the y-axis (equal to the FD) for each relevant retention index. In this way, AEDA focuses on the highest dilution at which a compound is detected whereas charm analysis also takes into account the time for which the odor is perceived [110].

AEDA has also been applied to the study of numerous food products, including olive oil, butter, Swiss cheese, meat, bread, beer, green tea, dill herb, and off-flavors [118], and wines [119].

c) Osme method

The word "osme" given to this method [120] derives from Greek and means smell (we have mentioned above the terms "anosmia", lack of sense of smell, and "normosmia", normal sense of smell). In contrast to the two techniques just described, osme measures perceived odor intensity and is not based on dilutions to odor detection thresholds. The subject uses a time-intensity tracking procedure to rate the intensity of each eluting odorant from the GC and, at the same time, provides verbal descriptions of the odor-active regions of the chromatogram [121]. Similar to charm analysis and AEDA, retention times for the odor peaks are converted into standardized retention indices to confirm the chemical identity of the odorants. In some cases, further confirmation is achieved by GC-MS [121].

Variations on the specific procedure of time-intensity odor tracking, for example a PC mouse moved on a 60-cm scale vs. a rheostat apparatus that measured finger span, were shown to make no significant difference on the odor peaks obtained [110]. Osme has been applied to the analysis of wines [121] and hop oils and beers [122].

Acknowledgments

References

[1] B. G. Green, J. R. Mason, and M. R. Kare in *Chemical Senses. Vol. 2: Irritation* (Ed.: B. G. Green, J. R. Mason, M. R. Kare), Marcel Dekker, Inc., New York, **1990**, v-vii.

[2] B. G. Green, and H. T. Lawless in *Smell and Taste in Health and Disease* (Ed.: T. V. Getchell, R. L. Doty, L. M. Bartoshuk, J. B. Snow Jr.), Raven Press, New York, **1991**, 235-253.

[3] G. H. Parker. *J. Acad. Nat. Sci. Phila.* **1912**, 15, 221-234.

[4] C. A. Keele. *Arch. Int. Pharmacodyn. Ther.* **1962**, 139, 547-557.

[5] J. E. Cometto-Muñiz in *Indoor Air Quality Handbook* (Ed.: J. D. Spengler, J. Samet, J. F. McCarthy), McGraw-Hill, New York, **2001**, 20.21-20.21.

[6] J. E. Cometto-Muñiz, W. S. Cain, and M. H. Abraham. *Exp. Brain Res.* **1998**, 118, 180-188.

[7] F. A. Fazzalari, eds. *Compilation of odor and taste threshold value data*. Baltimore:American Society for Testing and Materials; 1978.

[8] American Industrial Hygiene Association. *Odor thresholds for chemicals with established occupational health standards*. American Industrial Hygiene Association. **1989**.

[9] L. J. van Gemert. *Compilations of odour threshold values in air and water*. TNO Nutrition and Food Research Institute. **1999**.

[10] M. Devos, F. Patte, J. Rouault, P. Laffort, and L. J. van Gemert, eds. *Standardized Human Olfactory Thresholds*. Oxford:IRL Press; 1990.

[11] K. J. Rossiter. *Chem. Rev.* **1996**, 96, 3201-3240.

[12] M. Chastrette. *SAR QSAR Environ. Res.* **1997**, 6, 215-254.

[13] M. H. Abraham in *Indoor Air and Human Health. 2nd Edition* (Ed.: R. B. Gammage, B. A. Berven), CRC Lewis Publishers, Boca Raton, **1996**, 67-91.

[14] A. Dravnieks in *Flavor Quality: Objective Measurement (ACS Symposium Series, No. 51)* (Ed.: R. A. Scanlan), American Chemical Society, **1977**, 11-28.

[15] P. Laffort, and F. Patte. *J Chromatogr.* **1987**, 406, 51-74.

[16] P. A. Edwards, and P. C. Jurs. *Chem. Senses* **1989**, 14, 281-291.

[17] L. S. Anker, P. C. Jurs, and P. A. Edwards. *Anal. Chem.* **1990**, 62, 2676-2684.

