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ASSESSMENT OF UPPER RESPIRATORY TRACT AND OCULAR IRRITATIVE EFFECTS OF VOLATILE CHEMICALS IN HUMANS

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ABSTRACT

Accurate assessment of upper respiratory tract and ocular irritation is critical for identifying and remedying problems related to overexposure to volatile chemicals, as well as for establishing parameters of irritation useful for regulatory purposes. This paper (a) describes the basic anatomy and physiology of the human upper respiratory tract and ocular mucosa, (b) discusses how airborne chemicals induce irritative sensations, and (c) reviews practical means employed for assessing such phenomena, including psychophysical (e.g., threshold and suprathreshold perceptual measures), physiological (e.g., cytology), electrophysiological (e.g., event-related potentials), and imaging (e.g., magnetic resonance imaging) techniques. Although traditionally animal models have been used as the first step in assessing such irritation, they are not addressed here since (a) there are numerous reviews available on this topic and (b) many rodents and rabbits are obligate nose breathers whose nasal passages differ considerably from those of humans, potentially limiting generalization of animal-based data to humans. A major goal of this compendium is to inform the reader of procedures for assessing irritation in humans and to provide information of value in the continued interpretation and development of empirical databases upon which future reasoned regulatory health decisions can be made.

I. INTRODUCTION

The manufacture and use of volatile materials can result in exposures sufficient to cause sensory irritation in community and occupational environments. Public awareness of indoor and outdoor air pollution, including malodor arising from waste disposal plants, farms, pulp mills, and various industrial facilities, contributes to such concerns, and highlights the public consciousness of the potential adverse role of environmental chemicals on physical and mental health. Such concerns continue to increase as urban areas become more congested and the population increases. Modern regulatory agencies, in turn, have been hard pressed to set standards for acceptable levels of exposure to volatile agents within workplace or community air without, in many instances, the benefit of adequate empirical-based information regarding the nature of the hazards that are posed or the levels at which the involved chemicals produce adverse health effects. While such standards are obviously necessary, they should be based on empirical data, as they have far-reaching consequences for industrial enterprises and governmental agencies, and for consumers and taxpayers who must bear the burden of the cost of meeting the standards that are mandated.

The identification and remediation of sensory irritancy problems has commanded limited attention in the toxicology literature. Although histological procedures are available for assessing the effects of acute or chronic overexposure to volatile chemicals on the nasal epithelia of rodents and other small mammals (for review see Doty, R.L. & Hastings, L.M. (2001). Neurotoxic exposure and olfactory impairment. In M.L. Bleeker (Ed.), Clinics in Occupational and Environmental Medicine (Neurotoxicology), 2001 1: 547-575), these procedures are relatively difficult to perform and lack standardization. The degree to which concentration-response relationships from animal data are applicable to humans is also questionable, not only

in terms of biosynthetic pathways within the olfactory mucosa,[1] but because, unlike humans, most mice, rats, and rabbits are obligate nose breathers and have more complex nasal passages.[2,3] For example, the ethmoidal turbinates are greater in number and more complex in rodents than in humans, appearing in double, rather than single, rows. These differences are important, as the turbinates, in large measure, determine the pattern and nature of deposition of inhaled chemicals within the nasal passages.[4]

The shortage of reliable data for use by health professionals seeking to set exposure standards or assess chemical hazards is due, in part, to the perception by many that sensory irritation or smell sensations cannot be accurately quantified in humans. This is further complicated by the realization that a number of community complaints of sensory irritation or malodor reflect psychosocial, as well as sensory, factors. However, methods employing human beings do exist that can aid in (i) establishing concentration-response information for use in hazard and risk evaluations; (ii) explaining discrepancies in irritancy and odor databases, (iii) separating true adverse health effects from psychosocial factors, and (iv) providing insight into exposure-related factors that affect irritancy or odor responses.

In this paper we review the basic physiology of how airborne chemicals induce irritative sensations, and provide a state-of-the-art review of common and practical means employed to assess nasal and ocular irritative properties of chemicals in humans. The focus of the paper is mainly on irritative effects of chemicals, although some of the techniques that are described can be used to assess responsivity to odorants with little irritative properties, as well as to airborne particulates. While apparent strengths and weaknesses of various techniques are discussed, we have purposely refrained from providing or recommending specific tests at this time for practical applications, given the diversity of chemicals for which such information needs to be gleaned

and the variability in efficacy that can arise across different applications within various fields, including toxicology. This review does, however, provide a relatively comprehensive account of the practical techniques available to the toxicologist and others to assess irritative effects of chemicals in humans, along with a guide to those that seem most promising for future applications.

Organizationally, this article is divided into the four following sections: first, a section defining how the term irritation is used in this paper; second, a basic review of the anatomy and physiology of the neural systems within the nose, mouth, throat, and ocular regions that are responsive to volatile agents; third, a description of procedures for presenting irritants to subjects for assessment; and fourth, the presentation of techniques to quantitatively assess the irritative effects of airborne chemicals in the upper airways and eyes. The latter section is divided into four general categories of measurement: psychophysical, physiological, imaging (structural and functional), and psychological (e.g., assessment of community responses by questionnaires).

II. WHAT IS CHEMICAL SENSORY IRRITATION?

The word “irritation" can have different meanings. In the present article this word always refers to "*chemical sensory irritation.*" That is, the broad range of physiological responses (including sensory, secretory, respiratory, cellular, and biochemical) produced when airborne chemicals stimulate unspecialized free nerve endings (see Table 1). This is distinct from the chemical stimulation of the specialized olfactory or taste receptor cells. The free nerve endings from exposed mucosae, such as the ocular, nasal, oral, and upper respiratory tract mucosae, are very susceptible to being stimulated by chemicals, given that these sites show high accessibility and permeability.

INSERT TABLE 1 ABOUT HERE

Several terms have been employed that subsume the sensations evoked by chemicals that are typically viewed as irritative. For example, Parker (1912) introduced the concept of the "common chemical sense" to describe general mucosal sensitivity to chemicals.[9,10] More recently, the terms "chemesthesis" and "pungency" have been used to describe sensations evoked by chemicals that are not properly odors or tastes. Chemesthesis encompasses mucosal sensations, including those arising from the dermis,[11,12] whereas pungency refers to nasal and oral chemosensory responses that are mediated principally via the trigeminal nerve (Cranial Nerve V or CN V).[13] Among the pungent sensations are those of stinging, piquancy, burning, tingling, freshness, prickling, irritation, and the like. As these sensations grow in intensity, they all can be ultimately defined as irritative or painful. Note that chemesthesis and pungency include sensations beyond those simply viewed as irritative, the latter of which almost always carries a negative connotation as being unpleasant or unwanted.

In this article, nasal or ocular irritation is defined as localized and often unpleasant or annoying chemosensations such as burning, itching, and stinging, as well as associated physiologic (e.g., secretory) phenomena arising from selected mucosa or surrounding tissues (e.g., eyelid) of the target areas involved. Although, in many instances, its sensory referents are equivalent to those implied in the terms pungency and chemesthesis (e.g., at low stimulus levels the actual sensation being mediated by free nerve endings may be subtle and not articulated as irritating), sensory irritation is the preferred term of this review because of its universal general usage by toxicologists, air pollution researchers, and indoor air scientists and engineers.

III. ANATOMY AND PHYSIOLOGY OF SYSTEMS THAT MEDIATE CHEMICAL IRRITATION AND OTHER CHEMORESPONSES WITHIN THE NOSE, MOUTH, THROAT, AND EYES

Several sensory systems are responsive to airborne chemicals and mediate irritative responses; namely, those of the trigeminal, glossopharyngeal, and vagus nerves (Cranial nerves V, IX, and X, respectively).*[1] Branches of these nerves also innervate the specialized taste buds, which convey largely sweet, sour, bitter, and salty sensations induced by liquid-borne tastants. The sensory experiences elicited by the olfactory nerve or CN I (e.g., chocolate, smoke, strawberry, coffee, and lemon) are qualitatively distinct from the somatosensory sensations elicited by activation of the other nerves, which include such sensations as irritation, coolness, warmth, and sharpness.

Although the nose, naso-pharynx, and larynx are often described as separate sites of origin for respiratory tract reflexes and sensations, the boundaries of sensory innervation between these areas are rather diffuse (see Figure 1).[14] This redundancy or overlap complicates our ability to predict the irritancy of a chemical from the activity or response of a single afferent pathway, as many, if not most, vapor phase stimuli will act upon several sites and on several afferent pathways in the upper respiratory tract. Even though chemical solubility, together with flow rate, determines the dominant patterns of chemical deposition in the upper airways, most inhaled irritant vapors have the potential of contacting multiple sites of mucosal tissue and thereby directly eliciting sensory irritation via the trigeminal, glossopharyngeal or vagal nerves. Reflex responses to inhaled irritants can be elicited indirectly as well. For example, stimulation of the trigeminal fibers in the nose can elicit reflexes from the nasopharynx, while nasal mucus

*It should be noted that, in addition to trigeminal nerve afferents, two other neural or pseudoneural systems are present within the human nose – the nervus terminalis (CN O), a plexus of fine unmyelinated fibers of unknown function, and a rudimentary and presumably vestigial vomeronasal organ. Neither of these is believed to serve a sensory function in humans and they are not further mentioned in this review.[18-21]

that reaches the pharynx or larynx can elicit cough (even in the absence of direct stimulation by irritant vapors).[15,16]

INSERT FIGURE 1 ABOUT HERE

Despite the significant potential for contributions from the glossopharyngeal and vagus nerves to the sensation of upper airway irritation in humans, most of the studies of chemosensitivity of these regions have used animals and have focused on measurement of reflexes, not sensation.[17] Thus, little is known about the integrated sensory responses to irritants from these nerves and their relative sensitivity to volatile irritants, especially when compared with thresholds for trigeminally-mediated sensory irritation. In this section, the nerves that mediate irritative responses are described, following by a brief description of the olfactory nerve.

A. Trigeminal Nerve (CN V)

The trigeminal nerve (CN V) is the largest of the cranial nerves, being comprised of three major branches, as noted in detail below. One or more of these branches innervate the epithelia of the nose, forehead and face, nasal sinuses, oral cavity, teeth, eyelids, cornea, temporomandibular joint, the muscles of mastication, and large sectors of the cranial dura (Figure 2), and mediate physically- or chemically-evoked somatosensory sensations. Such sensations include pain, deep pressure, irritation, coolness, warmth, and sharpness, among others. Irritative sensations are most prominent within the CN V free nerve endings of the mucous membranes.

INSERT FIGURE 2 ABOUT HERE

1. Divisions and Subdivisions of CN V

The three divisions of the trigeminal nerve are the ophthalmic nerve, the maxillary nerve, and the mandibular nerve (Table 2; Figure 2). The ophthalmic division is purely sensory,

whereas the other two divisions contain both sensory and motor fibers. The cell bodies of all three divisions are found within the trigeminal ganglion (also termed the semilunar or Gasserian ganglion).

INSERT TABLE 2 ABOUT HERE

2. Fiber Classes and Receptor Mechanisms of CN V

In general, irritative and other chemesthetic responses arise, as will be noted below, from activation of polymodal nociceptors within free nerve endings.[38] A number of types of fibers have been found within CN V branches. For example, within the rat's infraorbital nerve, both myelinated and unmyelinated axons are present, with myelinated ones ranging from 0.8 to 14.9 μm in diameter and unmyelinated ones ranging from 0.3 to 1.5 μm in diameter.[39] More unmyelinated than myelinated axons are contained within the ethmoidal nerve.[40]

The fine unmyelinated C-fibers that innervate the nasal cavities contain substance P (SP) and, in many cases, associated calcitonin gene-related peptide (CGRP).[41] The unmyelinated C-fibers are most likely responsible for irritative reactions in the nasal and respiratory passages, as well as those from the epithelium in general, although small myelinated A-delta fibers may also be involved.[42,43] Chronic administration of capsaicin, which depletes SP from fine unmyelinated afferents, eliminates or severely reduces trigeminal nerve responses in rats, suggesting that the small unmyelinated and possibly some myelinated fibers subserve trigeminal pain reactions.[44] Polymodal nociceptors within the free nerve endings of axons belonging to C- and A-delta fibers have been proposed as the mediators of irritation.[45]

Although a few non-olfactory nerve fibers have been found that extend to the surface of the nasal epithelium,[43] electron microscopic studies suggest that the vast majority of CN V free nerve endings terminate within the lamina propria. However, a few trigeminal fibers do

terminate within 1 μm of the epithelial surface, just below the tight junctions.[41] For volatile chemicals to stimulate these nerve endings, they must (i) pass into the nasal cavity, (ii) partition into and diffuse through the mucus, and (iii) cross the epithelial membranes and/or intercellular tight junctions. Since many trigeminal stimulants are lipid soluble, such access is not difficult.

Several mechanisms have been proposed to explain how irritative chemicals initiate transduction at the surface of cell membranes,[46] although the nature of these processes is still poorly understood. Compounds that are chemically reactive (for discussion of reactive/nonreactive, see Alarie et al.)[47-50] can produce irritation directly by reacting with a receptor or indirectly by mucosal tissue damage via chemical reaction without the need to interact with any particular receptor.[46] In the latter case, damaged cells would release endogenous chemicals such as ATP, H^+, and bradykinin which, in turn, could act specifically upon ion channels to produce the neural response.[51-53]

Other compounds are likely to act on specific receptors. Eccles (1990), for example, suggests that menthol alters directly the calcium conductance of the trigeminal free nerve ending membranes.[54] As suggested by Jancso and associates,[42,55] it has been shown that a subset of sensory C-fibers expresses a receptor particularly sensitive to capsaicin, the pungent principle in hot peppers, and to structurally-related molecules known as vanilloids.[56] Interestingly, it has also been shown that this receptor can also be activated by noxious heat.[57] Results from recent electrophysiological studies in rats suggest that the irritant nicotine binds to a specific receptor on nasal trigeminal nerve endings.[58] In fact, electrophysiological studies in rats,[59] as well as psychophysical and electrophysiological studies in humans,[60] suggest the existence of a dose-dependent stereoselective activation of the trigeminal sensory system by S(-) and R(+)-nicotine. Stereoselectivity has been similarly noted for other agents. For example, rats studies employing

a decrease in respiratory rate as an index of sensory irritation have observed marked differences in potency between various pinene enantiomers (Kasanen et al., 1998).

That being said, however, the great majority of volatile substances found in indoor and outdoor air are common hydrocarbons with varied chemical functionalities such as alcohols, esters, ketones, carboxylic acids, aldehydes, and the like, including linear and branched, saturated and unsaturated, aliphatic and aromatic molecules.[61,62] Most of these compounds, at high enough concentrations, can trigger trigeminal sensory irritation.[63] Given their wide variety in chemical structure, it could be expected that their trigeminal impact rests heavily on general physicochemical parameters that govern the transfer of the irritant from the vapor phase to the trigeminal biophase where reception takes place. The applicability of a chemical model based on up to five such general physicochemical parameters to describe and predict human thresholds for nasal pungency[64] and for eye irritation[65] are in accord with this expectation, and is described in detail later in the paper. In addition, previous studies have shown the likely existence of a size-restriction for molecules of potential irritants to be able to actually evoke irritation.[66] The stimulus-size restriction manifested itself in the appearance of a "cut-off" point along homologous chemical series whereby members larger than a certain size would fail to evoke trigeminal sensory irritation in the nose or the eyes. This suggests a need to incorporate a size parameter in chemical models, such as the model just mentioned, to better account for the irritative effects of chemicals. For example, in a very recent study, a size-parameter has been found necessary to account for the odor potency of chemicals.[67]

B. Glossopharyngeal Nerve (CN IX)

The glossopharyngeal nerve (CN IX) is named for the main anatomical regions it innervates (glosso -- tongue; pharyngeal -- beginning of the alimentary canal). It possesses

chemosensitive nerve endings within the mucosal lining of the pharynx, except in the anterior portion of the nasopharynx (also termed the epipharynx, a part of the upper respiratory tract behind the soft palate), which is mostly innervated by CN V. This nerve also supplies the taste buds of the posterior tongue (which can respond to some volatile chemicals), and serves both visceral and general sensory functions.

The glossopharyngeal nerve supplies most of the sensory innervation to the naso-pharyngeal area, and both mechanical and chemical irritation of the nasopharyngeal mucosa can elicit the aspiration ("gag") reflex, repeated inspiratory efforts, and associated vagal reflexes.[14,15,68] Despite anecdotal evidence that sensations of pain, rawness, and irritation from the pharyngeal region follow chemical stimulation, there have been only a few studies that have determined thresholds or otherwise quantified irritant sensations in this area in response to vapor stimuli.[69-71]

C. Vagus Nerve (CN X)

Like the glossopharyngeal nerve, the free nerve endings of the vagus nerve also respond to some inhaled vapors. The internal branch of the superior laryngeal nerve supplies sensory fibers to the supraglottic region -- the area encompassing all laryngeal regions above the vocal cords. Various types of nerve endings have been identified in and under the laryngeal epithelium. Most are free nerve endings in the mucosa and submucosa that,[72,73] in animal studies, have been shown to respond to a wide variety of gasses and aerosols (e.g., ammonia, SO_2, cigarette smoke, and CO_2).[74,75] Although little systematic human research on vagal irritation from inhaled vapors has been conducted, recent studies examining oral exposure to liquid ibuprofen demonstrated that pharyngeal irritation can be quantified and that the pharyngeal area appears to be highly sensitive to certain chemical stimuli.[76,77] However,

chemical stimuli can elicit vagal activity through indirect pathways as well: irritation in the nose elicits vagal reflexes in the lower respiratory tract[14,16,78] and is important to understand as it may lead to laryngeal constriction or secretion of mucus in the lower respiratory tract.[79-81]

D. Olfactory Nerve (CN I)

The olfactory nerve is comprised of 6 million or so receptor cells whose cell bodies and dendritic extensions are located within the olfactory neuroepithelium at the roof of the nasal chambers. The axons of these cells extend from the nasal cavity into the brain (Figure 3). While it is generally believed that this nerve does not produce irritative sensations, per se, there is some evidence that stimulation of this nerve may influence irritative responses of other nerves, most notably the trigeminal nerve. Moreover, most irritants produce olfactory sensations. The olfactory neuroepithelium, which contains a number of cell types in addition to the bipolar receptor cells (e.g., basal cells, microvillar cells, sustentantacular or supporting cells), also harbors trigeminal free nerve endings. This epithelium is found within the region of the cribriform plate, as well as on the superior turbinate, superior septum, and sectors of the middle turbinate.[17] It is noteworthy that the olfactory epithelium loses its homogeneity postnatally, and as early as the first few weeks of life metaplastic islands of respiratory-like epithelia begin to appear, presumably as a result of insults from environmental agents such as viruses, bacteria, and toxins.[22] Such islands increase in extent and number throughout life. Surprisingly, the exact size of the olfactory neuroepithelium in humans is still not well established, and there is recent evidence that it may extend further onto the middle turbinate, at least in some individuals, than commonly believed.[23]

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INSERT FIGURE 3 ABOUT HERE

The CN I receptor cells are unique, in that they serve not only as a highly specialized receptor cell, but as the first order neuron, synapsing for the first time within the olfactory bulb that overlies CNS, just above the cribriform plate. The cilia of the olfactory receptor cells project into the overlying mucus, and differ from the cilia of the cells within the respiratory epithelium in being much longer and lacking dynein arms (hence, intrinsic motility). Odorant transport through the mucus to the cilia is aided by "odorant binding proteins." Approximately 1,000 classes of odorant receptors are now believed to exist,[31] reflecting the expression of the largest known vertebrate gene family – a family accounting for ~ 1% of all expressed genes. However, a large proportion of the odorant receptor genes are, in fact, pseudogenes. In general, the olfactory receptors are linked to the stimulatory guanine nucleotide-binding protein G_{olf}.[32] When stimulated, they activate the enzyme adenylate cyclase to produce the second messenger cyclic adenosine monophosphate (cAMP) and subsequent events related to depolarization of the cell membrane and signal propagation.[33] Although a given receptor cell seems to express only one type of receptor derived from a single allele,[34] each cell is electrophysiologically responsive to a wide, but circumscribed, range of stimuli.[35] This implies that a single receptor accepts a range of molecular entities, and odor coding occurs via a complex cross-fiber patterning of responses. The reader is referred elsewhere for more specific details of the anatomy and physiology of the olfactory system (Doty, R.L. (Ed.) *Handbook of Olfaction and Gustation*. 2nd Edition. New York: Marcel Dekker, 2003, 1112 pp.)

IV. PROCEDURES FOR PRESENTING CHEMICAL IRRITANTS FOR ASSESSMENT

A prerequisite for accurately assessing irritative responses to different concentrations of chemicals is having a means for quantitatively measuring and metering the stimuli that are presented. Stimulus generation and presentation procedures vary considerably, ranging from

rather simple squeeze or "sniff" bottles to elaborate devices employing computerized mass flow controllers that allow for generating and presenting mixtures of chemicals in known concentrations and ratios (Figure 4). The stimuli can be presented directly to the nares or eyes, or presented within a chamber or room where "whole body exposure" occurs. In cases where extended exposure is to be made, stimulus presentation can be done in the subjects' homes or offices using atomizers or other similar devices. *Dalton & Wysocki, 1996, The nature and duration of adaptation following long-term odor exposure, Perception & Psychophysics, 58, 781-792.*

INSERT FIGURE 4 ABOUT HERE

Stimulus generation devices for volatile agents are divided by some into two classes: "static," where the stimulus concentration arises from dilutions made in solvents (also termed diluents),[82,83] and "dynamic," where dilutions are derived from active mixing of an airstream containing the irritative substance with a non-odorized carrier airstream.[84] In some cases, the initial concentration of a stimulus is formed through a static dilution process and subsequent dilutions are performed dynamically. Purely static dilution techniques, however, are the most widely used, largely because of their practicality. In most static systems, a dilution series for a substance of interest is prepared in closed containers using a solvent with little or no odor. The containers can vary in volume from a few hundred milliliters to several liters, and the stimulus is either sniffed directly from each container after it is opened or is ejected or puffed from the containers into the nose[83,85,86] or into the eye.[87] Some investigators provide ocular exposure using goggles through which the stimulus flows.[88] A number of solvents have been used to produce the variations in concentration, depending upon the solubility characteristics of the stimuli involved, and include distilled/deionized water, USP grade light mineral oil (paraffin oil), diethyl

phthalate, and purified propylene glycol. The dilution factor is usually logarithmic, with most workers using binary volume dilution steps. The containers employed are usually glass, although some plastics have been employed [e.g., Teflon™, high density polyethylene (HDPE), and polypropylene (PP)]. Many plastics must be "cured" by lengthy preheating or chemical treatment to eliminate their odor or the odor left from molding oils before use, and care must be taken to assure that the plastics do not react with the stimuli to be employed.

In static systems, an equilibrium is ideally established between the liquid and vapor phases, although the time required for complete vapor saturation can be many minutes, depending upon the substance. At equilibrium, the concentration in the headspace (the actual stimulus) is proportional to that in the liquid. This factor varies among chemical stimuli, solvents, and stimulus-solvent pairs, and often deviates from Raoult's Law.[89] In general, the best assurance for an accurate delineation of the concentration of the vapor-phase stimulus is its direct measurement via an analytical instrument such as the gas chromatograph, although quantification of low concentrations may require collection procedures that extend for considerable periods of time. It is important to note that while a given concentration of agent may be presented near the nares or surface of the eye, the actual concentration reaching the epithelia can vary as a function of such idiosyncratic factors as the thickness or composition of the mucus or tear layer, and the shape and size of elements of the nasal chambers (e.g., turbinates), which influence airflow and sorption patterns. Furthermore, the final stimulus becomes diluted to varying degrees with surrounding air. The latter problem is more germane to nasal stimulation, since ocular stimulation is more passive and stimuli can be presented directly to the corneal or epithelial surfaces via goggles or other means. In the case of nasal stimulation, this problem can be mitigated to some degree by placing the orifice of the sniff or squeeze bottle inside of or over the

naris. However, because sniff volumes can be several liters, sniffs that outpace the restoration of saturated stimulus to the vessel become diluted with surrounding air. Thus, numerous empirical studies indicate that the volume of the vessel from which a stimulus is presented can influence, over a given range of volumes, sensitivity to volatiles, with larger vessels being associated with greater sensitivity.[86,90]

Dynamic dilution techniques are generally believed to provide a more accurate stimulus concentration than static procedures, although they require more complex equipment and also depend upon a stimulus airstream that is assumed to be saturated. Hence, the final concentration should be verified analytically. The reader is referred elsewhere for more specific information on dynamic stimulus presentation.[84,86,91-93]

While the output of most static and dynamic systems is directed to the proximity of the nares or ocular areas, in some cases the output is sent more generally into a room or environmental chamber, usually in an effort to mimic real-life exposures. In such situations, the subject's nose, respiratory tract, eye, and uncovered skin are concomitantly exposed to the chemical stimulus. Exposures in such situations can continue for hours while the subject rests comfortably and engages in such activities as reading or playing games, making them more amenable to evaluating the build-up of irritative or other responses; i.e., the "time" factor.[69,70,94-97] However, largely because of stimulus control issues (e.g., purging a stimulus before presenting another), experiments in rooms or environmental chambers cannot proceed at the pace of experiments in which the stimulus is directed more locally into the region of the nose or eyes.

V. QUANTITATIVE TECHNIQUES FOR ASSESSING IRRITATION

A. Psychophysical Measures

The science of psychophysics – the study of the relationship between perceptual responses and physical stimuli -- arose in the mid-19th Century and formed the backbone for much of 20th Century experimental psychology, audiology, and visual science. Today, psychophysical methods are commonly employed to assess chemosensory function in humans in academic, clinical, and industrial settings. As discussed below, psychophysical procedures can be divided into two categories, threshold procedures (where the goal is to find barely discernible stimuli) and suprathreshold procedures (where the employed stimuli are clearly discernible).

1. Threshold Procedures for Assessing Irritative Responses to Chemicals

Generally speaking, the lowest concentration of an irritant that can be discerned by sniffing or by ocular exposure is considered to be the threshold for irritation or, more simply, the irritation threshold. Such a threshold can vary considerably among individuals, and depends not only upon subject factors and the stimuli evaluated, but also upon the specific psychophysical procedure employed for its measurement.

There are numerous paradigms for operationally determining a threshold value (for reviews, see[99,100]). The "classic procedures" were formally developed by Fechner (1860), as outlined in his treatise, *Elemente der Psychophysik.*[98] More modern techniques employ fewer trials and forced-chice responses. Those that have received the most use in recent years are the ascending method of limits procedure (AML) and the single staircase procedure (SS). In the AML procedure, chemicals are presented sequentially from low to high concentrations and the point of transition between detection and no detection is estimated. In the SS method, the concentration of the stimulus is increased following trials on which a subject fails to detect the stimulus, and decreased following trials where correct detection occurs. An average of a number

of the up-down transitions ("reversals") is used to estimate the threshold value. The SS procedure is typically more reliable than the single series AML procedure, since a more thorough sampling of the perithreshold region is made.[99] On the other hand, the SS procedure is much more time consuming and for extremely irritating substances can be trying on the subject. In both the AML and SS procedures, the direction of initial stimulus presentation is made from weak to strong in an effort to reduce potential adaptation effects of prior stimulation. In most cases, a blank is paired with the stimulus at each stimulus concentration level, and the blank and the stimulus are successively presented in a counterbalanced order. The subject is required to report which one seems strongest, the first or the second. This "forced-choice" procedure produces a more stable threshold value than one obtained by simply asking a subject whether something is perceived or not, as it controls to a large degree subject response biases (e.g., liberalism or conservatism in reporting the presence or absence of a sensation in an uncertain situation). The reader is referred elsewhere for more detailed information about forced-choice testing.[99,101]

As a general rule, most volatile chemicals that are capable of eliciting irritative sensations (e.g., via the trigeminal nerve) can also elicit an odor (via CN I), and, furthermore, the odor is evoked at concentrations one or more orders of magnitude below those that evoke irritation.[63,102,103] Thus, when one wishes to establish the lowest concentration of a vapor that can be detected via non-CN I afferents, confusion can arise since the stimulus is already discernible by odor. This is problematic when one wishes to use forced-choice responses against a blank, since the stimulus, whether producing irritative sensations or not, will be apparent to the subject via its odor. To avoid this problem, and still allow for the use of forced-choice procedures, three strategies (see below) for assessing irritation thresholds have been devised: (a) to test subjects

lacking a functional sense of smell (i.e., anosmics) (Doty, 1975; Doty et al., 1978), (b) to test for ocular irritation (which is equivalent in sensitivity to nasal irritation for most volatiles; see Cometto-Muniz and Cain, 1995, 1998; Cometto-Muniz, Cain Abraham and Kumarsingh, 1998), and (c) to test for nasal localization or lateralization (von Skramlik, E. (1925) Uber die Lokalisation der Empfindungen bei den niederen Sinnen. *Zeitschrift fur Sinnesphysiologie* 56:69-140; Kobal G., Van Toller S. Hummel T. Is there directional smelling? Experientia. 45:130-2, 1989; *Wysocki, C.J., Dalton, P., Brody, M.J., & Lawley, H.J. (1997) Acetone odor and irritation thresholds obtained from acetone-exposed factory workers and from control (occupationally unexposed) subjects, American Industrial Hygiene Assoication Journal, 58, 704-712. Dalton, P., Dilks, D. & Banton, MI (2000) Evaluation of odor and sensory irritation thresholds for methyl isobutyl ketone in humans. American Industrial Hyginene Association Journal, 61, 340-50*; Cometto-Muniz and Cain, 1998). The latter strategy can be employed because, as noted below, irritative, but not olfactory, sensations can be localized to one or the other side of the nose.

a. Nasal Irritation Thresholds Determined in Anosmic Subjects

Anosmics detect many volatile chemicals intranasally via CN V.[104-107] Although anosmia can be due to a number of causes, cognitively normal individuals whose clinically-verified anosmia is due to head trauma or to the congenital lack of olfactory bulbs or tracts, are preferred for studies of intranasal CN V function since the anosmia is typically complete and permanent. Because anosmics cannot perceive any odor background, they can be tested for nasal detection of chemicals using forced-choice procedures employing blanks. Thus, nasal detection thresholds in

anosmics may represent relatively unbiased CN V thresholds that are independent of olfactory input.[2]

INSERT FIGURE 4 ABOUT HERE

b. Eye Irritation Thresholds

As noted in Section IIIA, the ocular mucosa, as well as the nasal mucosa, is innervated by CN V. Trigeminal chemosensitivity in the eyes can easily be measured in both normosmics (without olfactory interference) and anosmics. Numerous studies employing homologous n-alcohols, n-2-ketones, and alkylbenzenes, selected terpenes, butyl acetate, and toluene have reported intranasal and ocular irritation thresholds to be of equivalent magnitude, and for stimulus-response functions within the perithreshold region to be essentially equivalent for most volatiles, *see Cometto-Muñiz and Cain, 1995, 1998*).[108,109,111-114] Importantly, such studies have found that eye irritation thresholds do not meaningfully differ between anosmic and normal subjects, further validating the use of anosmics in establishing CN V-mediated irritation thresholds. Figure 5 illustrates the comparable irritation sensitivity shown by the ocular and nasal mucosae towards various vapor compounds.

INSERT FIGURE 5 ABOUT HERE

[2] Some normosmics reportedly detect chemesthetic sensations at concentrations below those that elicit such sensations in anosmics.[85,93] Whether this is due to physiologically different trigeminal sensitivities or a confusion between CN V- and CN I-mediated sensations is controversial. An investigation of irritation-induced reflex changes in respiration in mice found no influence of anosmia on trigeminal sensitivity to nasal irritation, and a number of human studies have noted equivalent nasal and ocular irritation thresholds in anosmic and normosmic subjects.[66,108,109] Nonetheless, one electrophysiological study found a marginally larger peak-to-peak amplitude in the early P1N1 response to CO_2 (a CN V stimulant with little or no odor) in normosmics than in hyposmics and anosmics.[110] The selection of anosmics for such studies may be critical. For example, viruses that induce anosmia conceivably produce, at least in some individuals, subtle changes in CN V function. In such anosmics, the decreased CN V function may have no physiological connection with the decreased CN I function, as such, emphasizing the need to choose subjects with congenital or head trauma-based anosmia.

c. Nasal Localization (i.e., Lateralization) Thresholds

It is well established that, when blank air is presented to one side of the nose and an irritating chemical to the other, most persons can readily identify the side of the presentation of the irritating chemical. Odorants that have no irritating or other somatosensory effects cannot be so localized,[115-117] contrary to what had been reported by von Bèkesy (1964), who employed odorants at concentrations that most likely had CN V activity.[118] This localization phenomenon provides the opportunity to test directly the chemosensitivity of the nasal trigeminal system in normosmics irrespective of the presence of background odorous sensations. Under this paradigm, two streams of air are directed into the nose, each entering one of the nostrils. One of these streams contains the chemical of interest, and the other not. The task of the subject is to decide which nostril experienced the stronger sensation, not to determine whether something was present or not; in other words, to localize the side of stimulus presentation.

Similar lateralization thresholds have not been performed for the eye, although theoretically establishing such thresholds would seem possible. Hempel-Jørgensen and colleagues (1999) have demonstrated that humans can distinguish which eye is most irritated when different concentrations of an irritating agent are presented separately to each eye simultaneously.[88] Moreover, they can match the degree of irritation produced in one eye to a reference concentration of CO_2 presented to the other eye (see section on intensity matching procedures below). Thus, it would seem straight-forward to establish ocular lateralization threshold values.

2. Suprathreshold Procedures for Assessing Irritative Responses to Chemicals

In order to obtain suprathreshold irritation ratings where odor plays no role, the same strategies described above for separating trigeminal from olfactory input at the threshold level need to be considered. As an alternative, normosmics can be instructed to either rate total nasal sensation,[119] or to rate separately the odor and irritative sensations.[93,105,120] This last option can have merit in applied studies where the interest is not to study the functional characteristics of the trigeminal chemosensory system, but to assess the overall adverse sensory impact of a chemical stimulus, including one composed of numerous unknown elements. In theory, psychophysical measurements of chemosensation are guided by the same principles as for any other sensory input.[121,122] Nevertheless, the specific characteristics of a chemical vapor and of a chemosensory system directly tuned to chemicals introduce practical limitations that are absent in such sensory systems as vision or hearing.[123-125]

a. Rating Scales

Since the intensity of an irritant is typically a function of its concentration, ratings or other measures of perceived intensity have been used to evaluate the degree of perceived irritation. Such measures have the advantage of being relatively brief, easy to administer, and less susceptible than threshold tests to subtle stimulus contamination. In chemosensory assessment, two types of rating scales are popular: *category scales*, where the relative amount of irritation is signified by indicating which of a series of discrete categories best describes the magnitude of the sensation, and *line scales* (also termed *visual analog or graphic scales*), where the strength of the sensation is indicated by placing a mark along a line that has descriptors (termed anchors) located at its extremes (e.g., very weak-very strong) and/or midpoint (Figure 4a and 4b). Due to their simplicity and ease of use, such scales are common in practical applications. Numerous studies have employed rating scales in studies of irritation. For example, visual analog scales

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have been used in studies of nasal[126] and ocular irritation to CO_2[127,128] and to other agents (Doty, R.L.: Intranasal trigeminal detection of chemical vapors by humans. Physiology & Behavior 14:492-496, 1975), [129] as well as in studies of ocular irritation to n-butanol, 1-octene, and various irritative mixtures.[88] A simple, unannotated line segment (22 cm) scale was employed in an investigation of the odor intensity of mixed and unmixed stimuli under environmentally realistic conditions.[130] The stimuli were chosen, in part, because of their frequent use in air fresheners. In another study, an annotated 26-cm line served to explore the psychophysical properties of two candidates, pyridine and cis-3-hexen-1-ol, for possible odorization of inert gases in occupational settings.[131] A peculiarity of this scale was that it had a mark placed 5 cm from its zero end (i.e., the no odor end), representing the perceived intensity of a comparison reference stimulus: the odor of a 57 ppm 1-butanol vapor presented via a squeeze bottle.

An example of a hybrid between a category and a visual analog scale is one that was used in studies of the odor and irritation of formaldehyde[69] and of tobacco smoke.[70] In these studies, in which the stimulus was presented within an environmental chamber, the scale had six categories. The upper boundary was labeled "None" and, below at equal intervals, the labels read "Slight," "Moderate," "Strong," "Very Strong," and "Overpowering" (at the lowest boundary). Participants used this scale to rate eye irritation, nose irritation, throat irritation, and odor and did so by marking the line at any point deemed appropriate (including between labels). The measurement of interest was the length, in cm, from the boundary labeled "None" to the place where the mark was made.

Several scales have been developed in which logarithmic elements have been incorporated into their design (Figure 6c and 6d) in an effort to overcome ceiling effects and to more closely mimic ratio-like properties of magnitude estimation, which is discussed in detail

below.[132,133] A currently popular scale is the *labeled magnitude scale* (LMS), which was initially employed to rate oral sensations, such as taste, chemesthesis, and temperature,[134] and was subsequently applied to taste and smell stimuli.[133] The LMS consists of six verbal labels arranged in a roughly logarithmic manner (Figure 6d).

INSERT FIGURE 6 ABOUT HERE

Psychophysical functions produced by the LMS and by magnitude estimation do not differ statistically for a number of chemosensory stimuli, suggesting that the LMS mimics the ratio-like properties of magnitude estimation scaling;[134-137] although it might also mimic the contextual effects that influence magnitude estimation.[138] The LMS has been successfully employed in a number of nasal and oral irritation studies.[5,76,139,140] The reader is referred elsewhere for discussions of the properties of rating scales, including the influence of category number on their psychometric properties.[141-143]

b. Intensity Matching Procedures

Intensity matching procedures have been used to assess how suprathreshold irritation increases as a function of stimulus concentration, with *cross-modal matching procedures* (e.g., *magnitude estimation*) being the most popular. In cross-modal matching, the relative magnitude of each member of a stimulus set is estimated by using some other sensory modality or cognitive domain. A key difference between this procedure and most rating scale procedures is that the ratio relations among the intensities of the different stimuli are sought, and the subject's responses are not confined to categories or a short response line. Continua commonly used in the cross-modal matching task termed magnitude estimation include number (e.g., assigning numbers proportionate to the degree of perceived nasal irritation) and distance (e.g., pulling a tape measure a distance proportional to the degree of such irritation).[122,144] When intensities of

sensations from two or more modalities are judged on a single common scale, the procedure is termed the *method of magnitude matching*.[145] Magnitude estimation and magnitude matching are among the most commonly used cross-modal matching procedures.

In the prototypical magnitude estimation paradigm, the subject assigns numbers relative to the magnitude of the sensations. For example, if the number 20 is used to indicate the intensity of an irritative response from one concentration of a stimulus, a concentration that seems four times as intense would be assigned the number 80. If another concentration is perceived to be half as strong as the initial stimulus, it would be assigned the value 10. The examinee can assign any range of numbers to the stimuli, so long as they reflect the relative magnitudes of the perceived intensities. In some cases, a standard for which a number has been preassigned (often the middle stimulus of the series) is presented to the subject in an effort to make his or her responses more reliable. In other cases, the individual is free to choose any number system he or she wishes, so long as the numbers are made proportional to the magnitude of the attribute (the "free modulus method"). For example, one subject may choose to assign the first stimulus the number 25, whereas another may choose to assign this same stimulus the number 5. If a second stimulus is perceived to be 10 times stronger than the first by each of these individuals, the first one would assign the number 250, whereas the second one would assign the number 50. The important point is that the absolute values of the numbers are not important; only the ratios between them are relevant.

To obtain an index of suprathreshold function, magnitude estimation data are most commonly plotted on log-log coordinates (log magnitude estimates on the ordinate and log odorant concentrations on the abscissa) and the best line of fit determined using linear regression. The resulting function,

$$\log \psi = ß \log \Phi + \log k \qquad (1)$$

where ψ = perceived intensity, log k = the Y intercept, Φ = stimulus concentration, and ß = the slope, can be represented in its exponential form as a power function,

$$\psi = k \Phi^{ß} \qquad (2)$$

where the exponent ß is the slope of the function on the log-log plot.[146] For nasal irritation, ß is typically > 1, whereas for olfaction it varies between 0.2 and 0.8.[144,147,148] Thus, for nasal irritation evoked by carbon dioxide (CO_2), one study found ß to be 1.6 in males and 2.2 in females.[13] CO_2 is one of the few examples of a volatile compound having almost exclusively irritative (CN V) effects with very little, if any, odor impact. For this reason it has been used quite often in nasal trigeminal chemosensory studies,[126,149-152] in addition to being used in ocular stimulation studies.[128,153] Unfortunately, most volatile substances elicit both odor and irritative responses. This can lead to departures from equation (2) as the predominant sensation evoked by the tested compound shifts from olfactory to trigeminal.[119,120,154]

It should be noted that procedural and subject factors can systematically influence or bias magnitude estimation measures, perhaps more so than measures from most other suprathreshold sensory procedures.[123,155] Magnitude estimation is a relatively complex task, in that accurate responses to a stimulus require a good memory for the prior stimulus. If too much time lapses between the presentation of stimuli, the memory of the prior stimulus fades. However, if the trials are spaced too closely together, adaptation can distort the relationship. Not all subjects can consistently provide ratio estimates of stimuli, and many do not understand the concept of producing ratios.[156,157]

The degree to which these and other potential shortcomings hinder the use of magnitude estimation procedures in applied settings is unknown; however, presumably such problems can

be minimized to a large degree by ensuring that the instructions, stimuli, and test procedures are carefully standardized and monitored. Comparative assessments of nine-point rating scales, line scales, magnitude estimation scales, and a hybrid of category and line scales suggest that, for untrained or mathematically unsophisticated subjects, category scales and line scales may be superior to magnitude estimation when such factors as variability, reliability, and ease of use are considered.[158,159] The labeled magnitude scale (LMS) appears to have similarly comparative utilitarian attributes as simple line and category scales.

Because the magnitude estimation function's intercept and distance above the origin depend to a large degree on idiosyncratic differences in the use of numbers and the specific magnitude estimation method employed (e.g., fixed vs. free modulus), only its slope has traditionally been used as an index of sensory function. In an attempt to gain additional information from the function's ordinate position, investigators have employed the *method of cross-modal magnitude matching*, which provides, at least theoretically, information about the perceived intensity of stimuli from the absolute position of the magnitude estimation function and corrects, to some degree, for differences among subjects in number usage (for a detailed discussion of this procedure, see Marks et al., 1988).[160] In the most common application of this method, judgments of the intensity of sensations from two modalities (e.g., loudness and the perceived degree of nasal irritation) are made on a common magnitude estimation scale.[161] Under the assumption that subjects experience stimuli on one of the continua (i.e., loudness) in a similar manner, differences among their loudness ratings would be expected to reflect differences in number usage. The irritation intensity continuum can then be adjusted accordingly. Such normalization allows, theoretically, for a direct comparison of scale values across subjects; thus, if the adjusted nasal irritation magnitude value for one subject is 10 and for another subject is 20

at the same concentration level, the second subject is presumed to experience twice the nasal irritation as the first subject.