[18] P. A. Edwards, L. S. Anker, and P. C. Jurs. *Chem. Senses* **1991**, 16, 447-465.

[19] L. M. Egolf, and P. C. Jurs. *Anal. Chem.* **1993**, 65, 3119-3126.

[20] Y. Alarie. *CRC Crit Rev Toxicol* **1973**, 2, 299-366.

[21] G. D. Nielsen. *CRC Crit. Rev. Toxicol.* **1991**, 21, 183-208.

[22] J. E. Cometto-Muñiz, and W. S. Cain. *Ann. N. Y. Acad. Sci.* **1992**, 641, 137-151.

[23] J. C. Stevens, W. S. Cain, and R. J. Burke. *Chem. Senses* **1988**, 13, 643-653.

[24] W. S. Cain, and J. F. Gent. *J. Exp. Psychol.: Hum. Percep. & Perform.* **1991**, 17, 382-391.

[25] J. E. Cometto-Muñiz, and W. S. Cain. *Physiol. Behav.* **1990**, 48, 719-725.

[26] W. S. Cain, J. E. Cometto-Muñiz, and R. A. de Wijk in *Science of Olfaction* (Ed.: M. J. Serby, K. L. Chobor), Springer-Verlag, New York, **1992**, 279-308.

[27] W. S. Cain. *Ear Nose Throat J.* **1989**, 68, 316-328.

[28] J. E. Cometto-Muñiz, and W. S. Cain in *Smell and Taste in Health and Disease* (Ed.: T. V. Getchell, R. L. Doty, L. M. Bartoshuk, J. B. Snow Jr.), Raven Press, New York, **1991**, 765-785.

[29] J. E. Cometto-Muñiz, and W. S. Cain. *Pharmacol. Biochem. Behav.* **1991**, 39, 983-989.

[30] J. E. Cometto-Muñiz, and W. S. Cain. *Arch. Environ. Health* **1993**, 48, 309-314.

[31] J. E. Cometto-Muñiz, and W. S. Cain. *Am. Ind. Hyg. Assoc. J.* **1994**, 55, 811-817.

[32] N. P. Franks, and W. R. Lieb. *Nature* **1985**, 316, 349-351.

[33] N. P. Franks, and W. R. Lieb. *Environ. Health Perspect.* **1990**, 87, 199-205.

[34] J. E. Cometto-Muñiz, W. S. Cain, M. H. Abraham, and J. M. R. Gola. *J. Appl. Toxicol. (submitted)* **2001**,

[35] J. E. Cometto-Muñiz, W. S. Cain, M. H. Abraham, and J. M. R. Gola. *Physiol. Behav.* **1999**, 67, 269-276.

[36] J. E. Cometto-Muñiz, W. S. Cain, M. H. Abraham, and J. M. R. Gola. *(in preparation)* **2001**,

[37] D. G. Guadagni, R. G. Buttery, S. Okano, and H. K. Burr. *Nature* **1963**, 200, 1288-1289.

[38] M. Q. Patterson, J. C. Stevens, W. S. Cain, and J. E. Cometto-Muñiz. *Chem. Senses* **1993**, 18, 723-734.

[39] J. E. Cometto-Muñiz, W. S. Cain, and H. K. Hudnell. *Percept. Psychophys.* **1997**, 59, 665-674.

[40] A. A. Rosen, J. B. Peter, and F. M. Middleton. *J. Water Pollut. Control Fed.* **1962**, 35, 7-14.

[41] R. A. Baker. *J. Water Pollut. Control Fed.* **1963**, 35, 728-741.

[42] M. Laska, and R. Hudson. *Chem. Senses* **1991**, 16, 651-662.

[43] B. Berglund, and M. J. Olsson. *Percept. Psychophys.* **1993**, 53, 475-482.

[44] W. S. Cain, F. T. Schiet, M. J. Olsson, and R. A. de Wijk. *Chem. Senses* **1995**, 20, 625-637.

[45] K.-O. Schnabel, H.-D. Belitz, and C. von Ranson. *Z Lebensm Unters Forsch* **1988**, 187, 215-223.

[46] M. Chastrette, and J. Y. d. Saint Laumer. *Eur. J. Med. Chem.* **1991**, 26, 829-833.

[47] G. M. Dyson. *Chem. Ind.* **1938**, 57, 647-651.

[48] R. H. Wright. *J. Theor. Biol.* **1977**, 64, 473-502.

[49] L. Turin. *Chem. Senses* **1996**, 21, 773-791.

[50] K. M. Hau, and D. W. Connelll. *Indoor Air* **1998**, 8, 23-33.

[51] N. M. Shvets, and A. S. Dimoglo. *Nahrung* **1998**, 42, 364-370.

[52] J. Muller, and G. Greff. *Food Chem. Toxicol.* **1984**, 22, 661-664.

[53] D. W. Roberts. *Chem. Biol. Interactions* **1986**, 57, 325-345.

[54] G. D. Nielsen, E. S. Thomsen, and Y. Alarie. *Acta Pharmacol. Nord.* **1990**, 1, 31-44.

[55] G. D. Nielsen, L. F. Hansen, and Y. Alarie in *Chemical, microbiological, health and comfort aspects of indoor air quality — State of the art in SBS* (Ed.: H. Knöppel, P. Wolkoff), Kluwer Academic Publishers, Dordrecht, **1992**, 99-114.

[56] K. M. Hau, D. W. Connell, and B. J. Richardson. *Toxicol. Sci.* **1999**, 47, 93-98.

[57] M. H. Abraham. *Pure Appl. Chem.* **1993**, 65, 2503-2512.

[58] M. H. Abraham. *Chem. Soc. Rev.* **1993**, 22, 73-83.

[59] M. H. Abraham, G. S. Whiting, Y. Alarie, J. J. Morris, P. J. Taylor, R. M. Doherty, R. W. Taft, and G. D. Nielsen. *Quant. Struct.-Act. Relat.* **1990**, 9, 6-10.

[60] M. H. Abraham, G. D. Nielsen, and Y. Alarie. *J. Pharm. Sci.* **1994**, 83, 680-688.

[61] M. H. Abraham, J. M. R. Gola, J. E. Cometto-Muñiz, and W. S. Cain. *Chem. Senses (for submission)* **2001**,

[62] M. H. Abraham, J. Andonian-Haftvan, J. E. Cometto-Muñiz, and W. S. Cain. *Fundam. Appl. Toxicol.* **1996**, 31, 71-76.

[63] M. H. Abraham, R. Kumarsingh, J. E. Cometto-Muñiz, and W. S. Cain. *Arch. Toxicol.* **1998**, 72, 227-232.

[64] M. H. Abraham, R. Kumarsingh, J. E. Cometto-Muñiz, W. S. Cain, M. Rosés, E. Bosch, and M. L. Díaz. *J. Chem. Soc. Perkin Trans. 2* **1998**, 2405-2411.

[65] J. E. Cometto-Muñiz, W. S. Cain, M. H. Abraham, and R. Kumarsingh. *Pharmacol. Biochem. Behav.* **1998**, 60, 765-770.

[66] J. E. Amoore, and B. G. Ollman. *Rhinology* **1983**, 21, 49-54.

[67] J. E. Cometto-Muñiz, W. S. Cain, T. Hiraishi, M. H. Abraham, and J. M. R. Jola. *Chem. Senses* **2000**, 25, 285-291.

[68] C. A. Elsberg, and I. Levy. *Bull. Neurol. Inst. N. Y.* **1935**, 4, 5-19.

[69] B. M. Wenzel. *Psychol. Bull.* **1948**, 45, 231-247.

[70] D. G. Laing. *Perception* **1983**, 12, 99-117.

[71] A. Dravnieks in *Methods in Olfactory Research* (Ed.: D. G. Moulton, A. Turk, J. W. J. Johnston), Academic Press, New York, **1975**, 1-58.

[72] H. R. Moskowitz, A. Dravnieks, W. S. Cain, and A. Turk. *Chem. Senses Flavor* **1974**, 1, 235-237.