A very illustrative way of expressing suprathreshold intensities of chemical sensations is to use a matching procedure employing a scale of "concrete chemical references." In this way, the perceptual scale can be reproduced by other investigators, and over time, retain its intrinsical meaning. A classical example of this type of scale is that provided by Dravnieks (1975) for the expression of odor intensities.[162] In this paradigm, a participant has to match the odor intensity of a presented stimulus to one of eight butanol concentrations, extending from 16 to 2,160 ppm by volume, delivered by an olfactometer. This procedure has been recommended as a standard method for referencing suprathreshold odor intensities.[163]

3. Modeling Perceived Chemical Sensory Irritation

A number of chemical features have been reported to correlate with sensory irritation. They include normal[164] and adjusted[165] boiling point, molecular weight,[105] molecular geometry,[105] saturated vapor pressure,[105,166] Ostwald solubility coefficient,[167] and other partition coefficients.[168] It has been pointed out that many of these Quantitative Structure-Activity Relationships (QSARs) provide limited chemical or mechanistic interpretations.[169] In contrast, a recently described QSAR model, capable of describing and predicting the perceptual irrigative effects of airborne chemicals in humans, does include such interpretations. This model is based on the general solvation equation of Abraham:[170,171]

$$\log SP = c + r \cdot R_2 + s \cdot \pi_2^H + a \cdot \sum\alpha_2^H + b \cdot \sum\beta_2^H + l \cdot \log L^{16} \qquad (3)$$

where SP is the dependent variable and represents some property (e.g., sensory irritation potency) of a series of chemical solutes (e.g., irritants) in a given (bio)phase solvent system (e.g., trigeminal nerve endings). In our case, SP is the reciprocal of the nasal pungency threshold

(1/NPT) or of the eye irritation threshold (1/EIT). The reciprocals are chosen simply because the larger the quantity, the more potent is the substance. This model considers the stimulus (i.e., the irritant) as a solute that is transported from the vapor phase (i.e., the air entering the nose or in contact with the eyes) into a solvent phase (i.e., the nasal mucus or the tear fluid) and is partitioned among a number of biophases, including the one responsible for the biological response (i.e., the free nerve endings of the trigeminal nerve). In other words, the model can best account for the sensory irritation potency of compounds acting maily via "transport" (or "transfer") processes and not those acting mainly via very specific "lock and key" stimulus-receptor interactions where well-defined restrictions in size, shape, and spatial configuration play a crucial role. The five independent variables are: excess molar refraction (R_2), dipolarity/polarizability (π_2^H), overall or effective hydrogen-bond acidity ($\sum\alpha_2^H$), overall or effective hydrogen-bond basicity ($\sum\beta_2^H$), and gas-liquid partition coefficient on hexadecane at 298 ^oK (L^{16}). The term "c" and the coefficient for each independent variable (r, s, a, b, and l) are obtained by multiple regression analysis. These coefficients have chemical and mechanistic meaning since they reflect the complementary properties that the biophase must possess in order to be receptive to the irritant stimulus. In this way, the independent variables (i.e., R_2, π_2^H, $\sum\alpha_2^H$, $\sum\beta_2^H$, and L^{16}) provide a physicochemical characterization of the stimulus (i.e., the irritant), whereas the corresponding coefficients (i.e., r, s, a, b, and l) provide a physicochemical characterization of the receptor area or biophase (e.g., trigeminal free nerve endings) likely to interact with that stimulus.[172] The r-coefficient measures the tendency of the biophase to interact with the irritant via polarizability-type interactions, mostly via π- and n-electron pairs. The s-coefficient reflects the biophase dipolarity/polarizability (since a dipolar irritant will interact with a dipolar biophase, and a polarizabile irritant will interact with a polarizable biophase). The a-

coefficient represents the complementary property to the irritant hydrogen-bond acidity, and, thus, is a measure of the biophase hydrogen-bond basicity (since an acidic irritant will interact with a basic biophase). Similarly, the b-coefficient is a measure of the biophase hydrogen-bond acidity (since a basic irritant will interact with an acid biophase). Finally, the l-coefficient is a measure of the biophase lipophilicity. The specific equation to describe and predict nasal pungency thresholds (NPTs) is:

$$\log (1/\mathrm{NPT}) = -8.519 + 2.154\,\pi_2^H + 3.522\,\sum\alpha_2^H + 1.397\,\sum\beta_2^H + 0.860 \log L^{16} \quad (4)$$

with $n = 43$, $r^2 = 0.955$, $SD = 0.27$, $F = 201$, where n is the number of compounds, r is the correlation coefficient, SD is the standard deviation and F is the F-statistic.[64,169,173] All other letters and symbols are as defined above for the general equestion (3). In the case of NPTs, the term $r \cdot R_2$ from equation (3) did not achieve signifiance and was omitted. In turn, the specific equation to describe and predict eye irritation thresholds (EIT) is:

$$\log (1/\mathrm{EIT}) = -7.918 - 0.482\,R_2 + 1.420\,\pi_2^H + 4.025\,\sum\alpha_2^H + 1.219\,\sum\beta_2^H + 0.853 \log L^{16} \quad (5)$$

with $n = 54$, $r^2 = 0.928$, $SD = 0.36$, and $F = 124$, where all letters and symbols are as defined above.[65,174]

In summary, the success of the general solvation equation (3) to describe and predict nasal and ocular irritation thresholds towards a broad range of nonreactive airborne chemicals suggests that transport processes, as defined above, are key components of the mechanism through which these compounds exert their chemoesthetic effect. For other substances, for example nicorine, the key component of such mechanism seems to rest on binding to a very specific receptor. [58,60]

4. Psychophysical Responses to Chemical Mixtures

The topic of the toxicology of chemical mixtures is drawing considerable attention nowadays, particularly in the field of risk assessment, given that many exposures in environmentally realistic situations involve the simultaneous presence of a number of substances.[175-179] (also Korpe, 1999; Schaper et al., 1995). Although most human studies addressing fundamental issues concerning function and mechanisms for the sensory irritation potential of volatiles have employed exposures to single chemicals, a few studies addressing issues of direct practical significance have employed complex mixtures. In the latter studies, the identity, number, and concentration of many individual components have remained unknown. Such mixtures included, for example, tobacco smoke,[70,71,180,181] body odor,[180,182] carpet emissions,[183] and building products emissions.[184,185] Studies on sensory reactions to indoor air have employed, in some instances, a model mixture of as many as 22 components[94,95,186,187] believed to be representative of indoor exposures.[188-190]

The bulk of the literature on chemosensory detection of chemicals mixtures by humans has focused on olfaction.[191-194] Until the various techniques for separating olfactory from trigeminal input were implemented (see section V. A. 2.a., b., and c.), studies on the detection of sensory irritation from mixtures relied on asking participants to ignore odor and focus on nasal pungency,[120] a procedure that, as discussed under V. A. 1., cannot control optimally for response biases. A study employing anosmics and including measurements of nasal pungency and eye irritation thresholds for mixtures having 3, 6, and 9 components, found various degrees of stimulus agonism that increased with number of components and with the lipophilicity of such components.[195] This work did not include complete detectability (i.e., concentration-response, also called psychometric)[114] functions and, thus, only allowed a restricted interpretation of the results. Later studies with binary mixtures included such functions and found support, as a first

approximation, to the notion of chemosensory agonism, in the sense of additivity between the components of the mixtures presented at perithreshold levels.[112,113] There are indications that the degree of sensory agonism decreases as the detectability of the mixtures approaches high values.[113] It would be a breakthrough to be able to predict the degree of sensory irritation agonism in mixtures based on the physicochemical and structural properties of the components via, for example, a model such as the solvation equation described under V. A. 3. Nevertheless, such possibility awaits the availability of results from additional and more complex mixtures where the components cover a wide range of properties and structures.

5. Factors that influence Psychophysical Measures of Irritation

a. Time of Exposure

Repetitive or chronic exposure to volatile irritants can result in either increases or decreases in perceived sensory irritation, depending upon the stimulus, time course, and nature of the exposure. Increases related to exposure are commonly termed sensitization, although in some cases a gradual increase in the accumulation of the chemical at the target site may explain the enhanced sensitivity or reactivity. Sensitization should not be confused with immunological sensitization, although it is conceivable that, in rare instances, immunological processes might become involved. Decreases in sensation reflect either sensory adaptation, which is often peripheral, or habituation, which can involve more central circuits. In general, habituation is more amenable to modulation from higher-order central nervous system processes, such as arousal or cognitive processes, than is adaptation.[196]

There are numerous examples of apparent sensitization to airborne irritants.[69,70,94] For example, Hudnell and others (1992) found, in a 2.75-hour-long chamber exposure, the intensity of nose, throat, and eye sensory irritation increased as a function of the duration of exposure to

volatile organic chemicals, with the perceived eye irritation being concentration-related.[94] More recently, Hempel-Jørgensen and others examined the time course of sensory eye irritation to n-butanol and 1-octene in 16 subjects, demonstrating consistent 10-fold increases in perceived irritation following 20 to 40 minutes of exposure, which thereafter remained relatively constant.[88] In the case of 1-octene, but not n-butanol, sensitization remained for some time after the removal of the stimulant.

While exposure-induced adaptation can produce dramatic reductions in nasal irritant sensations elicited by a volatile chemical, the time course of such reduction appears to be longer than for a primarily olfactory stimulus.[69] As in the case of olfaction, adaptation can be relatively specific to the compound to which an individual is exposed. For example, repetitive occupational exposure of textile workers to acetone elevated the nasal irritation threshold and and decreased the perceived magnitude of irritancy for acetone. These changes were not observed for butanol.[5,139] Similarly, the isopropanol irritation thresholds of phlebotomists who were regularly exposed to isopropanol in the workplace were elevated, but their irritant thresholds for butanol did not differ from unexposed, naïve controls.[197]

Cross-adaptation, defined as a decrease in sensitivity (or perceived intensity) to one chemical after exposure to a different chemical, has been interpreted, in the case of olfaction, as a measure of the degree to which stimuli share common sensory channels or stimulate overlapping subsets of receptors.[198] Although adaptation to one odor may generalize to a small subset of other chemicals that share structural or perceptual features with the adapting odorant,[199] the degree of cross-adaptation is almost always less than the degree of self-adaptation, providing the stimuli are equated for intensity and duration.[200] No adapting substance has been found to enhance the sensitivity to another substance. In addition, most cross-adaptation relationships are

non-reciprocal (i.e., A influences B more than B influences A). The magnitude of this effect can, in some cases, be mitigated to some degree – although rarely completely – by equating the perceived intensity of the two stimuli.

b. Subject Characteristics

i. Smoking Behavior

A few studies have looked into the issue of whether smoking affects the perception of sensory irritation.[201] Measurement of a transitory reflex apnea induced by the nasal pungency produced by CO_2 has shown that smokers are less sensitive, i.e., present thresholds for the reflex that are 29% higher than nonsmokers.[151] Nasal detection thresholds for CO_2 can be 44% higher in smokers than in nonsmokers.[202] Even immediately after short periods of smoking (6 to 10 min) smokers showed a further 12% decrease in sensitivity to this reflex,[150] indicating that, on top of a chronic reduction in nasal pungency sensitivity, smokers experience an acute desensitization right after smoking a cigarette. A recent study by Millquist and Bende[203] reports that coughing induced by capsaicin is decreased in smokers, in accord with this general notion. Use of the virtually odorless stimulus CO_2 in measurement of the transitory apnea or in a forced-choice detection task has been quite common for assessing nasal irritant sensitivity.[204]

ii. Gender

Again using CO_2 as stimulus and the reflex apnea as the outcome, it has been shown that females are 14 to 30% more sensitive than males to nasal pungency whether evoked unilaterally or bilaterally.[151,152] Nasal detection thresholds for CO_2 have also been found to be lower for females than for males.[205] Further experiments employing a magnitude matching technique (see section V. A. 3. b.) revealed that females produced steeper stimulus-response functions for nasally evoked CO_2 pungency and that they were actually experiencing between 50 and 67%

more nasal pungency from the same range of CO_2 concentrations than their male counterparts.[13] Interestingly, no differences of either kind (steepness of psychophysical function or relative perceived pungency) were seen between genders when CO_2 (i.e., carbonated water) was employed to produce buccal (i.e., oral) pungency; nor has evidence of differential gender sensitivity to ocular irritation from CO_2 been observed,[206] despite a higher prevalence of dry-eye syndrome in females that might predispose them to ocular irritation from volatile irritants.[207]

iii. Age

A few studies have shown a reduction in the perception of nasal irritation from CO_2 with aging.[206,208,209] This reduction revealed itself in the elderly as a strong elevation of the threshold for the reflex apnea (the average threshold for the elderly being 1.65 times that for the young) and as a marked weakening of suprathreshold nasal irritation (on the average any given stimulus seemed between 50 and 60% less intense to the elderly). The latter was clearly reflected in a shift to the right of the concentration response function but with no obvious change in slope. Unexpectedly, CO_2 detection thresholds were not significantly different between young and elderly. However, a study of nasal irritation thresholds from n-butanol, as determined by the nasal localization technique, found a significant decline in both olfactory and nasal irritant sensitivity as a function of age.[210] Also, eye irritation thresholds to CO_2 in the elderly averaged 61% higher than in younger subjects.[206]

iv. Health

Acute or chronic conditions that produce inflammation in the upper airways presumably can alter measures of irritation. However, at present the relationship between atopy and nasal irritant sensitivity is unclear and deserving of further investigation. Thus, some studies report greater reactivity to irritants (CO_2, n-propanol) among patients with seasonal allergic

rhinitis,[205,211] and others note no such phenomenon.[212,213] However, the increasingly common use of nasal steroidal sprays among the atopic population may well mask some of the predicted responses to nasal irritants and thus lead to variability even among the atopic population.[213] Kjaergaard and others[214] have presented evidence from chamber studies that atopic individuals may be more susceptible to the irritant effects of volatile organic compounds at low concentrations. They documented that atopic individuals describe more severe irritation at lower thresholds than do non-atopics.[95] Similarly, Shusterman and colleagues[205] found CO_2 irritation thresholds to be lower in atopic than in non-atopic individuals. Although some work suggests that individuals with building-related nasal complaints have increased nasal reactivity,[215,216] as a group such subjects do not demonstrate decrements in nasal volume.[217] Studies on asthma and odors, some of which presumably have irritative effects, suggest triggering is possible, conceivably involving allergy, irritation, or psychological correlates.[218]

Regarding ocular irritation, Franck suggested that individuals with building-related complaints had, as a group, more rapid tear film break-up time and were more likely to suffer from punctate conjunctivitis.[219, 220] However, it is unclear whether these effects represent a marker of susceptibility, a consequence of exposure, or a mechanism. Tsubota[221] has reviewed the physiology of tear film production and suggests two mechanisms by which underlying susceptibility might increase eye complaints. First, both decreased basal and reflex stimulation lead to dry-eye complaints. Second, decreased Meibomian gland lipid secretion will allow more rapid evaporation of tear fluid in the presence of enlarged exposed ocular surface during computer screen work, conceivably making such individuals more susceptible to irritation from airborne agents.

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v. Response Tendencies

Some psychological measures, such as ratings of intensity, pleasantness, or acceptability, can be influenced by response tendencies or biases of subjects. Such "tendencies" may be characteristic of either a person's individual characteristics or situational aspects. For example, Pennebaker has reviewed the construct of negative affectivity, a common characteristic of individuals with frequent complaints in indoor environments, in judging the nature of the chemical environment (Pennebaker, J. W. (1994). Psychological bases of symptom reporting: perceptual and emotional aspects of chemical sensitivity. Toxicol. Ind. Health 10: 497-511). Such individuals are more likely than others, even with the same perceptual experiences, to respond more negatively or to be more susceptible to group pressure or suggestion regarding the nature of the situation.[222] On the other hand, current discomfort may also affect responses.[223] As noted earlier, forced-choice psychophysical procedures, such as forced-choice threshold determinations, as well as measures derived from signal detection theory, where both false negatives and false positives are taken into account, minimize or eliminate the influences of such response tendencies on the perceptual measure.[123] In the case of rating scales, parallel scales can be administered on topics unrelated to the sensory measure at hand to identify or correct data from individuals who routinely provide extreme responses.[224] For example, the responses on the parallel non-sensory continua can be used as covariates in the statistical analyses. However, such corrective algorithms are less effective, and in some cases ineffective, for individuals whose extreme ratings are situation specific and less global.

It is important to realize that the perceived consequences of an irritant or odorant can bias an individual's intensity, hedonic, or acceptability ratings of that agent. Dalton and colleagues, for example, evaluated whether information about the health consequences of exposure to acetone would alter the ratings of odor and irritation during exposure and/or the frequency of

reported health symptoms following exposure.[225] Ninety subjects were exposed for 20 minutes to 200 ppm of phenyl ethyl alcohol (PEA; a relatively nonirritating rose-like smelling agent) and to 800 ppm acetone, an agent with more irritating properties. Subjects were assigned to three groups: *the positive bias group* was told they would be exposed to natural extracts that were commonly used in aromatherapy and may have beneficial effects on mood and health; *the negative bias group* was told they would be exposed to industrial solvents that purportedly caused health effects and cognitive problems following long-term exposure; and *the neutral bias group* was told they would be exposed to standard odorants commonly used and approved for olfactory research. The positive bias group exhibited, during the 20-minute exposure to acetone, the most adaptation or habituation and the lowest perceived intensity (as measured by repeated assessments using a labeled magnitude scale); following exposure they reported the fewest health symptoms. In contrast, the negative bias group rated the stimuli as more intense and, on average, reported the most overall irritation. Following exposure, they reported significantly more health symptoms than the other groups. Forced-choice detection thresholds to PEA performed before and after the exposure and bias group instructions did not differ significantly????CALL PAM ABOUT THIS????.

B. Physiological Measures

1. Cardiovascular Responses to Irritating Chemicals

While psychophysical procedures have been shown to be reliable and to correlate well with complaints of irritation, a number of investigators have sought to employ procedures not dependent upon verbal report. Since irritants lead to reactions that are mediated through reflex arcs involving the spinal cord and, in some instances, higher central nervous system brain regions, such psychophysiological measures as changes in heart rate and blood pressure have

been employed to assess relative degrees of irritation, particularly in non-human forms. However, many researchers have de-emphasized the cardiovascular system in the measurement of vegetative responses to sensory irritants in humans, although there is a small literature on responses to odorants[324] and a few papers describe the use of techniques to measure centrally-mediated human cardiovascular responses to airborne irritants.[322,323] The lack of interest in cardiovascular measures reflects, in part, the difficulty in interpreting cardiovascular responses, which can be relatively unspecific. Transient changes in heart rate and blood pressure are a common reflexive response to many kinds of novel environmental stimuli in real-life situations. Thus, if an individual in an office building is perceiving eye and nose irritation due to an airborne irritant mixture, a number of factors may result in what could loosely be described as a cardiovascular effect.

That being said, however, there is evidence of direct irritation-related effects on heart rate and blood pressure mediated by the sensory nerves of the cornea and nasal cavity. This may be maximal in the first few minutes of exposure, reflecting a rather general "arousal" phenomenon. However, this "direct" effect is complicated, being influence by such factors as duration and magnitude of exposure, individuals' beliefs about the effects of exposure, social context (e.g., are co-workers similarly affected and how do complaints from individuals affect the social milieu?), as well as somatic effects such as excessive eye blinking, eye tearing and nasal congestion. Inhalation of irritants may, with sufficient exposure time and dose, even result in pulmonary effects such as dyspnea and coughing or even bronchiolar and alveolar symptoms such as bronchospasm. The inhalation of respirable particles, acting as vectors for airborne irritants, further complicates the situation.[321] All of these factors can cause transient or more persistent

changes in cardiopulmonary status, so it is important to be aware that operationalization of the general term 'cardiovascular effect' needs to be precise in order to be interpretable.

There are several types of automatic devices to measure and record heart rate and blood pressure in a relatively unobtrusive fashion. Some are based on the sphygmomanometer; familiar from visits to the doctor's office. However, the periodic cuff inflations which cause arterial compression with consequent transient forearm and hand ischemia (often accompanied by paresthesia) lead to discomfort and may not be suitable for long duration recording of responses to sensory irritants.

The Finapres™ method is a photoplethysmographic technique applied noninvasively to the finger. Blood pressure is recorded beat-to-beat with an arterial pressure waveform that is typically indistinguishable from that recorded with an intra-arterial catheter.[325] This has been used in environmental chamber studies as an unobtrusive system to measure heart rate and blood pressure response to airborne irritants such as environmental tobacco smoke (ETS). Its accuracy is comparable to blood pressure measurement by arterial cannulation, which would be impractically invasive for many scientific experiments.

For the measurement of peripheral vascular changes in response to airborne irritants, the use of Doppler ultrasound equipment would likely be suitable. This involves the use of a small probe that is placed over an artery that is located just beneath the skin surface (for example, the radial artery at the inferior surface of the wrist). This probe then measures blood flow and perfusion of the peripheral vasculature can then be inferred. The literature on this application to sensory irritation is sparse, although this technique has extensive uses in cardiology and it is well recognized as being both safe and non-invasive.[326]

In summary, the paucity of published data on the cardiovascular response to irritants is a serious impediment to evaluating the sensitivity and utility of this measure. Other physiological responses such as breathing patterns,[246] EEG waveforms,[327] startle reflex,[328] and eye blink pattern[302,329] are much more widely used as responses indices to sensory irritation and at least one of these (breathing patterns) may be as sensitive as psychophysical measures, as described in section B. 3. below.

2. Electrophysiological Measures

The degree to which odorants activate CN I, and irritants activate CN V, can be determined electrophysiologically in some individuals. Such a determination can be made at the level of olfactory or respiratory epithelium or in terms of central synchronized changes in the EEG evoked in response to pulsed odorant or irritant stimuli.

a. Negative mucosal potentials (NMPs)

Peripheral local electrophysiological potentials to odorants and irritants can be determined within the nasal olfactory and respiratory mucosae, respectively, using surface macroelectrodes. Such potentials constitute, in a general sense, negative mucosal potentials (NMPs), although this term is used typically to refer to potentials derived only from the nasal respiratory epithelium. In general, such potentials correlate with the intensity of perceived irritation (Hummel, T., Kraetsch, H. G., Pauli, E. and Kobal, G. (1998). Responses to nasal irritation obtained from the human nasal mucosa. Rhinology 36: 168-172). When measured from the olfactory epithelium, the recording is commonly termed the electro-olfactogram (EOG). NMPs and EOGs are believed to reflect mainly summated generator potentials from activation of the CN V or CN I afferents, respectively. NMPs in response to irritants or other stimuli that produce pain likely reflect the activation of epithelial nociceptive (pain-transmitting) C-fibers

and/or A delta-fibers. (Hummel, T., Schiessl, C., Wendler, J. and Kobal, G. (1996). Neurosceicne Letters 212: 37-40). EOG responses to odorants reflect mainly the summated potentials from the olfactory receptor cells, correlating with c-AMP related processes (Ottoson, D. (1956). Analysis of the electrical activity of the olfactory epithelium. Acta Physiol. Scand. 35: 1-83; Doty, R.L., Kreiss, D.S. and Frye, R.E. (1990). Human odor intensity perception: correlation with frog epithelial adenylate cyclase activity and transepithelial voltage response. Brain. Res. 527: 130-134).