[73] E. ASTM, *Recommended practice for odor suprathreshold intensity referencing*, Am. Soc. Test. Materials, Philadelphia: **1975**.

[74] A. Dravnieks, and W. H. Prokop. *J. Air Pollut. Control Assoc.* **1975**, 25, 28-35.

[75] A. Dravnieks, W. H. Prokop, and W. R. Boehme. *J. Air Pollut. Control Assoc.* **1978**, 28, 1124-1130.

[76] W. S. Cain, and B. P. Leaderer. *Environ. Int.* **1982**, 8, 505-514.

[77] G. Kobal. *Pain* **1985**, 22, 151-163.

[78] T. Hummel, M. Knecht, and G. Kobal. *Brain Res.* **1996**, 717, 160-164.

[79] G. Kobal, and C. Hummel. *Electroenceph. clin. Neurophysiol.* **1988**, 71, 241-250.

[80] J. C. Walker, D. B. Kurtz, F. M. Shore, M. W. Ogden, and J. H. I. Reynolds. *Chem. Senses* **1990**, 15, 165-177.

[81] D. Warren, J. C. Walker, A. F. Drake, and R. Lutz. *Physiol. Behav.* **1992**, 51, 425-430.

[82] D. Warren, J. C. Walker, A. F. Drake, and R. Lutz. *Laryngoscope* **1994**, 104, 623-626.

[83] M. Kendal-Reed, J. C. Walker, W. T. Morgan, M. LaMacchio, and R. W. Lutz. *Chem. Senses* **1998**, 23, 71-82.

[84] D. G. Laing. *Perception* **1982**, 11, 221-230.

[85] D. G. Laing. *Physiol. Behav.* **1985**, 34, 569-574.

[86] W. S. Cain, B. P. Leaderer, R. Isseroff, L. G. Berglund, R. J. Huey, E. D. Lipsitt, and D. Perlman. *Atmos. Environ.* **1983**, 17, 1183-1197.

[87] W. S. Cain, T. Tosun, L.-C. See, and B. Leaderer. *Atmos. Environ.* **1987**, 21, 347-353.

[88] G. H. Clausen, P. O. Fanger, W. S. Cain, and B. P. Leaderer in *Indoor Air. Volume 3. Sensory and Hyperreactivity Reactions to Sick Buildings* (Ed.: B. Berglund, T. Lindvall, J. Sundell), Swedish Council for Building Research, Stockholm, **1984**, 437-441.

[89] J. C. Walker, P. R. Nelson, W. S. Cain, M. J. Utell, M. B. Joyce, W. T. Morgan, T. J. Steichen, W. S. Pritchard, and M. W. Stancill. *Indoor Air* **1997**, 7, 173-188.

[90] G. H. Clausen, P. O. Fanger, W. S. Cain, and B. P. Leaderer. *Environ. Int.* **1986**, 12, 201-205.

[91] H. K. Hudnell, D. A. Otto, D. E. House, and L. Mølhave. *Arch. Environ. Health* **1992**, 47, 31-38.

[92] S. K. Kjærgaard, L. Mølhave, and O. F. Pedersen. *Atmos. Environ.* **1991**, 25A, 1417-1426.

[93] L. Mølhave, B. Bach, and O. F. Pedersen. *Environ. Int.* **1986**, 12, 167-175.

[94] L. Mølhave, J. Grønkjær Jensen, and S. Larsen. *Atmos. Environ.* **1991**, 25A, 1283-1293.

[95] L. Mølhave. *Ann. N. Y. Acad. Sci.* **1992**, 641, 46-55.

[96] D. Otto, L. Mølhave, G. Rose, H. K. Hudnell, and D. House. *Neurotoxicol. Teratol.* **1990**, 12, 649-652.

[97] F. T. Schiet, and W. S. Cain. *Perception* **1990**, 19, 123-132.

[98] W. S. Cain, L. C. See, and T. Tosun in *IAQ'86. Managing Indoor Air for Health and Energy Conservation* (Ed.: American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., Atlanta, Georgia, USA, **1986**, 126-137.

[99] A. T. James, and A. J. P. Martin. *Biochem. J.* **1952**, 50, 679-690.

[100] T. E. Acree. *Anal. Chem. News & Features* **1997**, 170A-175A.

[101] Y.-W. Feng, and T. E. Acree. *Foods Food Ingredients J. Jpn.* **1999**, 179, 57-66.

[102] G. H. Fuller, R. Steltenkamp, and G. A. Tisserand. *Ann. N.Y. Acad. Sci.* **1964**, 116, 711-724.

[103] A. Dravnieks, and A. J. O'Donnell. *J. Agric. Food Chem.* **1971**, 19, 1049-1056.

[104] T. E. Acree, R. M. Butts, R. R. Nelson, and C. Y. Lee. *Anal. Chem.* **1976**, 48, 1821-1822.

[105] T. E. Acree, J. Barnard, and D. G. Cunningham. *Food Chem.* **1984**, 14, 273-286.

[106] P. Pollien, L. B. Fay, M. Baumgartner, and A. Chaintreau. *Anal. Chem.* **1999**, 71, 5391-5397.

[107] K. D. Deibler, T. E. Acree, and E. H. Lavin. *J. Agric. Food Chem.* **1999**, 47, 1616-1618.

[108] R. T. Marsili, and N. Miller. *J. Chromatogr. Sci.* **2000**, 38, 307-314.

[109] T. E. Acree, and J. Barnard in *Trends in Flavour Research* (Ed.: H. Maarse, D. G. van der Heij), Elsevier, Amsterdam, **1994**, 211-220.

[110] H. Guichard, E. Guichard, D. Langlois, S. Issanchou, and N. Abbott. *Z. Lebensm. Unters. Forsch.* **1995**, 201, 344-350.

[111] D. G. Cunningham, T. E. Acree, J. Barnard, R. M. Butts, and P. A. Braell. *Food Chem.* **1986**, 19, 137-147.

[112] P. A. Braell, T. E. Acree, R. M. Butts, and P. G. Zhou in *Biogeneration of Aromas* (Ed.: T. H. Parliment, R. Croteau), American Chemical Society, Washington, DC, **1986**, 75-84.

[113] T. E. Acree, E. H. Lavin, R. Nishida, and S. Watanabe in *Flavour Science and Technology* (Ed.: Y. Bessière, A. F. Thomas), Wiley & Sons, Geneva, **1990**, 49-52.

[114] A. B. Marin, T. E. Acree, J. H. Hotchkiss, and S. Nagy. *J. Agric. Food Chem.* **1992**, 40, 650-654.

[115] A. Bravo, J. H. Hotchkiss, and T. E. Acree. *J. Agric. Food Chem.* **1992**, 40,

[116] F. Ulrich, and W. Grosch. *Z. Lebensm. Unters. Forsch.* **1987**, 184, 277-282.

[117] W. Grosch. *Trends Food Sci. Technol.* **1993**, 4, 68-73.

[118] W. Grosch. *Flavour Fragr. J.* **1994**, 9, 147-158.

[119] Y. Kotseridis, A. Razungles, A. Bertrand, and R. Baumes. *J. Agric. Food Chem.* **2000**, 48, 5383-5388.

[120] M. R. McDaniel, R. Miranda-López, B. T. Watson, N. J. Micheals, and L. M. Libbey in *Flavor and Off-flavors (Proceedings of the 6th International Flavor Conference)* (Ed.: G. Charalambous), Elsevier Science, Amsterdam, **1990**, 23.

[121] R. Miranda-López, L. M. Libbey, B. T. Watson, and M. R. McDaniel. *J. Food Sci.* **1992**, 57, 985-993, 1019.