The NMP has several positive features in the nasal assessment of irritation. First, it is a measurement that is not dependent upon conscious behavior or responses of the subject and is easily quantifiable. Second, it reflects a chemically-induced response to a relatively circumscribed region of the nociceptive system. Third, it tends to correlate with the degree of nasal irritation reported by subjects. However, it also has several drawbacks. First, the placement of the recording electrode must be made by someone with experience with nasal endoscopy, as it is typically placed using endoscopic guidance. Second, activation of epithelial afferents (i.e., proprioceptors), such as pressure or temperature receptors, can evoke this potential or can confound its measurement, requiring care in the process of preparing and applying stimulants to ensure an accurate response. Third, these measurements are not easily determined in many subjects, since placing of the electrode is not always an easy task, anesthetics cannot be used, and the intrusion of foreign matter into the nose often leads to unacceptable pain, sneezing or excessive mucous discharge. Importantly, the degree to which a potential determined in one region of the nasal cavity reflects the responsiveness of other regions of the nasal cavity is debatable, particularly in the case of the EOG, where the underlying mucosa can be quite heterogeneous.

b. Chemosensory Event-Related Potentials (CERPs).

Event-related potentials are changes induced in electrical fields generated by large populations of neurons occurring before, during, or after a sensory or internal psychological event.[330] When chemosensory event-related potentials are recorded from the surface of the skull, their amplitudes are very small (< 50 μV). Thus, to be discerned from overall EEG activity, a number of stimulus-synchronous EEG records of 1-2 sec duration are digitized by computer and transformed into a sequence of numerical values. The averaging of the stimulus-locked array of numbers results in visualized waveforms, which represent responses of the synchronously reacting cortical neurons.

The major technical problem in obtaining CERPs is that steep stimulus onsets are needed to produce synchronous activation of a sufficient number of cortical neurons to result in a measurable potential, thereby requiring the use of sophisticated equipment for stimulus delivery.[99,331] Another problem is that of distinguishing between potentials evoked by activation of CN V and those evoked by activation of CN I. The shapes of evoked potentials elicited by chemical activation of these two nerves are indistinguishable. Fortunately, this is not the case with their onset latencies, which allows this problem to be largely overcome.[332]

A strength of the CERP paradigm is its lack of reliance on subject responses and reasonably reliable indices of CN V or CN I activity. Algorithms based upon assessment of temporal differences across a wide array of recording sites can help to localize the sources of such responses, although generally speaking EEG activity is best known for temporal, rather than spatial, resolution. Thus, unlike NMPs, CERPs are not very specific with regard to the location of factors that alter their magnitude.[333] Changes in CERPs amplitudes or latencies can reflect airway obstruction, or in the case of neural pathways, dysfunction anywhere from the nasal or

receptor mucosa to the primary or secondary sensory cortices. Since stimuli can only be presented every half minute or so (because of adaptation or sensitization problems), large numbers of trials cannot be practically collected in a given individual, in contrast to the case of the thousands of trials that can be obtained in a brief period of time in analogous visual and auditory paradigms.[333,334] Thus, the reliability of the data is suspect in cases where movement and other artifacts require elimination of a significant number of trial records. Since the quality of the late field potentials depends upon the alertness of the subject, uncooperative subjects who do not attend to an associated tasks during the chemosensory task at hand can produce misleading potentials.[335] Despite such shortcomings, however, CERPs have been successfully employed in a number of studies of irritation, and have been particularly of value in assessing the influences of analgesic drugs on mitigating pain responses induced by CN V activation.[336,337]

3. Breathing and Nasal Airflow Measures

a. Measures of Nasal Airflow/Patency/Resistance

A common complaint of individuals exposed to irritating chemicals, including urban pollution, is "nasal stuffiness." In addition to increasing, in some cases, the production of nasal mucus, irritative chemicals induce reflexes, largely via the autonomic nervous system, that can markedly increase the engorgement of the highly vascularized regions within the nose.[226] These vascularized regions, capable of circulating and accommodating a large quantity of blood, allow for the rapid passage of dissolved substances from the mucus and tissues to the blood vessels and vice versa. The nasal artery receives branches from the maxillary, ophthalmic, and facial arteries. Near the surface, these branches give rise to arterioles that end in capillary networks next to the respiratory epithelium or around the coils of glandular tissue. Most capillaries are found in portions of the mucosa that have the greatest exposure to the inspiratory airstream.

Thus, the nasal blood vessels are readily exposed to agents that are absorbed through the respiratory epithelium and fenestrated capillaries.

Despite the fact that one can roughly assess the degree of nasal congestion visually by employing anterior rhinoscopy (the traditional "head mirror" look into the nose opened by a speculum) or by endoscopic rhinoscopy (which employs fiber optic scopes), physiological measures of nasal airflow or resistance provide a more quantitative measure of nasal engorgement than simple clinical ratings of engorgement. Nasal peak inspiratory and expiratory flows have been measured employing *peak flow meters* to assess lung function. Peak nasal expiratory flows can be measured by connecting an oxygen type mask to a peak flow meter.[227,228] The subject takes a deep inspiration and forcibly exhales through the nose with the mask firmly covering the nose. This procedure has not gained much popularity because the procedure is thought to be messy, particularly when significant nasal secretions are present. To remedy this situation, a portable nasal inspiratory peak flow meter (NIPFM) has been developed (In-Check, Clement Clark), that measures maximum nasal *inspiratory* flow in liters per minute. Importantly, both methods have been shown not to be as sensitive as rhinomanometry, to be discussed below. Nonetheless, these procedures employ relatively simple devices that can be used to monitor changes in nasal patency of subjects at their home, at work, and away from the laboratory.[229]

Rhinomanometry is used to measure airflow and pressures during normal breathing. Two commonly used techniques are anterior and posterior rhinomanometry, which differ primarily in where the pressure measurement is made.[230] In *anterior rhinomanometry*, one nostril is occluded with an adhesive patch connected to a pressure transducer to measure nasopharyngeal pressure. Airflow is then measured simultaneously in the other nostril via a mask connected to a pneumotachometer. The measurement of resistance is a calculated value derived from plotting

the curve of airflow and pressure difference between atmospheric pressure and the pressure in the nasopharynx. Total nasal airway resistance, T_{NAR}, is calculated after measuring the left (LR) and right resistances (RR) by the following formula: $T_{NAR} = (LR * RR) / (LR + RR)$. The two nasal chambers can be considered or modeled as two resistors in parallel. In *posterior rhinomanometry*, the pressure sensing tubing is placed in the oral cavity to measure nasopharyngeal pressure. A facemask connected to a pneumotachometer is then used to measure airflow through both nostrils and the total nasal resistance is calculated from plotting the curve of airflow and pressure. This technique requires more training of the subject performing the breathing maneuver, and some individuals seemed unable to perform the test. For this reason, the active, anterior, uninasal rhinomanometric method is more practical,[231] and has been more widely employed in assessing acute and long-term exposure to various airborne irritants.[232-234]

A recent development in the field of rhinomanometry, termed *high resolution rhinomanometry* (HRR), was developed by Vogt and Hoffricter in Germany in 1994.[235] In this form of rhinomanometry, a computerized system divides the nasal breathing into four distinct phases: an inspiratory accelerating (phase 1), an inspiratory decelerating (phase 2), an expiratory accelerating (phase 3), and expiratory decelerating (phase 4). The HRR system measures nasal airflow at inspiratory pressures of -75 and -150 Pascals units, and at expiratory pressures of +75 and +150 Pascals. Normal adult airflow values for right or left nostril range between 300 and 500 cc^3/sec recorded at an intranasal pressure of –150 Pascal units. In resistance units the range is equivalent to 0.5 to 0.3 cm^3 H_2O/L/sec. In order to minimize any distortion of the nasal anatomy an adhesive patch has been developed (Rhino Patch, Rhino Diagnostics, San Diego, CA) to seal the nostrils while measuring intranasal pressure. The coefficient of variation of measurements done close to each other is small, less than 8%.[236] however, due to the nasal cycle and perhaps

other factors, normal resistance values can fluctuate as much as 30% as repeated measurements are performed in an 8-hour period.

While relatively easy to perform, the aforementioned rhinomanometry procedures provide little direct information regarding the specific anatomical or physiological nature of the intranasal factors responsible for influencing the changes in nasal resistance or airflow. In contrast to rhinomanometric procedures, *acoustic rhinometry* purports to assess directly changes in the cross-sectional area of the nasal cavity from the anterior nasal vestibule to the posterior nasopharynx.[237,238] In this procedure, sound waves are introduced into the nasal cavity and the reflected waves are picked up by a microphone and analyzed by a computer to calculate cross-sectional areas at various points within the nose. Specifically, a plot of the changes in cross-sectional area vs. distance from the naris is then generated from which it is possible to ascertain minimum cross-sectional areas and specific volumes. The procedure is brief, requiring little cooperation from the subject or patient, and therefore can be done in children and even babies. Animal studies are also possible using specially designed transmission tubes.

The location of a nasal obstruction can have profound changes on nasal airflow and acoustic rhinometry is, unfortunately, unable to tell us how this important physiologic variable is affected. Obstructions that are located anteriorly can interfere with the accuracy of measured changes analyzed by acoustic rhinometry. At best it can complement the physiologic measurement of airflow by rhinomanometry.[239] Although this method is widely employed due to its simplicity, it should merely be considered a tool to detect inflammation in the mucosa, as the swelling of the mucosa reduces the cross-sectional area and subsequently the volume of the nose.

b. Breathing Pattern Analysis

Irritants activate CN V-mediated reflexes which, in turn, can alter breathing patterns and, in extreme cases, halt inhalation for several seconds. However, only recently has this been quantified accurately in humans,[49] (wrong reference), despite the fact that breathing pattern analysis (BPA) has been employed for many years in animals and forms the basis of a widely used rodent assay for irritation. The rodent assay measures respiratory depression during exposure to irritants using the RD_{50} approach.[240]

The use of breathing patterns as an outcome measure in sensory irritation is attractive for several reasons. First, like rhinometric and rhinomanometric techniques, it is relatively non-invasive, requiring in the simplest case only a thermally sensitive resistor (thermistor) placed close to the anterior nares to permit basic measurements of inspiratory and expiratory flow, as well as breath frequency. Indeed, BPA recording systems can be made to be quite unobtrusive, to the point where the subject is unaware that inhalation and exhalation are being recorded. Second, breathing is the natural means by which volatile chemosensory stimuli are transported to the nasal cavity to activate CN I and CN V afferents. Therefore, breathing accounts for both stimulus delivery and an outcome measure that is readily recorded. Control of breathing is shared between voluntary muscle systems and the autonomic nervous system. Although the subject can consciously alter his or her breathing patterns (e.g., by sniffing, breath-holding, or deliberately changing inhalation/exhalation during the course of an experiment), careful attention to experimental design and the use of unobtrusive measurement devices can substantially reduce or eliminate this type of bias. A third advantage is the simplicity of the measurement. Since breathing is no more complex than a low frequency sinusoidal waveform (rarely exceeding 0.25 Hz while at rest), data acquisition is facilitated by computerized recording that simply generates

amplitude and frequency information on a single channel. Even a basic acquisition system can produce an easily understood waveform, as can be seen in Figure 7.

INSERT FIGURE 7 ABOUT HERE

Data analysis can also be straightforward when used to describe discrete events in the breathing cycle as well as changes in amplitude and frequency. This approach has been used with advantage by a small number of researchers who have documented "inspiratory pauses" (as well as other events) to relatively high, suprathreshold concentrations of irritants, such as carbon dioxide.[150,202,232,241-243] This is analogous to what is seen in the rodent paradigm, as noted above.

Although these basic techniques provide some information on breathing patterns in response to sensory irritants, other methods permit more fine-grain investigation. For example, Walker and his colleagues have combined the precision of dynamic dilution stimulus presentation procedures with the accuracy and unobtrusive operation of breathing measurement by pneumotachograph to refine BPA into a readily applicable technique.[244-246] A pneuomotachograph is a very low resistance in-line sensor that records bidirectional airflow by means of differential pressure measurements.[247] It is accurate and linear in response over the range of inhalatory and exhalatory volume airflow rates typically seen in resting breathing cycles (up to ~600 cm^3/sec). This physiological index of response to airborne irritants has been found to be both sensitive and reliable. In a study using propionic acid, normal subjects showed a clear change in one of the indices of breathing sensitivity (inhalation volume decline) which was paralleled by an increase in the perception of nasal irritation.[246] In contrast, another breathing pattern index (decrease in inhalation duration) is only altered in normal subjects by concentrations of odorant that are sufficiently high to elicit reports of nasal irritation in anosmic subjects.

Dynamic dilution stimulus presentation procedures provide the methodological advantages of rigorous control of stimulus and presentation parameters. For brief exposures up to about 30 seconds, this technique is unrivalled for precision and repeatability of stimulus presentation. However, real world exposures to irritants may be lengthier, with typical durations of tens of minutes or hours. A method is therefore required that approximates environmentally realistic exposure durations (minutes and hours), concentrations (extremely low to moderately detectable), and stimulus complexity (binary to multiple components). Furthermore, it is important to duplicate the "whole body exposures" (eyes, nose, mouth and upper respiratory tract) that are typical of everyday indoor air situations while maintaining a high degree of stimulus control.

Breathing pattern measurements can be recorded for periods of over an hour on multiple testing sessions by means of the Respitrace™ system.[248] This method uses chest and abdominal belts, worn over light clothing, that contain transducers that convert the chest wall movements of inhalation and exhalation into voltages. Once calibrated for each subject before, during and after every testing session, a very accurate measure of normal unimpeded breathing can be made. This can be continuous or intermittent, as desired. The main disadvantage of this method is the need to frequently and scrupulously calibrate the system output with known air volumes to ensure the integrity of the data. The chest and abdominal bands require careful application and monitoring for stability of position; this means that the subjects usually have to be stationary. Subjects that are very obese do not usually produce good data, but persons of normal build and stature tolerate the breathing measurements well.

In summary, BPA is a non-invasive, sensitive and informative method of assessing physiological responses. Although it is currently used by relatively few of the small number of

laboratories that investigate sensory irritation, the simplicity and utility of this method makes it an attractive method that is slowly gaining acceptance as a valid index of human response.

4. Local Physiological and Cytological Measures

Inflammation of the nasal mucosa (rhinitis) and of the conjunctival mucosa (conjunctivitis) due to allergies, infection, or chemically-induced irritation can be assessed in a number of ways in addition to those noted above. These include, in the case of the nasal mucosa, analysis of ciliary beat frequency (which presumably reflects direct compromise to the epithelium), visual inspection of the redness of the eyes or nose (an index of vascular activity), the assessment of local changes in nasal or ocular blood flow using sophisticated equipment, and sampling of secretions or epithelial tissue for detailed cytological or biochemical analysis. Since exogenous stimuli, such as irritants or dusts, can elicit a variety of local vascular reactions in the nasal mucosa, there is considerable interest both from clinicians and researchers in obtaining accurate measures of the local vascular response in the nasal mucosa and using changes in vasoactivity as an objective measure of reactivity to irritants. Changes in vasoactivity may, in fact, precede sneezing, itching, swelling, increases or decreases in nasal secretions, and the subsequent release of inflammatory mediators (such as IgE, leukotrienes, eosinophils).

a. Ciliary Beat Frequency

Ciliated columnar epithelial cells line the tracheobronchial tree, the nose, and paranasal sinuses and their main function is to transport the mucus blanket that covers the entire epithelial surface and discharge it into the oropharynx. The mucus is propelled by the beating of the cilia, which move rapidly forward and slowly backward, with a coordinated beat of approximately 10 Hz. The cilia are quite resistant to most conditions encountered in normal life. The one factor that is most damaging to the ciliated cells is dehydration;[249] however, various chemical agents can also

affect ciliary function, such as the neurotransmitter neuropeptide Y,[250] beta2-adrenergic agents,[251] the preservative benzalkonium chloride,[252] and such irritants as tobacco smoke, sulfur dioxide, and nitrogen dioxide.[253,254]

Bronchial or nasal mucociliary clearance is a complex process involving not only cilia function but also the viscoelastic properties of the mucous blanket. This blanket is composed of two layers. The superficial layer is mucus (gel layer) secreted by the glands and goblet cells. The layer below is a water transudate (sol layer) probably emanating from the fenestrated capillaries below. The blanket absorbs gases and traps particulate matter from the inspired air for their removal and serves as an important host defense function. It is clear then that disease or agents that disrupt this system could be detrimental to pulmonary and nasal functions.

A useful test to objectively measure the interaction between ciliary beating and removal of mucus secretion from the nose is to measure saccharin transport time (STT).[255] A small particle of saccharin is placed on the inferior turbinate, approximately 0.5 cm past its most anterior portion, and the time that it takes for the subject to taste the bitter-sweetness of the saccharin is measured.[234] The method used was developed to minimize any irritation caused by the placement of the saccharin unto the nasal mucosa, which could increase mucus production and cause transport time to vary. Using a similar technique, Mackay and others[256] reported that healthy subjects had STT of 13.5 ± 1.2 (SEM) minutes, while patients with chronic nasal and sinus disease have a much delayed STT of more than 20 minutes. Kleischmidt and Witt used another modification of the saccharin method to study 48 healthy volunteers. Using a saccharin liquid test (SLT), they reported a STT of 10.4 (4.2 SD) minutes.[257] The time and speed of mucociliary transport has been found to be related to the solubility of the tracer, with the

insoluble substance having a faster transport.[258] These investigators reported a mean transport time for saccharin of 17.4 ± 5.5 min in normal persons.

A variety of in vivo and in vitro methods exist to measure the cilia beat frequency. In vitro measurements require that samples be taken from the nasal mucosa, either by scraping the superficial epithelial layer with a curette or with a cytology brush, or by taking a biopsy sample of the inferior turbinate (see next section). The tissue obtained is placed in culture media and observed microscopically right away or cultured to form a monolayer of ciliated epithelial cells, which can be maintained alive for months and studied under a variety of conditions. The beating frequency of the cilia can be measured by high-speed video microscopy. The recorded beating of the cilia is then played back at a speed that allows counting of the beats.[259] A photoelectric device was used by Karnitzki et al. [260] to measure beat frequency in biopsies from the ciliated epithelium and the results compared to those measured by the saccharin test. No correlation was found and the authors concluded that these methods measure two different aspects of the mucociliary system.

In vivo measurements of ciliary beat frequency are more difficult to perform. Lindberg and Runer[261] measured mucociliary activity in the human nose using a photoelectric method. This method has the disadvantage of being sensitive to the movements of the subject and the operator's hand. More recently, Paltieli and others described a method using a laser light scattering instrument that provides real-time in vivo measurement of mucociliary beat frequency unaffected by motion artifacts.[262] Mucociliary beat frequency was measured in 16 normal subjects. The mean ± SEM of 36 measurements was 7.7 ± 0.5 Hz. The instrument described by these investigators may prove to be useful in studying mucociliary function in the human nose.

b. Visual Indices of Irritation

The degree of chemical irritation of the nose or eye can be assessed using straight-forward visual procedures or endpoints that do not require collection of samples of mucus, tears, or cells for analysis. In the case of nasal irritation, the redness of the nasal mucosa can be noted and quantified, and scales of redness have been developed to allow for rough assessment of the degree of inflammatory activity.[263] Although such procedures as reflectance colorimetry are theoretically applicable in such investigations, they have rarely been employed to this end. Ideally, pictures of the epithelium before and after irritant challenge should be employed to enhance the validity of any colorimetric approach.

The redness of the ocular mucosa reflects the filling of conjunctival and scleral arteries and veins. Although correlated with irritation, other factors, such as age, blood pressure (BP), and time of day, can also influence the amount of redness.[153] The time-of-day factor likely reflects, to a large degree, diurnal fluctuations in levels of hormones associated with the inflammatory response (e.g., catecholamines). Although naked eye visualization of the ocular mucosa can be performed, better evaluation and quantification is obtained using the slit-lamp microscope accompanied by photography. The eye (the bulbar conjunctiva) is photographed in a standardized manner while the subject is looking outward and slightly upward. By comparing pictures taken before and after exposure, one can evaluate changes in eye redness objectively and experimental bias can be eliminated by employing double blind designs. This method makes it possible to evaluate even small differences between pre- and post exposure in experiments, or to follow inflammatory changes over time in epidemiological studies. Quantification of eye redness typically employs methods borrowed from microcirculation research, and includes counting the number of visible vessels touching or crossing specific figures, or the employment of computerized techniques to measure absolute redness.[153,206]

Recently changes in the swelling of the surface of the nasal mucosa in response to challenges with allergens or chemicals have been quantitatively assessed using *rhinostereometry*.[264-266] This procedure employs a binocular microscope focused on the tissue of interest. The whole microscope can be fixed in a tri-dimensional position, which can be registered and recorded using three micrometers. The basic idea is to measure changes in the inferior nasal turbinate swelling by the help of the ocular scale, which can detect changes down to 1/10 of a millimeter. To ensure that the subject is not just changing position, the head is typically positioned using a bite board. While this procedure has promise, the angle of the microscope relative to the tissue assessed can vary from subject to subject, and idiosyncratic factors can play a role in adding to the variance of the measurements. As with the case of other measurements of nasal function, the accuracy of this method is dependent upon achieving a baseline where the influences of temperature, humidity, and other factors known to influence nasal engorgement are stabilized. This method has been applied most commonly to detect changes in sensitivity to histamine.[216,267]

c. Indices of Submucosal Blood Flow

More sophisticated means of assessing blood flow to nasal tissues are available than simple observations of mucosal color.[268,269] In animals, radioactive microspheres[270] and vessel cannulation[271] have been used to directly measure nasal blood flow, but the degree of invasiveness makes these techniques less feasible for human studies. Currently, direct measurement of nasal mucosal blood flow (NMBF) in humans can be more easily employed using either the laser-Doppler velocimetry (LDV) or the Xenon washout technique.

i. Laser-Doppler Velocimetry

The method of laser-Doppler velocimetry (LDV) utilizes the properties of laser light to monitor the velocity of moving red blood cells within an illuminated sample of tissue. Calculating the mean Doppler shift multiplied by the fraction of Doppler-shifted light yields an output signal that is related to the flux of cells, which is considered to reflect blood flow in a specific tissue volume. The signal is linearly related to the product of the number of moving cells and their velocity and is unaffected by oxygen content in the tissue. The advantage of this technique over other methods for assessing nasal mucosal blood flow is that it permits continuous noninvasive assessment of local microcirculatory vasoactivity.