[122] N. Sanchez, C. L. Lederer, G. Nickerson, L. M. Libbey, and M. R. McDaniel in *Food Science and Human Nutrition* (Ed.: G. Charalambous), Elsevier Science, Amsterdam, **1992**,

Figure Legends

Figure 1. Thresholds for odor (empty squares) and nasal pungency (filled squares) along homologous chemical series of alcohols, acetate esters, ketones, alkylbenzenes, aliphatic aldehydes, and carboxylic acids. Only primary and unbranched homologs are joined by a line. The segment of dotted lines on nasal pungency shows those homologs for which pungency begins to "cut-off" (see text). Bars (sometimes hidden by the symbol) indicate standard deviation.

Figure 2. Psychometric function for the odor (empty symbols) and nasal pungency (filled symbols) detection of butyl acetate (diamonds), 2-heptanone (circles), toluene (triangles), and 1-butanol (squares).

Figure 3. Left. Olfactory testing of a subject via plastic squeeze bottles and caps with pop-up spouts. Right. Olfactory testing of a subject via glass vessels with Teflon nosepieces.

Figure 4. Top: Drawing illustrating some of the principles in the Dravnieks Binary Dilution Olfactometer (from [71]). Bottom: A perspective drawing of the same olfactometer (from [14]).

Figure 5. Schematic representation of the test station for measurement of sensory responses and breathing parameters. (From [83].)