LDV can simultaneously measure multiple microcirculatory parameters: the concentration of moving blood cells, the velocity of the flow, and the perfusion or flow, which is the product of concentration of moving blood cells and velocity. The first site where the laser-Doppler technique was used to assess blood flow was in skin; since then the technique has been widely applied in different parts of the body, including the nasal mucosa. In the nose, the inferior turbinate has been the primary choice of recording site, as it generally exhibits higher sensitivity to drugs and other challenges and has a more uniform vascular structure over the surface than does the septum, for example.[272] Use of the Doppler probe in the nose requires some important modifications, however, including a special probe, a stable support system and restraints in order to ensure that measurements assess the same microvascular field.[273] Because spontaneous fluctuations in NMBF or habituation-like decreases of 10-15% can occur, Druce (1984) has recommended that only NMBF changes in excess of 15% be considered reliable.[274]

Although LDV holds considerable promise as an objective technique for measuring local irritant responses in the nose, most studies to date employing LDV have examined changes in NMBF following exposure to pharmacologic agents or histamines.[272,274] The few studies that

have used LDV to measure changes in nasal blood perfusion from exposure to volatile chemicals in humans have found that NMBF can be altered by exposure to irritants (i.e., trigeminal stimulants) but not by pure olfactory stimuli.[275,276] Electrophysiological studies in rats have confirmed that persistent vasodilatation in the nasal mucosa is mediated by stimulation of capsaicin-sensitive trigeminal fibers.[277]

ii. The Xenon Washout Technique

The Xenon (Xe) washout technique for monitoring changes in nasal blood flow involves the injection of a radioactive isotope into the nasal mucosa (typically the anterior part of the inferior turbinate), using a small gauge needle without anesthesia. Results from a direct comparison of Xe washout and LDV indicated that the two techniques measured different components of microcirculatory blood flow: Xe washout appeared to measure blood flow in the deeper vessels while LDV measured the more relevant (for trigeminally-mediated sensory irritation) superficial flow.[278]

d. Measures of Nasal and Ocular Cytology and Biochemical Markers for Irritation

A number of physiological indices of irritation or inflammation are available from sampling the mucosal surface or secretions from the nose and eye. At the simplest level, the amount of nasal or ocular (tear) secretion elicited per unit time can be assessed during a period of chemical challenge relative to baseline secretion rates. More commonly, analysis of the contents of mucus, tears, sloughed cells, or biopsies of affected tissue is made. Because the method of collection can markedly influence the findings, we describe in detail below the most common techniques for obtaining nasal and ocular specimens. Subsequently we describe a number of cytological and biomarkers that have been shown to correlate with response to irritation.

i. Methods for Collecting Secretions or Cells

(a) Nasal Secretions

The *method of blown secretions* consists of blowing nasal secretions onto a collecting funnel, vessel, dish, or sheet of wax paper or plastic film.[279-281] The collected materials can be weighed or, in some paradigms, a sample of the secretions can be transferred from the collection media onto a glass slide, allowed to air dry, followed by staining and analysis of the cells present in the sample. The specimen can also be analyzed for the presence of various biomarkers as noted in detail later in this section. Major drawbacks of this technique are that nasal secretions must be present in sufficient quantities at the time of sampling, and that only cells present in the secretions can be evaluated

The collection of secretions via *nasal lavage* can be performed using several different techniques. The most widely used is that of introducing a bolus of 5 mL of isotonic saline (0.9 %) warmed to 37 ^{o}C or Ringer's lactate in each nostril with a plastic graduated pipette or syringe, while the subject tilts the head back and closes the palate against the posterior pharyngeal wall for ten seconds. The subject then bends the head forwards and expels the lavage fluid into a basin.[282] The fluid collected in the basin is transferred into a graduated test tube and the volume recorded. A portion of the sample can be used to measure the total number of cells collected in the lavage fluid. For the measurement of biomarkers, it is recommended that the specimen be placed on ice or refrigerated immediately until centrifugation at 4^{o}C. The supernatant is divided into aliquots and placed in cryotubes and frozen at –20 to -80 ^{o}C until measurements of the selected biomarkers are made. The cell pellet can be re-suspended in buffer and a small volume placed in a cyto-centrifuge. The prepared slide is then fixed, stained with the desired stain, and

analyzed microscopically. A differential count can be performed and the total number and percentage of each cell type calculated.

Other means of introducing known quantities of lavage fluid have been proposed, such as the use of a compressible plastic container, referred to as a "nasal pool."[283] Wålinder and colleagues[284] used a 20-ml plastic syringe attached to a nose olive to flush the nasal cavity with 5 mL of saline. Other investigators have used a disposable meter-dose inhaler to deliver a known volume of isotonic saline solution into each nostril.[285] A variation of this technique is to use hand pump spray devices to deliver the saline and then placing a soft rubber 8F urethral catheter along the floor of the nasal cavity to suction the secretions with a syringe attached to the catheter, or by having the subject expell the fluid into a dish.[286]

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Nasal lavage has been employed extensively in research to quantify changes in mediator levels, cells, glandular secretions and vascular exudates after challenge procedures. Four or five lavages are performed before the challenge procedure begins, to establish a stable baseline and to clear the nose of secretion. This procedure appears to cause minimal to no irritation. It is difficult however to determine how much of the lavaging solution is being diluted by the unknown amount of secretions present in the nasal cavity. Attempts have been made to introduce markers in the lavage, such as inulin[287] or radiolabeled albumin,[288] to permit calculation of the amount of fluid recovered. These methods are impractical outside of a well established research facility. Repeated lavages potentially have the effect of removing the blanket of mucus covering the epithelial lining and it is not known if this can affect the dynamic flux of fluid across the mucosal barrier. Other drawbacks of this relatively simple technique are that the small concentration of certain mediators and marker are being diluted to the point where they become difficult to measure, even utilizing ultrasensitive assay methods. Additionally, the lavage cannot

provide information as to where the cells or mediators are coming from since the technique samples the whole nasal cavity and nasopharynx.

Various *aspiration or absorption techniques* have been employed in an effort to correct some of the problems associated with nasal lavages, in particular the dilution problem noted above. In 1991 Biewenga and colleagues described an aspiration system in which secretions from the middle meatus and from the floor of both nasal cavities are aspirated into a pre-weighed plastic sampling tube.[289] This was followed by aspiration of 1.0 mL of phosphate buffered saline (PBS) (pH 7.4), containing 10% of a mucolytic agent. Okuda described a technique for the microsuction of nasal secretions using standard capillary glass tubes used to collect capillary blood samples. The capillary tubes are inserted into the nose at different distances along the inferior turbinate to aspirate the secretions.[290] Bernheim and colleagues developed an improved micro-suction method in 1995 to collect nasal secretions atraumatically for cytologic examination.[291] The method has been used to study the kinetics of cell influx into the nasal mucosa after allergen challenge. Aspiration techniques provide very small volumes of secretions and appear to cause some discomfort to the subjects.

Several materials have been used to collect small quantities of undiluted nasal secretions: filter paper discs, absorbent foam rubber samples, cotton, gauze, and more recently cellulose sponge discs. All these methods limit the number of biomarkers that can be assayed. A standardized technique was described by Knowles and colleagues using filter paper.[292] A similar technique has been used by Baroody and colleagues not only to collect small amounts of secretions, but also to perform nasal challenges.[293,294] In order to increase the amount of secretions collected other investigators have used samples of absorbent foam rubber.[295] Pieces of foam rubber, 14 x 14 x 4 mm, were placed with bayonet pincers under visual inspection between

the septum and the head of the inferior turbinate on both sides of the nose, and were left for 10 minutes. The samples are then removed and transferred to centrifuge tube with a sieve. During centrifugation the secretions are squeezed to the bottom of the tube, its volume measured and then transferred to a cryotube for shock freezing in liquid nitrogen and storage at –80 ^{o}C until the specimen is assayed.

Pre-weighed pieces of cotton or gauze have also been used to absorb nasal secretions, however, these methods have not gained much popularity. Recently, discs manufactured from cellulose sponges (Rhino Diagnostics, Inc.,San Diego, CA), but similar in size to the filter paper discs have been used to collect nasal secretions. This combined with the development of centrifugal filter devices by Millipore Corp. for volumes less than 0.5 mL have made it easier to collect secretions and elute biomarkers that may be present in them.

If any of these methods are to be used successfully in the laboratory, one must perform recovery assays on every biomarker being tested. It has been found, for example, that albumin binds to filter paper discs and that only 40-60 percent of known amounts of albumin placed unto the filter paper can be recovered (Jalowayski, unpublished data). This does not occur if cellulose sponges are used. However, Eosinophilic Cationic Protein (ECP) does bind to cellulose sponges (and other plastics), due to its high ionic charge. In order to maximize the amount of ECP that can be eluted from this material, special buffer solutions containing high levels of proteins and detergents are needed.

(b) Nasal Epithelial Cells

Although epithelial cells are found, among other things, in nasal secretions collected by the methods mentioned in the previous section, techniques for harvesting larger numbers of nasal epithelial cells have been devised. Bryan and Bryan[296] described the use of a cotton-tipped

applicator for directly sampling the superficial epithelial layer. Another method of sampling the superficial epithelium is by scraping either with a cytology brush (Cytobrush Plus; Medscand, Malmö, Sweden)[297] or with a plastic curette (Rhino-probe, ASI, Springville, UT).[298] Advantages of these methods include specificity of the sampling site (scraping the surface on the middle third of the inferior turbinate), minimal trauma with no need for anesthesia, ease of repetition, and adequacy of specimen at any age and in all nasal conditions. The Rhino-probe scraping technique when compared to other methods has been found to provide a better quality specimen and a more reproducible method of quantifying the cellular changes in rhinitis.[297,299-301]

(c) Ocular Secretions and Other Measures

Ocular secretions (tears) are produced by the main and the accessory tear glands located in the upper tarsus. A basal rate of tearing is maintained to moisten the eye and to allow the eye lids to function properly. Tearing in response to irritative stimuli (termed "reflexive tearing") serves a protective role to limit the concentration of the offending agent and to clear it from the region to minimize tissue damage. Tear flow rate, blinking, tear film stability, and irritation are all interrelated in a system where blinking induces tear secretion or tear film breakup, which may then induce irritation and blinking.

A number of procedures have been developed for assessing responses of the eye to irritating agents. It should be noted, however, that some of these procedures have inherent problems and, in some cases, may themselves confound the results of subsequent measurements, particularly if recovery periods are absent or short. Thus, a number of factors unrelated to irritation can influence some measures. For example, decreased blinking may lead to desiccation and subsequent irritation unrelated to the exposure. Blinking frequency is also affected by the mental state of the test subject and by other types of environmental disturbances, so it is difficult

to use this measure as a correlate of irritation. With experimental designs that take the psychophysiological nature of eye blink rate into account, this measure is useful as a non-verbal correlate of eye irritation. A number of environmental chamber studies with second-hand smoke support the idea that blink rate is elevated reliably with contaminant concentrations sufficiently high to show a progressive worsening of reported eye irritation with prolonged exposure.[302]

Secretions and tears from the eye can be collected in a number of ways. The classic clinical measurement of tear production is the Schirmer 1 test, where the tear production is established by inserting a small piece of filter paper in the lower tarsus of the eye for a brief period.[303] The tear flow is estimated from the length of the wetted part of the paper after a specific time span, or by weighing the paper. The Schirmer 1 test can be performed with and without topical anesthesia. Anesthesia is commonly employed to reduce the amount of reflex tearing to get a score for the basal tear production. However, as the eyelids are difficult to fully anesthetize, some reflex tearing may still be present. The Schirmer 2 test includes pain stimulation of the nose to activate the naso-lachrymal reflex.[304] Other procedures employ absorption of the tears unto cellulose sponges, or by suction capture into glass capillary tubes fired polished at one end so as to avoid injury to the conjunctival mucosa. Capillary tubes, which can be calibrated with known volumes of solution prior to use, are individually placed in the lower conjunctival sac to vacuum the conjunctival fluids. Proud et al. collected tears by placing cellulose sponges in the inferior fornix of the eye for a period of 1 minute.[305] Biomarkers of allergy, such as histamine, tryptase and ECP were measured following specific allergen challenge of the conjunctival mucosa. Bacon and colleagues used a similar technique to measure tear inflammatory mediators after challenging the eyes with grass or dust mite allergens.[306] Basic

tear flow has been estimated using colorometric methods,[307] as well as radioactive isotopes like Tc^{124}.[308] The colorometric methods include the fluorescein elimination or simple dye dilution.

ii. Common Measures of Nasal Cytology

The nasal cavity may be thought of as having three compartments: a variable space containing mostly air and some secretions and cells, a superficial epithelial layer, and a deeper submucosal layer. Cytologic techniques have been developed over the years to document the dynamic cellular changes that may take place in each of the compartments when the nose is exposed to allergens, infectious organisms or irritants.[296,298,309-311]

After collection, the sampled specimen is typically placed on a microscope slide, fixed, and then stained, and, for example, the number of inflammatory cells is counted in each of ten high power fields. The mean number of neutrophils, eosinophils and basophilic cells per ten high power fields are typically established and reported. Nasal lavages and direct aspirations are centrifuged and the cell pellet is resuspended in a small volume of media. Total cell count is performed with a hemocytometer and the cell differential determined on cytospin slide. For a complete description of the various techniques and documentation of the advantages and disadvantages of each method, the reader is referred to recent review articles on this subject.[312,313]

A quantitative method for determining the number of cells per 10 high power fields (HPF) has been devised to monitor cellular changes before and after nasal challenge.[298] Using this technique, the normal superficial nasal mucosa has been found to have the following cytologic profile per HPF: neutrophils 0-10.5, eosinophils 0-0.45, and basophilic cells 0-0.2. When subjects have neutrophils ranging from 16-20 cells/HPF or greater, and/or eosinophils ranging from 1.1-5.0 or greater, and/or basophilic cells from 0.4-1 or greater, a clinically

significant inflammatory condition usually exists. This information is important when screening subjects to identify asymptomatic nasal mucosal inflammation before a subject can participate in a research study involving the nose.

McKenna and Connell independently reported cytological studies of the nasal mucosa and submucosa from tissue biopsies.[311,314]. The most common site of biopsy is the lower edge of the inferior turbinate. A disadvantage of the biopsy procedure is that it is somewhat traumatic, requiring anesthesia, and also difficult to perform in a serial fashion. The major advantage of the technique is that it allows examination of not only the superficial epithelium, but also the submucosal layer as well as providing the ability to quantify non-neuronal nicotinic cholinergic receptors.[315]

iii. Common Inflammatory Biomarkers

The list of biomarkers that have been assayed to assess mucosal inflammatory changes is long. The selection of which biomarker to use for a particular study must be done having knowledge or at least an educated guess as to the mechanism of action of a particular irritant. For example, if irritation is allergic in nature and IgE mediated, then biomarkers associated with an allergic reaction should be studied, such as influx of inflammatory cells, and release of products from these cells, e.g., histamine, tryptase, PGD2 and ECP, to name a few. If, however, the mechanism is neurogenic in nature, then biomarkers released by nerve endings, such as neuropeptides, should be the ones to be evaluted. When the mechanism of action is unknown, then the objective measures can help to elucidate the etiology of the process in question.

Some specific examples illustrate how the methods for collecting nasal secretions, tears, and conjunctival fluid described earlier have been used to measure biomarkers in human irritation research. Myeloperoxidase (MPO) is an enzyme present in the granules of

polymorphnuclear leukocytes (PMNs). Human exposure to ozone causes an influx of PMNs to the nasal mucosa and the levels of MPO in nasal lavages increase proportionally.[316] ECP is present primarily in the granules of eosinophil leukocytes, which can be attracted and activated at the site of inflammation. The release of ECP can be damaging to epithelial cells and its measurement has been shown to correlate highly with the numbers of eosinophils present in collected secretions.[317] Tryptase is an enzyme present primarily in mast cells and not in basophil leukocytes. In allergic inflammation, stimulation of mast cells release tryptase, histamine and prostaglandin that promote the cardinal signs and symptoms of nasal and eye allergy, such as sneezing, runny nose, nasal congestion, watery and itchy eyes.[318] The levels of these mediators again correlate with the number of mast cells present in secretions or tissue samples from the nose and conjunctiva. More recently cytokines and chemokines have been recognized as playing a significant role in allergy and inflammation. Inteleukin-8, IL-8, is a chemokine which attracts neutrophils to, and activate eosinophils at, a site of inflammation. Soluble intercellular adhesion molecule, sICAM, is a cytokine whose primary function is to facilitate eosinophil and neutrophil infiltration across endothelial and epithelial cells. Both IL-8 and sICAM levels have been shown to be elevated in allergic rhinitis and conjunctivitis.[319,320] Albumin is a non-specific marker that leaks across the mucosal layer during an inflammatory process. The concentration of albumin and other biomarkers can be measured by enzyme-linked immunosorbent assays (ELISA) methods using commercially available kits.[234]

Proud collected tears by placing cellulose sponges in the inferior fornix of the eye for a period of 1 minute.[305] Biomarkers of allergy, such as histamine, tryptase, and ECP were measured following specific allergen challenge of the conjunctival mucosa. Bacon et al. used a

similar technique to measure tear inflammatory mediators after challenging the eyes with grass or dust mite allergens.[306]

C. Imaging Techniques

Major advances in imaging the nasal and sinus cavities, as well as activation of brain structures employing functional imaging techniques, have occurred over the last few years. A clear distinction must be made, in the context of this article, between the use of such techniques in identifying loci or magnitude of mucosal irritation within the sinonasal cavities, and the employment of imaging technology to study functionally central brain pathways activated by irritative chemicals. While few studies have employed the latter category of functional imaging techniques in mapping irritation responses, we would be remiss if we did not briefly mention this rapidly evolving technology and its potential application to the study of irritation in this article.

1. Structural Imaging Techniques

a. Computerized Axial Tomography (CAT)

Computerized axial tomography (CAT; also known as computed tomography or CT), is a useful and cost-effective screening tool for evaluating sinonasal tract inflammatory disorders.[338] CAT employs an x-ray source or sources that move in a circular path around the individual being scanned. The detection system consists of either stationary or movable multiple x-ray detectors. The configuration of the x-ray emitters and detectors allows for a thin slice or planar volume (1 to 10 mm) of tissue to be evaluated for each picture, minimizing blur from superimposition of adjacent tissues. The individual being screened is moved by the same increment as the slice thickness for contiguous images. A computer algorithm is used to reconstruct the radiographic data, increasing the signal to noise ratio via filtering processes.

Since CAT scanning is as sensitive to soft tissue inflammatory responses as to bony

changes, it is well suited for evaluating the sinonasal cavities. The typical CAT scan employed for assessing nasal inflammation or responses to irritation includes all of the nasal cavity, paranasal sinuses, hard palate, anterior skull base, orbits, and nasopharynx. Axial and coronal planes are usually assessed to best view the complex paranasal anatomy.[338]

b. Magnetic Resonance Imaging (MRI)

Structural MRI has the advantage over CAT in not subjecting the subject or patient to ionizing radiation. Its resolution is generally superior to CAT, although it is not always as effective in detecting inflammation due to irritating agents as CAT.[339] MRI takes advantage of the fact that, within the body, the nuclei of atoms with an odd number of protons and/or neutrons have a positive charge. These nuclei spin, resulting in the production of a local magnetic field that makes each nucleus behave like a tiny bar magnet. Biologically significant examples of such elements include ^{1}H, ^{13}C, ^{31}P, ^{23}Na, and ^{39}K, with ^{1}H being perhaps the most important. The numbers and density of such nuclei differ from structure to structure, being nearly absent in air and very numerous in bone, for example. In the absence of a magnetic field stronger than the terrestrial one, orientation of the charged nuclei is essentially random. When an intense magnetic field is applied to them, they orient in the direction of the magnetic field and reach a state of equilibrium. When radiofrequency energy is then transmitted to the protons while they are within the magnetic field, they "flip" their orientation in relation to the magnetic field (i.e., turn 90 or 180 degrees, depending upon the amount of energy transmitted) and are considered to be "excited." When this energy is turned off, they give up all of the energy that they have absorbed, spontaneously returning back to their original orientation within the field, a process termed "relaxation." During relaxation the protons emit energy at the same frequency as the excitation and such energy can be measured by specialized receiver coils or antennae. The

differences in relaxation time among the brain structures are the source of contrast in the structural MRI image. The time required for the protons to orient back to that of the static field is termed the T1 relaxation time, and the time required for the relaxation of the proton's spin from the transverse orientation to the static field is termed the T2 relaxation period. It should be emphasized that T1 and T2 measures, as well as measures of proton density, are tissue dependent and not under the control of the experimenter.

2. Functional Imaging Techniques

During the last several decades, in vivo functional imaging procedures for assessing, in humans, regions of the brain activated by sensory, motor, or even mental activation have been developed and widely employed. The strength of these procedures is that they can provide relatively non-invasive in vivo assessments of brain regions activated by irritative or other sensory agents. Their weaknesses arise from methodological issues that limit their sensitivity in some brain regions, as well as both their spatial and temporal resolution.[340] Nonetheless, a considerable amount of new information about sensory systems has been obtained through their application, and in a few instances such techniques have been employed to assess pain-related brain activity induced by experimental manipulations of the trigeminal system; e.g., by injecting capsaicin subcutaneously in the skin of the forehead[341] or by looking at volatiles that activate both olfactory and trigeminal intranasal afferents.[342]

a. Functional Magnetic Resonance Imaging (fMRI)

Functional magnetic resonance imaging (fMRI) is the most popular means for in vivo functional imaging.[343] Unlike positron emission tomograph or PET (see next section), fMRI is non-invasive, requiring no injection of radioactive or other materials into the circulatory system, and can be performed in most hospital or medical center settings where cyclotrons needed for

PET isotopes are absent and MRI machines are readily available. Importantly, since MRI itself allows for accurate identification of brain structures, there is no need for additional scanning to map activity to identifiable brain regions, as is the case with PET. fMRI has the further advantages of relatively high spatial and temporal resolution.

fMRI is based upon the basic principals of structural MRI described above. The most widely employed fMRI procedure -- brain oxygen level dependent (BOLD) fMRI – capitalizes on the fact that local changes in neural activity induce local changes in the amount of oxygen in tissue (i.e., in the ratio of oxyhemoglobin to deoxyhemoglobin) which can be detected by MRI.[344] Since changing the amount of oxygen carried by hemoglobin influences the degree to which hemoglobin disturbs the magnetic field, T2 relaxation times differ relative to the amount of deoxyhemoglobin in the blood. Hence, a signal is generated that indirectly reflects the amount of neural activity within the more activated brain regions.[345] Other fMRI methodologies employ contrast agents that increase the signal to noise ratio in the target regions.

fMRI does, however, have some technical limitations that should be noted. First, stimulus presentation equipment that contains metal cannot be brought near the MRI magnet, requiring the use of non-ferrous instruments for stimulus presentation. To present odorants or irritants to subjects in this situation, usually plastic lines are employed, with the switching and dilution devices maintained outside the MRI room. Second, a number of brain regions are not, with current technology, able to be assessed with fMRI. For example, structures such as the olfactory bulbs at the skull base, where contrast or susceptibility artifacts are prevalent, cannot be clearly examined in fMRI studies. A discussion of means for overcoming some of these types of artifacts in other brain regions is presented elsewhere.[344]

b. Positron Emission Tomography (PET)

Positron emission tomography (PET) is a sensitive and quantitative indirect measure of brain function that relies upon changes in relative blood flow or uptake of glucose in brain regions that are undergoing increased (or decreased) neural activity.[346] A typical PET study is initiated by injecting into the subject's blood stream water or some other substance (e.g., butanol) tagged with an unstable neutron-deficient radioactive tracer such as ^{15}O, ^{77}Kr, or ^{11}C.[347] The amount of accumulation of the tracer is increased in brain regions with higher blood flow (i.e., those with greater neural activity), as might be induced by exposure to an irritating chemical to the nose. As the unstable tracer decays, it emits positrons that are annihilated by negatively charged electrons within the tissue. This results, in turn, in the emission of two photons per tracer molecule from the brain in exactly opposite directions from the point of annihilation. An array of radiation detectors around the skull, coupled through coincidence circuits, allows for establishing the location of the brain regions emitting the photons, with representation proportionate to the amount of blood flow. Typically an MRI is also made of the subject's brain to allow for mapping of the regions of the most PET activity to actual brain structures or regions. Since, in the case of ^{15}O, which is widely used because it is easy to synthesize, the half-life of the tag is relatively short (~ 2 minutes), the subject's brain can be scanned repeatedly in a single session with minimal radiation exposure consequence. While this procedure allows for a determination of brain regions activated by stimulants, it nonetheless involves invasive injection of radioactive isotopes and complex and expensive equipment, including a cyclotron to produce the isotopes. Importantly, the temporal and spatial resolution of the images are limited. Relative to the neural events, which occur in the time course of milliseconds, PET scans at best require the integration of signals over tens of seconds. Furthermore, even under the most optimal of circumstances, images with no greater than ~ 3

mm^3 of spatial resolution can be obtained. Most commonly, the resolution is, in fact, four to five times this size. Compared to fMRI, PET has the disadvantage of being somewhat invasive, in that infusions of radioactive materials must be made. Furthermore, its image resolution both temporally and spatially is less refined, and the need for additional MRI scanning to allow a template for localization of activated structures more than doubles the time required for measurement in a given subject. Nonetheless, PET has several advantages over fMRI, including more easily obtained whole-brain data and activation of structures at the skull base that are not beset with contrast or susceptibility artifacts.

c. Single Photon Emission Computerized Tomography (SPECT)

SPECT differs from PET in a number of ways. Although both SPECT and PET require radioactive tracer (radionuclide), SPECT scans use radionuclides that emit a single photon with lower energy (about 140 keV) than those employed in PET. A special lens known as a *collimator* is used to acquire image data and this results in much lower spatial resolution than PET, by a factor of 3 or 4. Since SPECT is technically a somewhat simpler imaging method (the radioactive tracers do not need to be generated on-site in a cyclotron), the cost is lower. This advantage is to some extent nullified by relatively long image acquisition periods and prolonged bioactivity of the tracer chemicals, which results in longer test periods and re-test intervals. Consequently, rather few studies have investigated human chemosensation with this method. Nonetheless, work by Di Nardo and others has shown that this semi-quantitative method has value in assessing cortical perfusion in responses to odor (lavender water).[348] Data from normosmics were compared with results from anosmic patients, who showed much lower degrees of perfusion change in response to the odor stimulus. This type of chemoreception study

may well be superseded by fMRI studies, however, which do not require the use of radionuclides and the attendant risks of exposure to ionizing radiation.

D. Psychological and Medical Measures of Chemical Irritation and Annoyance in Working and Living Environments

Airborne irritants often have other adverse health effects, but typically generate irritation in humans at levels below where irreversible somatic damage occurs. Sensory irritation thresholds commonly fall above those of odor thresholds; hence, odor annoyance complaints typically occur at relatively lower vapor concentrations. Irritation is itself, however, considered an adverse effect. In its 1988 Permissible Exposure Limits Project, the Occupational Safety and Health Administration considered irritation an adverse health effect to be regulated. Some standards, such as the formaldehyde standard, require surveillance for overt irritation in workers exposed to this chemical. Most importantly, irritants have been associated with a range of adverse health outcomes, including pulmonary function deficits in children in the absence of atopy,[349] the *de novo* development of allergies,[350] and the triggering of effects in "susceptible populations" at levels different from "normal" populations.[205,206] In addition to direct nasal or ocular irritative effects, many chemicals – including ones accompanied by irritation – have odorous properties. Thus, situations arise in home, workplace, and community settings where release or presence of an odor, even at sub-irritation levels, can lead to somatic complaints in some individuals.[351] The most widespread is that of annoyance. As noted by First:[352]

> Some nontoxic substances are of public-heath concern solely because they have odors that cause annoyance to some members of the exposed population. For susceptible persons, annoyance (vexation, irritation, etc.) may increase to the point of nuisance (harm or injury usually with reference to a continuing or repeated annoyance). In general, the odors in question here are those which can be described as bothersome, unpleasant, offensive, disgusting, noxious, loathsome, or irritating … Annoyance reactions are emotional reactions and involve all major organ systems of the body. Most odorous substances in the

> atmosphere that evoke complaints to air-pollution control agencies belong to this category; they do not produce dire physical symptoms, but a sizable fraction of the exposed population cannot live with them in comfort. …
>
> The effects of annoyance, irritation, and inconvenience "are difficult to measure but [are] nonetheless, real and important. They include sensory perceptions, such as … odors, and irritation of the eyes, nose and throat which are not accompanied by demonstrable organic injury or disease. Such reactions … can be serious nuisances and interfere with performance without causing physical illness or shortening of life. Sensory perceptions and various physiologic responses … can be precisely measured, but their clinical significance is unknown (President's Science Advisory Committee, 1965)."

Odor annoyance is a vexing problem. Although covered by the Clean Air Act, no widely agreed-upon adverse health effects have been defined from odors. Some anecdotal data suggest triggering of asthma attacks in known asthmatics can occur at stimulus concentrations several orders of magnitude below those at which irritation occurs. The phenomenon of multiple chemical sensitivity is considered by some to represent a conditioned response to odors,[353] though this topic goes far beyond the limits of this review. The definitions of "adverse health effect" and physiology are the subject of numerous reviews.[354-356]

There is presently no widely-accepted standardized measuring instrument for assessing the degree of perceived irritation or odor annoyance of airborne chemicals in field situations. Questionnaires, however, have been used in a variety of air quality investigations, as well as in investigations of disorders associated with exposure to airborne chemicals. Such instruments are the most common, and often only, outcome measure in indoor chemical environmental investigations, and have been widely employed in community studies of air quality and in evaluating such medical symptoms as hypersensitivity pneumonitis, rhinitis, or asthma. The latter assessment includes their qualitative manifestations, severity or intensity, frequency, and temporal pattern. Symptom intensity is typically assessed using rating scales, such as those

described earlier in this article. The most widely used questionnaire format is exemplified by the instruments developed by the Environmental Protection Agency (EPA) and the National Institute of Occupational Safety and Health (NIOSH), and in various United Kingdom (UK), Dutch, Swedish, and Danish studies.[357-360] These specific questionnaires seek to define symptoms in a discrete frequency over some defined period of time. For example, one month was used in the EPA Building, Assessment Survey and Evaluation (BASE) and the NIOSH building investigations.[361,362] Other studies have used longer periods, some as long as a year.

The decision to collect questionnaire data requires thoughtful consideration of the setting, the goal for the utilization of the data, and the constraints surrounding their application. In general, self-administered questionnaires generally produce a higher frequency of complaints than interviewer-administered instruments.[363] The selection of items within a questionnaire is crucial to its success and, depending upon the nature of the questionnaire, one may wish to measure more than one construct. Although there are advocates for attempting to increase the power or stability of an individual item by including "double-" or "triple-barreled" questions that ask about two or three similar symptoms at the same time,[364] this approach has drawbacks. First, if the constructs or symptoms are highly correlated, then there would be no gain in sensitivity or validity of the item, since one construct could be substituted for another. Second, the *a priori* assumption that the symptoms or constructs are similar may not be correct, and an empirical determination may be required. If, in fact, the constructs are dissimilar to any degree, then the item has the potential for ambiguity or variation in interpretation among individuals, resulting in a decrease in its specificity and, hence, accuracy. As a general rule, a given questionnaire item should be clear and focus on a single dimension or concept, although different items within the questionnaire can obviously be disparate.

Post-hoc determination of the number of different constructs being measured by a questionnaire can, in some cases, be established using factor or principal component analysis.[143] These procedures examine the correlations among the test items and establish subsets of items that are most highly correlated with one another. By use of an algorithm, such analyses attempt to establish orthogonal factors or principal components that have little in common with one another. The underlying construct of a given factor or principal component can often be identified for such subset by carefully evaluating the items involved. Using such data, the developer of the questionnaire can omit items that are not measuring the dimension or dimensions of interest, and optimize the amount of variance accounted for by the included items. Importantly, statistical procedures exist to determine how well individual items correlate with the overall questionnaire, as well as how much unique variance individual items contribute to the total.[365]

Several studies have sought to determine the number of factors present in odor annoyance surveys given to members of the community who live near factories or other locations where air pollution is common or exacerbated. Interestingly, even when the same items are used, different studies may produce different numbers of factors, although usually several common factors emerge. The reasons for this are multiple, and can include the types of statistical analyses that are performed (e.g., parametric vs. non-parametric) and types or locales of the respondents. Seffelaar subjected the Dutch "WKJ"-odor-annoyance survey, which was administered throughout the Netherlands, to principal components analysis using both parametric and non-parametric solutions.[366] The former yielded four meaningful factors, whereas the latter only two. An overlap of factor structure appeared to be present to some degree with earlier surveys using common items (the German Winneke and Kastka surveys).[367,368]

Commonly questionnaire items simply assess the frequency or point prevalence of a symptom or construct. Examples of such items are:

1. How often do you have a headache? 1) almost every day; 2) several times a week; 3) several times a month; 4) less than monthly

2. Do /did you have a headache today /now? yes no

As noted above, severity or degree of a construct can be established using rating scales such as those described in the psychophysics section of this paper. The metric of the scale depends upon the nature of what is being asked. In some cases, particularly when definite categories or graded descriptions exist for the construct, simple categories are most appropriate for assessing the magnitude of the percept. However, many constructs vary on continua that can only artificially be broken into categories, and visual analog scales are more appropriate for their assessment. In some cases, the underlying dimension is not linear, but more logarithmic, and the design of the scale should take this into account (e.g., the LMS scale described earlier in the paper may be useful in this regard). This is particularly true in the case of perceptual categories (e.g., intensity). An example of a category scale, where discrete levels of the construct to be assessed are available, is as follows:

3. Please rate the quality of the air in this room as you experience it now

very un-acceptable	unacceptable	somewhat unacceptable	somewhat acceptable	acceptable	very acceptable

In general, one seeks to divide the dimension into as many categories as possible, particularly when the optimal continuum is continuous, although for practical and other reasons category scales rarely have more than nine categories. Five- or seven-point ordinal scales using

terms such as acceptable, comfortable, etc. are commonly used in thermal comfort literature to provide language referents. This makes it possible to easily communicate the results of research to individuals and organizations in a straight-forward manner. For example, a statement such as "35% more of the occupants designated the environment unacceptable for more than 10 minutes" is clearly interpretable to funding agencies, engineers, managers, and subjects.

Multiple items are needed within a questionnaire and, ideally, such items should tap the same construct or sets of constructs. Importantly, the reliability of the questionnaire for a given construct is a function of the number of items related to that construct. Too few items lead to an unreliable questionnaire, whereas too many items make it impractical and unwieldy. Since the relationship between reliability and questionnaire length can be mathematically determined using, for example, the Spearman Brown Prophesy Formula,[369] a questionnaire developer can establish the questionnaire length that optimizes both reliability and practicality.

It is important that the reliability of a questionnaire be established before it is administered to subjects, either in the laboratory or the field. This is commonly established by administering the questionnaire to the same set of subjects twice and determining a correlation coefficient (termed the "test-retest" reliability coefficient), although internal reliability (also termed internal consistency) can be established by splitting the questionnaire into two sets of items (e.g., odd vs. even) and correlating them (termed the "split-half reliability coefficient"). In this case, the correlation coefficient would be corrected for test length using the Spearman Brown formula.[143] Questionnaire validity (i.e., does the questionnaire measure what it is intended to measure) is much more difficult to establish, since it is often difficult to obtain independent indices of the end-point that is being assessed.

There are several types of validity.[370] For example, *face validity* is a more or less qualitative determination as to whether the instrument looks like it measures what it portends to measure, and is closely associated with *content validity*. *Criterion-related validity*, on the other hand, is a measure that can be empirically determined, reflecting the relationship of the test score with some independently measurable aspect of the construct under consideration. For example, symptoms of irritation are considered common in buildings and often attributed to the complex mixture of volatile organic compounds found indoors.[371] Hence, a strong correlation between responses to questionnaire items related to the intensity of irritation and measured levels of a given volatile organic compound would indicate that the questionnaire is valid for assessing this specific environmental situation. There are a number of studies that have, in fact, sought to establish concentration-response relationships for eye and nose irritation for a range of individual chemicals and complex mixtures.[95,96,372-374] Indeed, studies have confirmed that such effects, initially documented only in the laboratory, are also measureable in field study populations. For example, Hodgson identified relationships between symptoms and volatile organic compounds measured with a screening device (photoionization detector) although such relationships were markedly weaker when measured with a photoacoustic detector.[375] Ten Brinke and colleagues identified relationships between classes of compounds released by groups of products and symptoms indoors.[376] Sundell and others found that symptoms increased as more VOCs were "lost" between air supply and exhaust louvers in the same room,[377] suggesting that formation of de novo agents might explain some symptoms.[378] Some such field studies have included physiologic measures of eye irritation or nasal effects,[284,379-380] reporting that defined exposures are in fact associated with measurable adverse health effects in populations.

As noted in detail under subject characteristics earlier in this paper, an important issue in studies of community complaints of odor or irritation is the degree to which an individual's responses reflect true sensory perceptions or response-criterion related psychological factors (e.g., the tendency to complain, suggestibility, etc.). Clearly, individuals differ considerably in regards to what level of symptoms they feel must be experiencing before they voice complaints, and some of this variation reflects somatic and psychological factors, including personality styles.[381] Thus, perceptions of work stress are consistently associated with higher symptom levels.[382] A number of studies find that women are more likely than men to voice complaints at any given level of discomfort, a phenomenon well documented in medical settings. In the building-related symptom literature, this gender-symptom excess is consistently related to excess work stress[383,384] and strongly associated with irritation symptoms in all buildings in which a relationship was sought. It should be emphasized that surveys in which volunteers are sought through advertisements are susceptible to numerous selection biases (e.g., a disproportionate number of dissatisfied individuals) than surveys based upon random or stratified sampling strategies.

Several studies have applied questionnaire instruments to assess community influences of airborne pollutants.[385-387] What such instruments reflect, however, may be rather complex. As noted by First (p. 451), "The results <of these studies> pointed to the risk of relying on voluntary complaints for enforcement purposes as the volume of complaints received may reflect not only the amount of discomfort experienced by the exposed population, but also its social-class composition and degree of community organization.[388] This has become a truism of community odor control, but it is not known whether it reflects a lower annoyance threshold, a greater degree

to achieve a goal, or better knowledge of how to register complaints among citizens who are better educated and in a higher social class.[352]

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REFERENCES

1. **Gervasi, P. G., Longo, V., Naldi, F., Panattoni, G., and Ursino, F.,** Xenobiotic-metabolizing enzymes in human respiratory nasal mucosa, *Biochem. Pharmacol*., 41, 177, 1991.

2. **Kimbell, J. S., Gross, E. A., Joyner, D. R., Godo, M. N., and Morgan, K. T.,** Application of computational fluid dynamics to regional dosimetry of inhaled chemicals in the upper respiratory tract of the rat, *Toxicol. Appl. Pharmacol.,* 121, 253, 1993.

3. **Subramaniam, R. P., Richardson, R. B., Morgan, K. T., and Kimbell, J. S.,** Computational fluid dynamics simulations of inspiratory airflow in. the human nose and nasopharynx, *Inhal. Toxicol.,* 10, 91, 1998.

4. **Negus, V.,** *The Comparative Anatomy and Physiology of the Nose and Paranasal Sinuses*, E. & S. Livingstone Ltd, Edinburgh, Scotland, 1958.

5. **Wysocki, C. J., Dalton, P., Brody, M. J., and Lawley, H. J.,** Acetone odor and irritation thresholds obtained from acetone-exposed factory workers and from control (occupationally unexposed) subjects, *Am. Ind. Hyg. Assoc. J.*, 58, 704, 1997.

6. **Hummel, T., Dalton, P., and Dilks, D. D.,** Effects of exposure to irritants, *Soc. Neurosci. Abstr.,* 25, 2187, 1999 (abstract).

7. **Morgott, D. A.,** Acetone. In: *Patty's Toxicology*, Bingham, E., Cohrssen, B., and Powell, C. H., Eds., 5th ed., Vol. 6, Chapter 74, John Wiley & Sons, New York, 2001, 1.

8. **Matsushita, T., Goshima, E., Miyakaki, H., Maeda, K., Takeuchi, Y., and Inoue, T.,** Experimental studies for determining the MAC value of acetone. 2. Biologic reactions in the "six-day exposure" to acetone, *Jpn. J. Ind. Health*, 11, 507, 1969.

9. **Keele, C. A.,** The common chemical sense and its receptors. *Arch. Int. Pharmacodyn. Ther.*, 139, 547, 1962.

10. **Parker, G. H.,** The reactions of smell, taste, and the common chemical sense in vertebrates, *J. Acad. Nat. Sci. Phila.*, 15, 221, 1912.

11. **Green, B. G. and Lawless, H. T.,** The psychophysics of somatosensory chemo-reception in the nose and mouth, in *Smell and Taste in Health and Disease,* Getchell, T. V., Doty, R. L., Bartoshuk, L. M., and Snow J. B., Jr., Eds., Raven Press, New York, 1991, 235.

12. **Green, B. G., Mason, J. R., and Kare, M. R**., Preface, in *Chemical Senses,* Vol.. 2, Green, B. G., Mason, J. R., and Kare, M. R., Eds., Marcel Dekker, New York, 1990, v.

13. **Cometto-Muñiz, J. E. and Noriega, G.,** Gender differences in the perception of pungency, *Physiol. Behav.*, 34, 385, 1985.

14. **Henkin, R. L,** The definition of primary and accessory areas of olfaction as the basis for a classification of decreased olfactory ability, in *Olfaction and Taste II*, Hayashi, T., Ed., Pergamon Press, Oxford, 1967, 235.

15. **Korpas, J. and Tomori, Z.,** *Cough and Other Respiratory Reflexes,* S. Karger, Basel, 1979.

16. **Widdicombe, J. G.,** Reflexes from the upper respiratory tract, in *Handbook of Physiology, Section 3: The Respiratory System*, Vol. II, Cherniak, N. S. and Widdicombe, J. G., Eds., Oxford University Press, New York, 1986, 363.

17. **Widdicombe, J. G.,** Sensory innervation of the lungs and airways, in *Visceral Sensation, Progress in Brain Research,* Cervero, F. and Morrison, J. F. B., Eds., Vol. 67, Elsevier Science Publishers, New York, 1986, 49.

18. **Bhatnagar, K. P., Kennedy, R. C., Baron, G., and Greenberg, R. A**., Number of mitral cells and the bulb volume in the aging human olfactory bulb: A quantitative morphological study, *Anat. Rec.,* 218, 73, 1987.

19. **Bhatnagar, K. P. and Meisami, E.,** Vomeronasal organ in bats and primates: Extremes of structural variability and its phylogenetic implications, *Microsc. Res. Tech.,* 43, 465, 1998.

20. **Doty, R. L.,** Olfaction, *Ann. Rev. Psychol*., 52, 423, 2001.

21. **Wirsig-Wiechmann, C. R.,** Nervus terminalis lesions. II. Enhancement of lordosis induced by tactile stimulation in the hamster, *Physiol. Behav.,* 61, 867, 1997.

22. **Nakashima, T., Kimmelman, C. P., and Snow, J. B., Jr.,** Structure of human fetal and adult olfactory neuroepithelium, *Arch. Otolaryngol.,* 110, 641, 1984.

23. ***Leopold, D. A., Hummel, T., Schwob, J. E., Hong, S. C., Knecht, M.,* and *Kobal, G.,*** *Anterior distribution of human olfactory epithelium*, *Laryngoscope, 110*, *417*, 2000.

24. **Huard, J. M., Youngentob, S. L., Goldstein, B. L., Luskin, M. B., and Schwob, J. E.,** Adult olfactory epithelium contains multipotent progenitors that give rise to neurons and non-neural cells, *J. Comp. Neurol.,* 400, 469, 1998.

25. **Menco, B. P. M. and Morrison, E. E.,** Morphology of the mammalian olfactory epithelium: Form, fine structure, function and pathology, in *Handbook of Olfaction and Gustation*, 2nd ed., Doty, R. L., Ed, Marcel Dekker, New York, 2003, (in press).

26. **Moran, D. T., Rowley, J. C., III, Jafek, B. W., and Lovell, M. A**., The fine structure of the olfactory mucosa in man, *J. Neurocytol.,* 11, 721, 1982.

27. **Getchell, M. L. and Getchell, T. V.,** Fine structural aspects of secretion and extrinsic innervation in the olfactory mucosa, *Microsc. Res. Tech.,* 23, 111, 1992.

28. **Lewis, J. L. and Dahl, A. R.,** Olfactory mucosa: composition, enzymatic localization, and metabolism, in *Handbook of Olfaction and Gustation*, Doty, R.L., Ed., Marcel Dekker, New York, 1995, 33.

29. **Mackay-Sim, A. and Kittel, P. W.,** On the life span of olfactory receptor neurons, *Eur. J. Neurosci.,* 3, 209, 1990.