Figure 6. Example of a "charm" response chromatogram produced from the relationship $c = d^{n-1}$, where d is the dilution constant and n is the number of coincident responses at any given retention index. (From [105].).
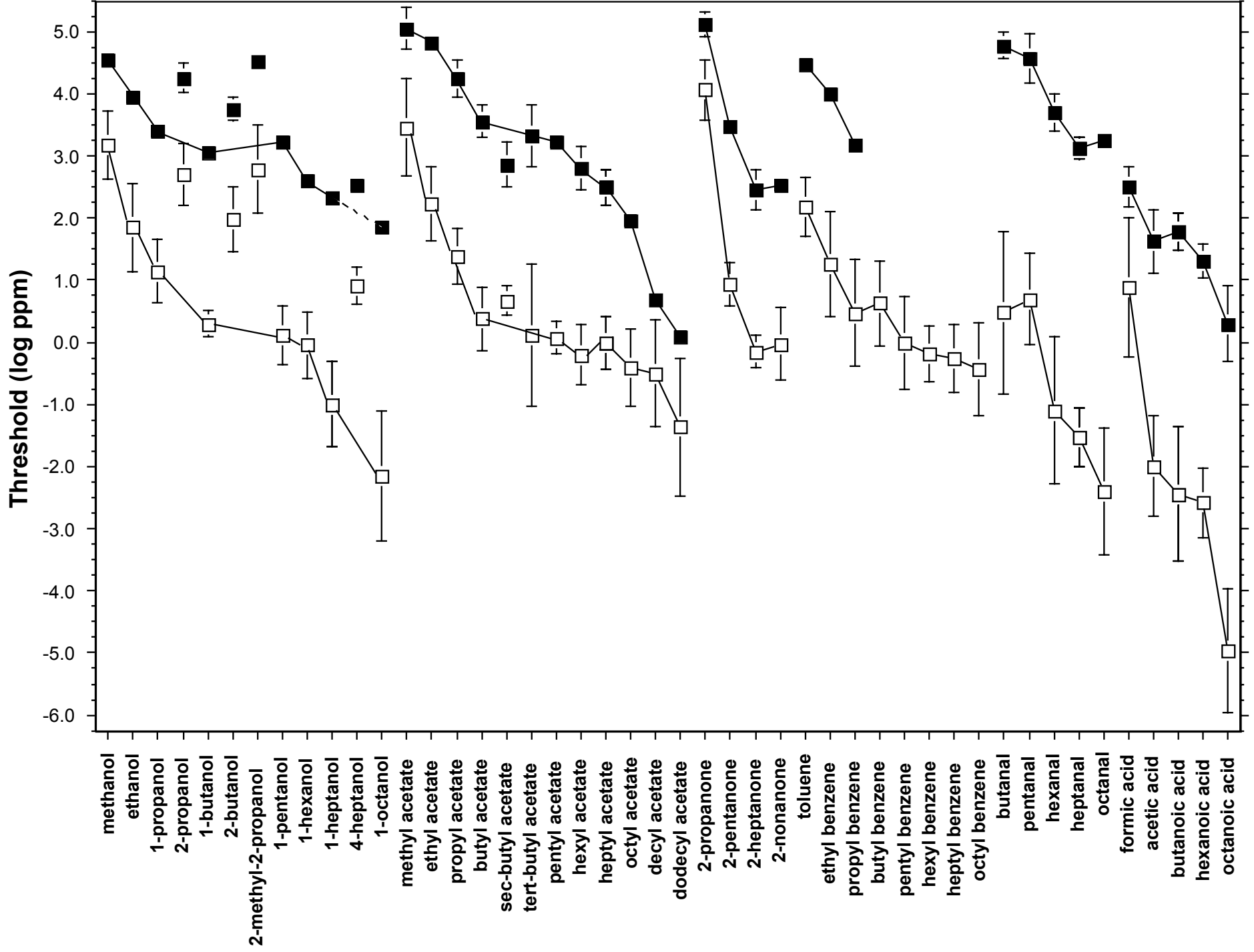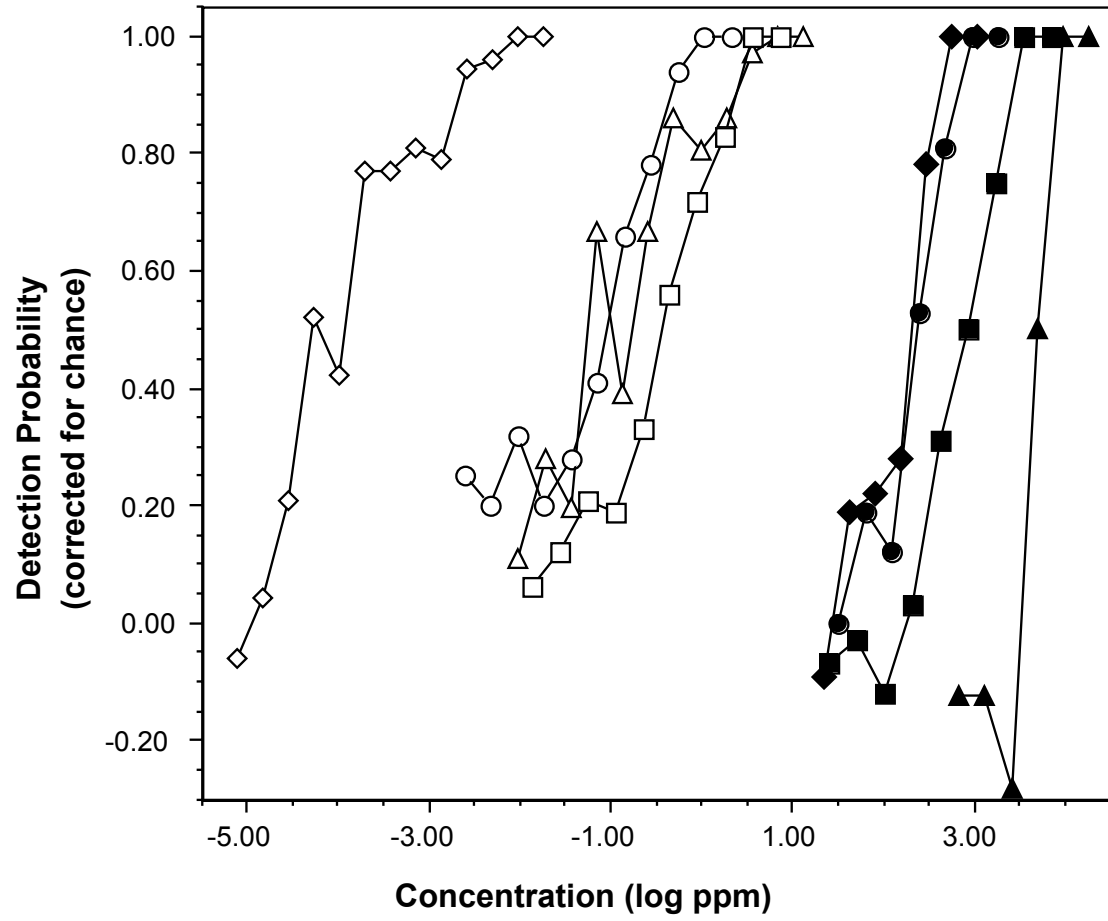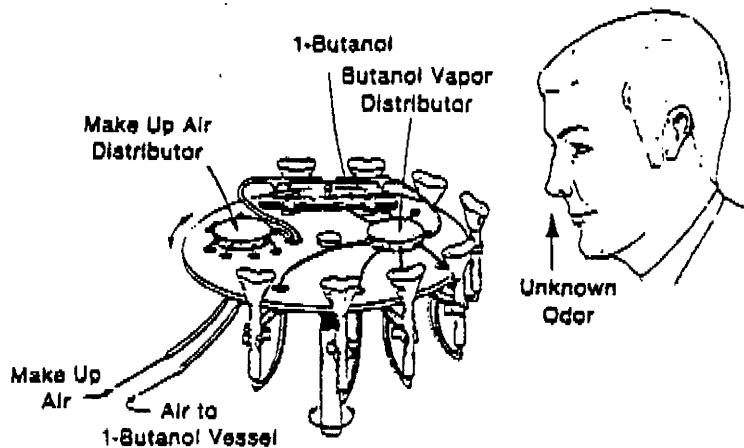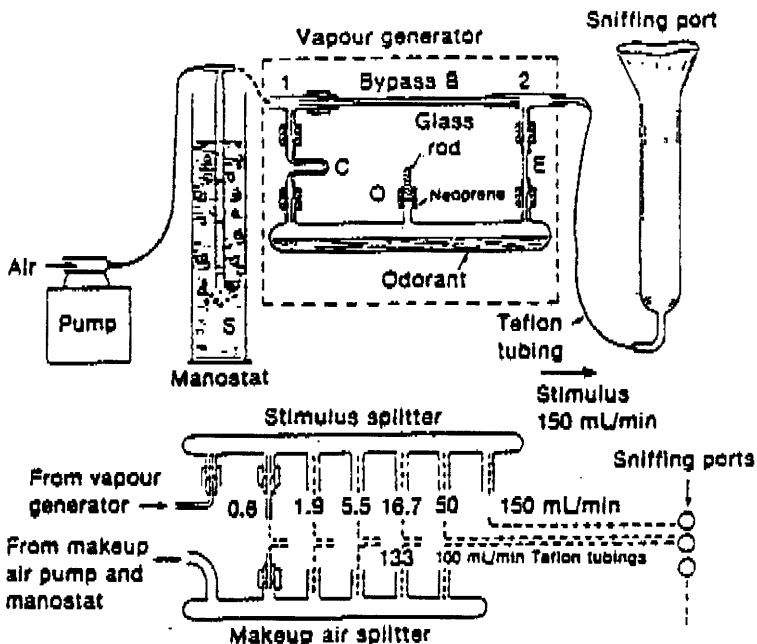
FIGURE 2

FIGURE 3

Vapour generator

Sniffing port

1 Bypass B 2

Glass rod

C Neoprene E

Odorant

Air

Pump

Manostat S

Teflon tubing

Stimulus 150 mL/min

Stimulus splitter

From vapour generator →

0.8 1.9 5.5 16.7 50 150 mL/min

Sniffing ports

From makeup air pump and manostat

133 100 mL/min Teflon tubings

Makeup air splitter

Make Up Air Distributor

1-Butanol

Butanol Vapor Distributor

Make Up Air

Air to 1-Butanol Vessel

Unknown Odor

HEADPHONES (WHITE NOISE)

RATINGS COMPUTER

TRUE EXHAUST

PARTITION

PNEUMO-TACHOGRAPH

FACEMASK SEAL (MONITORED)

FLOW VALVE

CLEAN AIR

HUMIDIFIER

EXHAUST

OLFACTO-METER

P.I.D

CLEAN OR ODORIZED AIR

$$c = d^{n-1}$$