30. **Mackay-Sim, A.,** Neurogenesis in the adult olfactory neuroepithelium, in *Handbook of Olfaction and Gustation*, 2nd ed., Doty, R. L., Ed, Marcel Dekker, New York, 2002, (in press).

31. **Buck, L. and Axel, R. A.,** A novel multigene family may encode odorant receptors: a molecular basis for odor recognition, *Cell,* 65, 175, 1991.

32. **Jones, D. T. and Reed, R. R.,** G_{olf}: an olfactory neuron specific-G protein involved in odorant signal transduction, *Science,* 244, 790, 1989.

33. **Lowe, G., Nakamura, T., and Gold, G. H.,** Adenylate cyclase mediates olfactory transduction for a wide variety of odorants, *Proc. Nat. Acad. Sci. USA,* 86, 5641, 1989.

34. **Chess, A., Simon, I., Cedar, H., and Axel, R.,** Allelic inactivation regulates olfactory receptor gene expression, *Cell,* 78, 823, 1994.

35. **Holley, A., Duchamp, A., Revial, M. F., and Juge, A.,** Qualitative and quantitative discrimination in the frog olfactory receptors: analysis from electrophysiological data, *Ann. N.Y. Acad. Sci.,* 237, 102, 1974.

36. **Kratskin, I.,** Functional anatomy, central connections, and neurochemistry of the mammalian olfactory bulb, in *Handbook of Olfaction and Gustation*, Doty, R. L., Ed., Marcel Dekker, New York, 1995, 103.

37. **Bryant, B. and Silver, W. L.,** Chemesthesis: the common chemical sense, in *The Neurobiology of Taste and Smell*, Finger, T. E., Silver, W. L., and Restrepo, D., Eds., 2nd ed., Wiley-Liss, New York, 2000, 73.

38. **Silver, W. L. and Finger, T. E.,** The trigeminal system, in *Smell and Taste in Health and Disease*, Getchell, T. V., Doty, R. L., Bartoshuk, L. M., and Snow, J. B., Jr., Eds., Raven Press, New York, 1991, 97.

39. **Jacquin, M. F., Hess, A., Yang, G., Adamo, P., Math, M. F., Brown, A., and Rhoades, R. W.,** Organization of the infraorbital nerve in rat: a quantitative electron microscopic study, *Brain Res.*, 290, 131, 1984.

40. **Biedenbach, M. A., Beuerman, R. W., and Brown, A. C.,** Graphic-digitizer analysis of axon spectra in ethmoidal and lingual branches of the trigeminal nerve, *Cell Tissue Res.,* 157, 341, 1975.

41. **Finger, T.E., St. Jeor, V., Kinnamon, J.C., and Silver, W.L.,** Ultrastructure of substance P- and CGRP-immunoreactive nerve fibers in the nasal epithelium of rodents, *J. Comp. Neurol.,* 294, 293, 1990.

42. **Jansco, N., Jansco-Gabor, A., and Szolcsanyi, J.,** Direct evidence for neurogenic inflammation and its prevension by denervation and by pretreatment with capsaicin, *Br. J. Pharmacol. Chemother.,* 31, 138, 1967.

43. **Lunblad, L., Saria, A., Lundberg, J. M., and Anggard, A.,** Increased vascular permeability in rat mucosa induced by substance P and stimulation of capsaicin-sensitive trigeminal neurons, *Acta Otolaryngol.,* 96, 479, 1983.

44. **Silver, W. L., Mason, J. R., Marshall, D. A., and Maruniak, J. A.,** Rat trigeminal, olfactory and taste responses after capsaicin desensitization, *Brain Res.,* 333, 45, 1985.

45. **Martin, J. H. and Jessell, T. M.,** Modality coding in the somatic sensory system, in *Principles of Neural Science,* 3rd ed., Kandel, E. R., Schwartz, J. H., and Jessell, T. M., Eds., Elsevier Science Publishers, New York, 1991, 341.

46. **Nielsen, G. D.,** Mechanisms of activation of the sensory irritant receptor by airborne chemicals, *Crit. Rev. Toxicol.,* 21, 183, 1991.

47. **Alarie, Y., Nielsen, G. D., and Abraham, M. H.,** A theoretical approach to the Ferguson principle and its use with non-reactive and reactive airborne chemicals, *Pharmacol. Toxicol.,* 83, 270, 1998.

48. **Alarie, Y., Neilsen, G. D., Andonian-Haftvan, J., and Abraham, M. H.,** Physicochemical properties of nonreactive volatile organic chemicals to estimate RD50: Alternatives to animal studies, *Toxicol. Appl. Pharmacol.,* 134, 92, 1995.

49. **Alarie, Y., Schaper, M., Nielsen, G. D., and Abraham, M. H.,** Estimating the sensory irritating potency of airborne nonreactive volatile organic chemicals and their mixtures, *SAR QSAR Environ. Res.,* 5, 151, 1996.

50. **Alarie, Y., Schaper, M., Nielsen, G. D., and Abraham, M. H.,** Structure-activity relationships of volatile organic chemicals as sensory irritants, *Arch. Toxicol.,* 72, 125, 1998.

51. **Cesare, P. and McNaughton, P.,** Peripheral pain mechanisms, *Curr. Opin. Neurobiol.*, 7, 493, 1997.

52. **McCleskey, E. W. and Gold, M. S.,** Ion channels of nociception, *Ann. Rev. Physiol.*, 61, 835, 1999.

53. **Steranka, L. R., De Haas, C. J., Vavrek, R. J., Stewart, J. M., Enna, S. J., and Snyder, S. H.,** Antinociceptive effects of bradykinin antagonists, *Eur. J. Pharmacol.,* 136, 261, 1987.

54. **Eccles, R., Jawad, M. S., and Morris, S.,** The effects of oral administration of (-)-menthol on nasal resistance to airflow and nasal sensation of airflow in subjects suffering from nasal congestion associated with the common cold, *J. Pharm. Pharmacol.*, 42, 652, 1990.

55. **Jansco, N.,** Role of the nerve terminals in the mechanism of inflammatory reactions, *Bull. Millard Filmore Hosp.,* 7, 53, 1960.

56. **Szallasi, A.,** The vanilloid (capsaicin) receptor: Receptor types and species differences, *Gen. Pharmacol.,* 25, 223, 1994.

57. **Caterina, M. J., Schumacher, M. A., Tominaga, M., Rosen, T. A., Levine, J. D., and Julius, D.,** The capsaicin receptor: a heat-activated ion channel in the pain pathway, *Nature,* 389, 816, 1997.

58. **Alimohammadi, H. and Silver, W. L.,** Evidence for nicotinic acetylcholine receptors on nasal trigeminal nerve endings of the rat, *Chem. Senses,* 25, 61, 2000.

59. **Walker, J. C., Kendal-Reed, M., Keiger, C. J., Bencherif, M., and Silver, W. L.,** Olfactory and trigeminal responses to nicotine, *Drug Develop. Res.,* 38, 160, 1996.

60. **Thürauf, N., Kaegler, M., Dietz, R., Barocka, A., and Kobal, G.,** Dose-dependent stereoselective activation of the trigeminal sensory system by nicotine in man, *Psychopharmacology,* 142, 236, 1999.

61. **Brown, S. K., Sim, M. R., Abramson, M. J., and Gray, C. N.,** Concentrations of volatile organic compounds in indoor air — A review, *Indoor Air*, 4, 124, 1994.

62. **Wolkoff, P. and Wilkins, C. K.,** Desorbed VOC from household floor dust. Comparison of headspace with desorbed dust, method for TVOC release determination, in *Proceedings of the 6th International Conference on Indoor Air Quality and Climate, Indoor Air '93*, 2, 287, 1993.

63. **Cometto-Muñiz, J. E.,** Physicochemical basis for odor and irritation potency of VOCs, in *Indoor Air Quality Handbook,* Spengler, J. D., Samet, J., and McCarthy, J. F., Eds., McGraw-Hill, New York, 2001, 20.1.

64. **Abraham, M. H., Kumarsingh, R., Cometto-Muñiz, J. E., and Cain, W. S.,** An algorithm for nasal pungency thresholds in man, *Arch. Toxicol.,* 72, 227, 1998.

65. **Abraham, M. H., Kumarsingh, R., Cometto-Muñiz, J. E., and Cain, W. S.,** Draize eye scores and eye irritation thresholds in man can be combined into one quantitative structure-activity relationship, *Toxicol. In Vitro,* 12, 403, 1998.

66. **Cometto-Muñiz, J. E., Cain, W. S., and Abraham, M. H.,** Nasal pungency and odor of homologous aldehydes and carboxylic acids, *Exp. Brain Res*., 118, 180, 1998.

67. **Abraham, M. H., Gola, J. M. R., Cometto-Muniz, J. E., and Cain, W. S.,** A model for odor thresholds, *Chem. Senses,* 2002 (in press).

68. **Tomori, Z. and Widdicombe, J. G**., Muscular, bronchomotor, and cardiovascular reflexes elicited by mechanical stimulation of the respiratory tract, *J. Physiol.,* 200, 25, 1969.

69. **Cain, W. S., See, L. C., and Tosun, T.,** Irritation and odor from formaldehyde: Chamber studies, in *Managing Indoor Air for Health and Energy Conservation*, American Society of Heating, Refrigerating and Air-Conditioning Engineers, Atlanta, 1986, 126.

70. **Cain, W. S., Tosun, T., See, L.-C., and Leaderer, B.,** Environmental tobacco smoke: Sensory reactions of occupants, *Atmos. Environ*., 21, 347, 1987.

71. **Walker, J. C., Nelson, P. R., Cain, W. S., Utell, M. J., Joyce, M. B., Morgan, W. T., Steichen, T. J., Pritchard, W. S., and Stancill, M. W.,** Perceptual and psychophysiological responses of non-smokers to a range of environmental tobacco smoke concentrations, *Indoor Air,* 7, 173, 1997.

72. **Hatakeyama, S.,** Histological study of the nerve distribution in the larynx in the cat, *Arch. Jpn. Histol.,* 19, 369, 1969.

73. **Lewis, D. J. and Prentice, D. E.,** The ultrastructure of rat laryngeal epithelia, *J. Anat.,* 130, 617, 1980.

74. **Andrew, B. L. A.,** A functional analysis of the myelinated fibres of the superior laryngeal nerve of the rat, *J. Physiol*., 133, 420, 1956.

75. **Boushey, H. A., Richardson, P. S., and Widdicombe, J. G.,** The response of laryngeal afferent fibres to mechanical and chemical stimuli, *J. Physiol*., 224, 501, 1974.

76. **Breslin, P. A. S., Gingrich, T. N., and Green, B. G.,** Ibuprofen as a chemesthetic stimulus: Evidence of a novel mechanism of throat irritation, *Chem. Senses*, 26, 55, 2001.

77. **Rentmeister-Bryant, H. and Green, B. G.,** Perceived irritation during ingestion of capsaicin or piperine: Comparison of trigeminal and non-trigeminal areas, *Chem. Senses*, 22, 257, 1997.

78. **Eccles, R.,** Neurological and pharmacological considerations, in *The Nose: Upper Airway Physiology and Atmospheric Environment*, Proctor, D. F. and Andersen, I. B., Eds., Elsevier Biomedical Press, Amsterdam, 1982, 192.

79. **Phipps, R. J.,** The airway mucociliary system, in *Respiratory Physiology*, Vol. III, Widdicombe, J. G., Ed., University Park Press, Baltimore, 1981, 213.

80. **Richardson, P. S. and Phipps, R. J.,** The anatomy, physiology, pharmacology and pathology of tracheobronchial mucus secretion and the use of expectorant drugs in human disease, *Pharmacol. Ther.*, 3, 441, 1978.

81. **Widdicombe, J. G. and Wells, U. M.,** Airway secretions, in *The Nose: Upper Airway Physiology and the Atmospheric Environment*, Proctor, D. F. and Anderson, I. B., Eds., Elsevier Biomedical Press, Amsterdam, 1982, 215.

82. **Amoore, J. E. and Ollman, B. G.,** Practical test kits for quantitatively evaluating the sense of smell, *Rhinology*, 21, 49, 1983.

83. **Doty, R. L.,** *The Smell Threshold Test™ Administration Manual*, Sensonics, Inc., Haddon Heights, New Jersey, 2000.

84. **Prah, J. D., Sears, S. B., and Walker, J. C.,** Modern approaches to air dilution olfactometry, in *Handbook of Olfaction and Gustation*, Doty, R. L., Ed., Marcel Dekker, New York, 1995, 227.

85. **Cometto-Muñiz, J. E. and Cain, W. S.,** Thresholds for odor and nasal pungency, *Physiol. Behav.,* 48, 719, 1990.

86. **Doty, R. L., Gregor, T., and Settle, R. G.,** Influences of intertrial interval and sniff bottle volume on the phenyl ethyl alcohol olfactory detection threshold, *Chem. Senses*, 11, 259, 1986.

87. **Cometto-Muñiz, J. E. and Cain, W. S.**, Nasal pungency, odor, and eye irritation thresholds for homologous acetates, *Pharmacol. Biochem. Behav.*, 39, 983, 1991.

88. **Hempel-Jørgensen, A., Kjærgaard, S. K., Mølhave, L., and Hudnell, H. K.,** Time course of sensory eye irritation in humans exposed to *n*-butanol and 1-octene, *Arch. Environ. Health*, 54, 86, 1999.

89. **Haring, H. G.,** Vapor pressures and Raoult's Law deviations in relation to odor enhancement and suppression, in *Human Responses to Environmental Odors,* Turk, A., Johnston, J. W., Jr., and Moulton, D. G., Eds., Academic Press, New York, 1974, 199.

90. **Cometto-Muniz, J. E., Cain, W. S., Hiraishi, T., Abraham, M. H., and Gola, J. M. R.,** Comparison of two stimulus-delivery systems for measurement of nasal pungency thresholds, *Chem. Senses*, 25, 285, 2000.

91. **Cain, W. S., Cometto-Muñiz, J. E., and de Wijk, R. A.,** Techniques in the quantitative study of human olfaction, in *Science of Olfaction*, Serby, M. J. and Chobor, K. L., Eds., Springer-Verlag, New York, 1992, 279.

92. **Dravnieks, A.,** Instrumental aspects of olfactometry, in *Methods in Olfactory Research*, Moulton, D. G., Turk, A., and Johnston, J. W. Jr., Eds., Academic Press, New York, 1975, 1.

93. **Kendal-Reed, M., Walker, J. C., Morgan, W. T., LaMachio, M., and Lutz, R. W.,** Human responses to propionic acid: I. Quantification of within- and between-participant variation in perception by normosmics and anosmics, *Chem. Senses,* 23, 71, 1998.

94. **Hudnell, H. K., Otto, D. A., House, D. E., and Mølhave, L.,** Exposure of humans to a volatile organic mixture. II. Sensory, *Arch. Environ. Health*, 47, 31, 1992.

95. **Kjærgaard, S. K., Mølhave, L., and Pedersen, O. F.,** Human reactions to a mixture of indoor air volatile organic compounds, *Atmos. Environ*., 25A, 1417, 1991.

96. **Mølhave, L., Bach, B., and Pedersen, O. F.**, Human reactions to low concentrations of volatile organic compounds, *Environ. Int.*, 12, 167, 1986.

97. **Otto, D., Mølhave, L., Rose, G., Hudnell, H. K., and House, D.,** Neurobehavioral and sensory irritant effects of controlled exposure to a complex mixture of volatile organic compounds, *Neurotoxicol. Teratol.*, 12, 649, 1990.

98. **Fechner, G. T.,** *Elemente der Psychophysik*, Breitkopf and Harterl, Leipzig, 1860.

99. **Doty, R. L. and Kobal, G.,** Current trends in the measurement of olfactory function, in *Handbook of Olfaction and Gustation*, Doty, R. L., Ed., Marcel Dekker, New York, 1995, 191.

100. **Woodworth, R. S. and Schlosberg, H.,** *Experimental Psychology*, Holt, Reinhart and Winstona, New York, 1965.

101. **Blackwell, H. R.,** Studies of psychophysical methods for measuring visual thresholds. *J. Opt. Soc. Am.*, 42, 606, 1952.

102. **Cometto-Muñiz, J. E. and Cain, W. S.,** Physicochemical determinants and functional properties of the senses of irritation and smell, in *Indoor Air and Human Health*, 2nd ed., Gammage, R. B. and Berven, B. A., Eds., CRC Lewis Publishers, Boca Raton, 1996, 53.

103. **Cometto-Muñiz, J. E. and Cain, W. S.,** Perception of odor and nasal pungency from homologous series of volatile organic compounds, *Indoor Air*, 4, 140, 1994.

104. **Doty, R. L.,** Intranasal trigeminal detection of chemical vapors by humans, *Physiol. Behav.*, 14, 855, 1975.

105. **Doty, R. L., Brugger, W. E., Jurs, P. C., Orndorff, M. A., Snyder, P. F., and Lowry, L. D.,** Intranasal trigeminal stimulation from odorous volatiles: Psycho-metric responses from anosmic and normal humans, *Physiol. Behav.*, 20, 175, 1978.

106. **Cometto-Muñiz, J. E. and Cain, W. S.,** Efficacy of volatile organic compounds in evoking nasal pungency and odor, *Arch. Environ. Health,* 48, 309, 1993.

107. **Cometto-Muñiz, J. E. and Cain, W. S.,** Sensory reactions of nasal pungency and odor to volatile organic compounds: The alkylbenzenes, *Am. Ind. Hyg. Assoc. J.,* 55, 811, 1994.

108. **Cometto-Muñiz, J. E. and Cain, W. S.,** Trigeminal and olfactory sensitivity: Comparison of modalities and methods of measurement, *Int. Arch. Occup. Environ. Health,* 71, 105, 1998.

109. **Cometto-Muñiz, J. E., Cain, W. S., Abraham, M. H., and Kumarsingh, R.,** Trigeminal and olfactory chemosensory impact of selected terpenes, *Pharmacol. Biochem. Behav*., 60, 765, 1998.

110. **Hummel, T., Barz, S., Lötsch, J., Roscher, S., Kettenmann, B., and Kobal, G.,** Loss of olfactory function leads to a decrease of trigeminal sensitivity, *Chem. Senses*, 21, 75, 1996.

111. **Cometto-Muñiz, J. E. and Cain, W. S.,** Relative sensitivity of the ocular trigeminal, nasal trigeminal, and olfactory systems to airborne chemicals, *Chem. Senses*, 20, 191, 1995.

112. **Cometto-Muñiz, J. E., Cain, W. S., Abraham, M. H., and Gola, J. M. R.,** Chemosensory detectability of 1-butanol and 2-heptanone singly and in binary mixtures, *Physiol. Behav.,* 67, 269, 1999.

113. **Cometto-Muñiz, J. E., Cain, W. S., Abraham, M. H., and Gola, J. M. R.,** Ocular and nasal trigeminal detection of butyl acetate and toluene presented singly and in mixtures, *Toxicol. Sci.,* 63, 233, 2001.

114. **Cometto-Muñiz, J. E., Cain, W. S., Abraham, M. H., and Gola, J. M. R.,** Psychometric functions for the olfactory and trigeminal detectability of butyl acetate and toluene, *J. Appl. Toxicol*., 2002, (in press).

115. **Kobal, G., Van Toller, S., and Hummel, T.,** Is there directional smelling?, *Experientia,* 45, 130, 1989.

116. **Schneider, R. A. and Schmidt, C. E.,** Dependency of olfactory localization on non-olfactory cues, *Physiol. Behav.*, 2, 305, 1967.

117. **von Skramlik, E.,** Über die Lokalisation der Empfindungen bei den niederen Sinnen, *Z. Sinnesphysiol.,* 56, 69, 1925.

118. **von Bèkesy, G.,** Olfactory analogue to directional hearing, *J. Appl. Physiol.,* 19, 369, 1964.

119. **Cometto-Muñiz, J. E., García-Medina, M. R., and Calviño, A. M.,** Perception of pungent odorants alone and in binary mixtures, *Chem. Senses,* 14, 163, 1989.

120. **Cometto-Muñiz, J. E. and Hernández, S. M.,** Odorous and pungent attributes of mixed and unmixed odorants, *Percept. Psychophys*., 47, 391, 1990.

121. **Stevens, S. S.,** Mathematics, measurement, and psychophysics, in *Handbook of Experimental Psychology*, Stevens, S. S., Ed., Wiley, New York, 1951, 1.

122. **Stevens, S. S.,** The psychophysics of sensory function, *Am. Scient.,* 48, 226, 1960.

123. **Doty R. L.,** Olfactory system, in *Smell and Taste in Health and Disease,* Getchell, T. V., Doty, R. L., Bartoshuk, L. M., and Snow J. B., Jr., Eds., Raven Press, New York, 1991, 175.

124. **Cain, W. S.,** The odoriferous environment and the application of olfactory research, in *Handbook of Perception,* Carterette, E. C. and Friedman, M. P., Eds., Academic Press, New York, 1978, 277.

125. **Cain, W. S. and Moskowitz, H. R.,** Psychophysical scaling of odor, in *Human Responses to Environmental Odors*, Turk, A., Johnston, J. W., Jr., and Moulton, D. G., Eds., Academic Press, New York, 1974, 1.

126. **Anton, F., Euchner, I., and Handwerker, H. O.,** Psychophysical examination of pain induced by defined CO_2 pulses applied to the nasal mucosa, *Pain,* 49, 53, 1992.

127. **Chen, X., Gallar, J., Pozo, M. A., Baeza, M., and Belmonte, C.,** CO_2 stimulation of the cornea: A comparison between human sensation and nerve activity in polymodal nociceptive afferents of the cat, *Eur. J. Neurosci.,* 7, 1154, 1995.

128. **Hempel-Jorgensen, A., Kjærgaard, S. K., and Molhave, L.,** Integration in human eye irritation, *Int. Arch. Occup. Environ. Health,* 69, 289, 1997.

~~129.~~ **THIS SHOULD BE REFERENCE 104** ~~**Doty, R. L., Shaman, P., and Dann, M.,** Development of the University of Pennsylvania smell identification test: A standardized microencapsulated test of olfactory function, *Physiol. Behav.*, 32, 489, 1984.~~

130. **Schiet, F. T. and Cain, W. S.,** Odor intensity of mixed and unmixed stimuli under environmentally realistic conditions, *Perception,* 19, 123, 1990.

131. **Cain, W. S., Leaderer, B. P., Cannon, L., Tosun, T., and Ismail, H.,** Odorization of inert gas for occupational safety: Psychophysical considerations, *Am. Ind. Hyg. Assoc. J.,* 48, 47, 1987.

132. **Neely, G., Ljunggren, G., Sylven, C., and Borg, G.,** Comparison between the Visual Analogue Scale (VAS) and the Category Ratio Scale (CR-10) for the evaluation of leg exertion, *Int. J. Sports Med.,* 13, 133, 1992.

133. **Green, B. G., Dalton, P., Cowart, B., Shaffer, G., Rankin, K., and Higgins, J.,** Evaluating the 'Labeled Magnitude Scale' for measuring sensations of taste and smell, *Chem. Senses,* 21, 323, 1996.

134. **Green, B. G., Shaffer, G. S., and Gilmore, M. M.,** Derivation and evaluation of a semantic scale of oral sensation magnitude with apparent ratio properties, *Chem. Senses,* 18, 683, 1993.

135. **Intranuovo, L. R. and Powers, A. S.,** The perceived bitterness of beer and 6-*n*-propylthiouracil (PROP) taste sensitivity, *Ann. N.Y. Acad. Sci.,* 855, 813, 1998.

136. **Kurtz, D. B. and White, T. L.,** Metrics of odorant dissimilarity. Labeled magnitude scale vs magnitude estimation, *Ann. N.Y. Acad. Sci.,* 855, 638, 1998.

137. **Lucchina, L. A., Curtis, O. F., Putnam, P., Drewnowski, A., Prutkin, J. M., and Bartoshuk, L. M.,** Psychophysical measurement of 6-*n*-propylthiouracil (PROP) taste perception, *Ann. N.Y. Acad. Sci.,* 855, 816, 1998.

138. **Lawless, H. T., Horne, J., and Spiers, W.,** Contrast and range effects for category, magnitude and labeled magnitude scales in judgments of sweetness intensity, *Chem. Senses*, 25, 85, 2000.

139. **Dalton, P., Wysocki, C. J., Brody, M. J., and Lawley, H. J.,** Perceived odor, irritation, and health symptoms following short-term exposure to acetone, *Am. J. Ind. Med.,* 31, 558, 1997.

140. **Dalton, P., Dilks, D. D., and Banton, M. I.,** Evaluation of odor and sensory irritation thresholds for methyl iso-butyl ketone (MiBK) in humans, *Am. Ind. Hyg. Assoc. J.*, 61, 340, 2000.

141. **Anderson, N. H.,** Functional measurement and psychophysical judgement, *Psychol. Rev.,* 77, 153, 1970.

142. **Doty R. L.,** Olfactory system, in *Smell and Taste in Health and Disease,* Getchell, T. V., Doty, R. L., Bartoshuk, L. M., and Snow J. B., Jr., Eds., Raven Press, New York, 1991, 175.

143. **Guilford, J. P**., *Psychometric Methods*, 2nd ed., McGraw Hill, New York, 1954.

144. **Berglund, B., Berglund, U., Ekman, G., and Engen, T.,** Individual psychophysical functions for 28 odorants, *Percept. Psychophys.,* 9, 379, 1971.

145. **Stevens, J. C. and Marks, L. E.,** Cross-modality matching functions generated by magnitude estimation, *Percept. Psychophys.,* 27, 379, 1980.

146. **Stevens, S. S.,** On the psychophysical law, *Psychol. Rev.,* 64, 153, 1957.

147. **Doty, R. L.,** An examination of relationships between the pleasantness, intensity, and concentration of 10 odorous stimuli, *Percept. Psychophys.,* 17, 492, 1975.

148. **Patte, F., Etcheto, M., and Laffort, P.,** Selected and standardized values of suprathreshold odor intensities for 110 substances, *Chem. Senses Flav*., 1, 283, 1975.

149. **Cain, W. S. and Murphy, C. L.,** Interaction between chemoreceptive modalities of odour and irritation, *Nature,* 284, 255, 1980.

150. **Cometto-Muñiz, J. E. and Cain, W. S**., Perception of nasal pungency in smokers and nonsmokers, *Physiol. Behav.,* 29, 727, 1982.

151. **Dunn, J. D., Cometto-Muñiz, J. E., and Cain, W. S.,** Nasal reflexes: Reduced sensitivity to CO_2 irritation in cigarette smokers, *J. Appl. Toxicol.,* 2, 176, 1982.

152. **García-Medina, M. R. and Cain, W. S.,** Bilateral integration in the common chemical sense, *Physiol. Behav.*, 29, 349, 1982.

153. **Kjærgaard, S., Taudorff, E., Mølhave, L., and Pedersen, O. F.,** Assessment of changes in eye redness, *Int. Arch. Occup. Environ. Health,* 62, 133, 1990.

154. **Cometto-Muñiz, J. E. and Cain, W. S.,** Temporal integration of pungency, *Chem. Senses*, 8, 315, 1984.

155. **Marks, L. E.,** *Sensory Processes*, Academic Press, New York, 1974.

156. **Baird, J. C.,** A cognitive theory of psychophysics. I. Information transmission, partitioning and Weber's Law, *Scand. J. Psychol.,* 11, 35, 1970.

157. **Moskowitz, H. R., Dravnieks, A., and Klarman, L. A.,** Odor intensity and pleasantness for a diverse set of odorants, *Percept. Psychophys.,* 19, 122, 1976.

158. **Lawless, H. T. and Malone, G. J.,** The discriminative efficiency of common scaling methods, *J. Sensory Stud.,* 1,85, 1986.

159. **Lawless, H. T. and Malone, G. J.,** A comparison of rating scales: Sensitivity, replicates, and relative measurement, *J. Sensory Stud.,* 1,155, 1986.

160. **Marks, L. E.,** Magnitude estimation and sensory matching, *Percept. Psychophys.,* 43, 511, 1988.

161. **Marks, L. E., Szczesiul, R., and Ohlott, P.,** On the cross-modal perception of intensity, *J. Exp. Psychol. Hum. Percept. Perform.,* 12, 517, 1986.

162. **Dravnieks, A. and Prokop, W. H.,** Source emission odor measurement by a dynamic forced-choice triangle olfactometer, *J. Air Pollut. Control Assoc.,* 25, 28, 1975.

163. **ASTM,** Standard practices for referencing suprathreshold odor intensity, in *Annual Book of Standards.* Designation: E544-75, American Society for Testing and Materials. Philadelphia, 1988, 21.

164. **Muller, J. and Greff, G.,** Recherche de relations entre toxicite de molecules d'interet industriel et proprietes physico-chimiques: Test d'irritation des voies aeriennes superieures applique a quatre familles chimiques, *Food Chem. Toxicol*., 22, 661, 1984.

165. **Roberts, D. W.,** QSAR for upper-respiratory tract irritation, *Chem. Biol. Interact.,* 57, 325, 1986.

166. **Nielsen, G. D., Thomsen, E. S., and Alarie, Y.,** Sensory irritant receptor compartment properties, *Acta Pharmacol. Nord.,* 1, 31, 1990.

167. **Nielsen, G. D., Hansen, L. F., and Alarie, Y.,** Irritation of the upper airways. Mechanisms and structure-activity relationships, in *Chemical, Microbiological, Health and Comfort Aspects of Indoor Air Quality — State of the Art in SBS*, Knöppel, H. and Wolkoff, P., Eds., Kluwer Academic Publishers, Dordrecht, 1992, 99.

168. **Hau, K. M., Connell, D. W., and Richardson, B. J.,** Quantitative structure-activity relationships for nasal pungency thresholds of volatile organic compounds, *Toxicol. Sci.,* 47, 93, 1999.

169. **Abraham, M. H.,** The potency of gases and vapors: QSARs — Anesthesia, sensory irritation, and odor. In *Indoor Air and Human Health*. 2nd ed., Gammage, R. B. and Berven, B. A., Ed. CRC Lewis Publishers, Boca Raton, 1996, 67.

170. **Abraham, M. H.,** Application of solvation equations to chemical and biochemical processes, *Pure Appl. Chem.,* 65, 2503, 1993.

171. **Abraham, M. H.,** Scales of solute hydrogen-bonding: Their construction and application to physicochemical and biochemical processes, *Chem. Soc. Rev.,* 22, 73, 1993.

172. **Abraham, M. H., Andonian-Haftvan, J., Cometto-Muñiz, J. E., and Cain, W. S.,** An analysis of nasal irritation thresholds using a new solvation equation, *Fundam. Appl. Toxicol.,* 31, 71, 1996.

173. **Abraham, M. H., Kumarsingh, R., Cometto-Muñiz, J. E., Cain, W. S., Rosés, M., Bosch, E., and Díaz, M. L.,** The determination of solvation descriptors for terpenes, and the prediction of nasal pungency thresholds, *J. Chem. Soc. Perkin Trans.,* 2, 2405, 1998.

174. **Abraham, M. H., Kumarsingh, R., Cometto-Muñiz, J. E., and Cain, W. S.,** A quantitative structure-activity relationship (QSAR) for a Draize eye irritation database, *Toxicol. In Vitro,* 12, 201, 1998.

175. **Cassee, F. R., Groten, J. P., van Bladeren, P. J., and Feron, V. J.,** Toxicological evaluation and risk assessment of chemical mixtures, *Crit. Rev. Toxicol.,* 28, 73. 1998.

176. **El-Masri, H. A., Reardon, K. F., and Yang, R. S. H.,** Integrated approaches for the analysis of toxicologic interactions of chemical mixtures, *Crit. Rev. Toxicol.,* 27, 175, 1997.

177. **Groten, J. P., Feron, V. J., and Sühnel, J.,** Toxicology of simple and complex mixtures, *Trends Pharmacol. Sci.,* 22, 316, 2001.

178. **Groten, J. P., Tajima, O., Feron, V., and Schoen, E. D.,** Statistically designed experiments to screen chemical mixtures for possible interactions, *Environ. Health Perspect.,* 106, 1361, 1998.

179. **Yang, R. S. H.,** Some current approaches for studying combination toxicology in chemical mixtures, *Food Chem. Toxicol.*, 34, 1037, 1996.

180. **Cain, W. S., Leaderer, B. P., Isseroff, R., Berglund, L. G., Huey, R. J., Lipsitt, E. D., and Perlman, D.,** Ventilation requirements in buildings. I. Control of occupancy odor and tobacco smoke odor, *Atmos. Environ.,* 17, 1183, 1983.

181. **Clausen, G. H., Fanger, P. O., Cain, W. S., and Leaderer, B. P.,** Stability of tobacco smoke in enclosed spaces, in *Indoor Air, Sensory and Hyperreactivity Reactions to Sick Buildings*, Vol. 3, Berglund, B., Lindvall, T., and Sundell, J., Eds., Swedish Council for Building Research, Stockholm, 1984, 437.

182. **Clausen, G. H., Fanger, P. O., Cain, W. S., and Leaderer, B. P.,** Stability of body odor in enclosed spaces, *Environ. Int.,* 12, 201, 1986.

183. **Dietert, R. and Hedge, A.,** Toxicological considerations in evaluating indoor air quality and human health: Impact of new carpet emissions, *Crit. Rev. Toxicol*., 26, 633, 1996.

184. **Knudsen, H. N., Kjaer, U. D., Nielsen, P. A., and Wolkoff, P.,** Sensory and chemical characterization of VOC emissions from building products: Impact of concentration and air velocity, *Atmos. Environ.,* 33, 1217, 1999.

185. **Wolkoff, P.,** How to measure and evaluate volatile organic compounds emissions from building products. A perspective, *Atmos. Environ.,* 227, 197, 1999.

186. **Mølhave, L., Jensen, J. G., and Larsen, S.,** Subjective reactions to volatile organic compounds as air pollutants, *Atmos. Environ.,* 25A, 1283, 1991.

187. **Mølhave, L., Liu, Z., Jørgensen, A. H., Pedersen, O. F., and Kjærgaard, S. K.,** Sensory and physiological effects on humans of combined exposures to air temperatures and volatile organic compounds, *Indoor Air*, 3, 155, 1993.

188. **Kostiainen, R.,** Volatile organic compounds in the indoor air of normal and sick houses, *Atmos. Environ.,* 29, 693, 1994.

189. **Mølhave, L. and Nielsen, G. D.,** Interpretation and limitations of the concept "total volatile organic compounds" (TVOC) as an indicator of human responses to exposures of volatile organic compounds (VOC) in indoor air, *Indoor Air*, 2, 65, 1992.

190. **Rothweiler, H. and Schlatter, C.,** Human exposure to volatile organic compounds in indoor air-A health risk?, *Toxicol. Environ. Chem.,* 40, 93, 1993.

191. **Berglund, B. and Olsson, M. J.,** Odor-intensity interaction in binary and ternary mixtures, *Percept. Psychophys.,* 53, 475, 1993.

192. **Laska, M. and Hudson, R.,** A comparison of the detection thresholds of odour mixtures and their components, *Chem. Senses,* 16, 651, 1991.

193. **Olsson, M. J.,** An interaction model for odor quality and intensity, *Percept. Psychophys.,* 55, 363, 1994.

194. **Patterson, M. Q., Stevens, J. C., Cain, W. S., and Cometto-Muñiz, J. E.,** Detection thresholds for an olfactory mixture and its three constituent compounds, *Chem. Senses,* 18, 723, 1993.

195. **Cometto-Muñiz, J. E., Cain, W. S., and Hudnell, H. K.,** Agonistic sensory effects of airborne chemicals in mixtures: Odor, nasal pungency, and eye irritation, *Percept. Psychophys.,* 59, 665, 1997.

196. **Thompson, R. F. and Spencer, W. A.,** Habituation: A model phenomenon for the study of neuronal substrates of behavior, *Psychol. Rev.,* 73, 16, 1966.

197. **Smeets, M. A. and Dalton, P.,** Effects of occupational exposure on perceived odor and irritation of isopropanol, *Int. Arch. Occup. Environ. Health*, 2001, (submitted).

198. **Cain, W. S. and Polak, E. H.,** Olfactory adaptation as an aspect of odor similarity, *Chem. Senses,* 17, 481, 1992.

199. **Pierce, Jr. J. D., Wysocki, C. J., Aronov, E. V., Webb, J. B., and Boden, R. M.,** The role of perceptual and structural similarity in cross-adaptation, *Chem. Senses*, 21, 223, 1996.

200. **Köster, E. P.,** Adaptation and cross-adaptation in olfaction, doctoral dissertation, Bronder-Offset, N.V., Rotterdam, 1971.

201. **Cometto-Muñiz, J. E. and Cain, W. S.,** Influence of airborne contaminants on olfaction and the common chemical sense, in *Smell and Taste in Health and Disease,* Getchell, T. V., Doty, R. L., Bartoshuk, L. M., and Snow J. B., Jr., Eds., Raven Press, New York, 1991, 765.

202. **Shusterman, D. and Balmes, J.,** Measurement of nasal irritant sensitivity to pulsed carbon dioxide: a pilot study, *Arch. Environ. Health*, 52, 334, 1997.

203. **Millqvist, E. and Bende, M.,** Capsaicin cough sensitivity is decreased in smokers, *Respir. Med.,* 95, 19, 2001.

204. **Shusterman, D., and Balmes, J.,** A comparison of two methods for determining nasal irritant sensitivity, *Am. J. Rhinol.,* 11, 379, 1997.

205. **Shusterman, D., Murphy, M. A., and Balmes, J.,** The influence of sex, allergic rhinitis, and test system on nasal sensitivity to airborne irritants: A pilot study, *Environ. Health Perspect.*, 109, 15, 2001.

206. **Kjærgaard, S. K., Pederson, O. F., and Molhave, L.,** Sensitivity of the eyes to airborne irritant stimuli: Influence of individual characteristics, *Arch. Environ. Health,* 47, 45, 1992.

207. **Jensen, A. A., Higginbotham, E. J., Guzinski, G. M., Davis, I. L., and Ellish, N. J.,** A survey of ocular complaints in postmenopausal women, *J. Assoc. Acad. Minor. Phys.,* 11, 44, 2000.

208. **Stevens, J. C. and Cain, W. S.,** Aging and the perception of nasal irritation, *Physiol. Behav.,* 37, 323, 1986.

209. **Stevens, J. C., Plantinga, A., and Cain, W. S.,** Reduction of odor and nasal pungency associated with aging, *Neurobiol. Aging,* 3, 125, 1982.

210. **Wysocki, C. J., Cowart, B. J., and Radil, T.,** Localizing inhaled stimuli: A normative study of nasal-trigeminal chemosensitivity, *Percept. Psychophys.,* 2002 (in press).

211. **Shusterman, D. J., Murphy, M. A., and Balmes, J. R.,** Subjects with seasonal allergic rhinitis and nonrhinitic subjects react differentially to nasal provocation with chlorine gas, *J. Allergy Clin. Immunol.,* 101, 732, 1998.

212. **Wilhelmsson, B. and Holmstrom, M.,** Possible mechanisms of formaldehyde-induced discomfort in the upper airway, *Scand. J. Work Environ. Health*, 18, 403, 1992.

213. **Woskie, S. R., Eisen, E. E., Wegman, D. H., Hu, X., and Kriebel, D.,** Worker sensitivity and reactivity: indicators of worker susceptibility to nasal irritation, *Am. J. Ind. Med.,* 34, 614, 1998.

214. **Kjaergaard, S., Mölhave, L., and Pedersen, O. F.,** Changes in human sensory reactions, eye physiology, and performance when exposed to a mixture of 22 indoor air volatile organic compounds, in *Proceedings of the 5th International Conference on Indoor Air Quality and Climate, Indoor Air '90*, 1, 319, 1990.

215. **Ohm, M., Juto, J. E., and Andersson, K.,** Nasal hyperreactivity and sick building syndrome, in *Proceedings from the ASHRAE/ACGIH/AIHA, IAQ 92 Conference, Environment for People*, San Francisco, CA, 1992, 41.

216. **Ohm, M., Juto, J. E., Andersson, K., and Bodin, L.,** Nasal histamine provocation of tenants in a sick-building residential area, *Am. J. Rhinol.,* 11, 167, 1997.

217. **Apter, A., Hodgson, M., Lueng, W.-Y., and Pichnarcik, L.,** Nasal symptoms in the "Sick Building Syndrome", *Ann. Allergy Asthma Immunol.,* 78, 152, 1997 (abstract).

218. **Shim, C. and Williams, Jr., W. H.,** Effect of odors in asthma, *Am. J. Med.,* 80, 18, 1986.

219. **Franck, C, and Skov, P.,** Foam at inner eye canthus in office workers, compared with an average Danish population as control group, *Acta Ophthalmol. Scand.,* 67, 1, 1989.

220. **Franck, C., Bach, E., and Skov, P.,** Prevalence of objective eye manifestations in people working in office buildings with different prevalences of sick building syndrome compared with the general population, *Int. Arch. Occup. Environ. Health*, 65, 65, 1993.

221. **Tsubota, K.,** Tear dynamics and dry eye, *Prog. Retin. Eye Res.,* 17, 565, 1998.

222. **Pennebaker, J. W.,** Psychological bases of symptom reporting: perceptual and emotional aspects of chemical sensitivity, *Toxicol. Ind. Health,* 10, 497, 1994.

223. **Stellman, J. M., Klitzman, S., Gordon, G. C., and Snow, B. R.,** Air quality and ergonomics in the office: survey results and methodologic issues, *Am. Ind. Hyg. Assoc. J.,* 46, 286, 1985.

224. **Doty, R. L.,** Food preference ratings of congenitally anosmic humans, in *The Chemical Senses and Nutrition,* Kare, M. R. and Maller, O., Eds., Academic Press, New York, 1977, 315.

225. **Dalton, P., Wysocki, C. J., Brody, M. J., and Lawley, H. J.,** The influence of cognitive bias on the perceived odor, irritation and health symptoms from chemical exposure, *Int. Arch. Occup. Environ. Health,* 69, 407, 1997.

226. **McCaffrey, T. V. and Kern, E. B.**, Clinical evaluation of nasal obstruction, *Arch. Otolaryngol.,* 105, 542, 1979.

227. **Taylor, G., MacNeil, A. R., and Frud, D. L. J.,** Assesing degree of nasal patency by measuring peak expiratory flow rate through the nose, *J. Allergy Clin. Immunol.,* 52, 193, 1973.

228. **Larsen, K. and Kristensen, S.,** The peak flow nasal patency index, *Ear Nose Throat J.,* 71, 23, 1991.

229. **Terrien, M.-H., Rahm, F., Fellrath, J.-M., and Spertini, F.,** Comparison of the effects of terfenadine with fexofenadine on nasal provocation tests with allergen, *J. Allergy Clin. Immunol.*, 103, 1025, 1999.

230. **Lai, V. W. S. and Corey, J. P.,** The objective assessment of nasal patency. *Ear Nose Throat J.,* 72, 395, 1993.

231. **Clement, P. A. R.,** Committee report on standardization of rhinomanometry, *Rhinology,* 22, 151, 1984.

232. **Doty, R. L., Deems, D. A., Frye, R. E., Pelberg, R., and Shapiro, A.,** Olfactory sensitivity, nasal resistance, and autonomic function in patients with multiple chemical sensitivities, *Arch. Otolaryngol. Head Neck Surg.,* 114, 1422, 1988.

233. **Jalowayski, A. A., Yuh, Y. S., Koziol, J. A., and Davidson, T. M.,** Surgery for nasal obstruction – Evaluation by rhinomanometry, *Laryngoscope,* 93, 341, 1983.

234. **Meltzer, E. O., Jalowayski, A. A., Orgel, A., and Harris, A. G.,** Subjective and objective assessments in patients with seasonal allergic rhinitis: Effects of therapy with mometasone furoate nasal spray, *J. Allergy Clin. Immunol.,* 102, 39, 1998.

235. **Vogt, K., Hoffrichter, H., and Hasse, W.,** Hochauflösende Rhinomanometrie: Theorie, Modell und Praxis, *Annual Convention of the Working Group Clinical Immunologie, Allergologie and Environmental Medicine*, German Society for Neck, Nose, Ear Medicine, Head and Neck Surgery, Davos, Switzerland, 1999 (abstract).

236. **Cole, P.,** Toronto rhinomanometry: Laboratory, field and clinical studies, *J. Otolaryngol.,* 17, 331, 1988.

237. **Hilberg, O. and Pedersen, O. F.,** Acoustic rhinometry: recommendations for technical specifications and standard operating procedures, *Rhinol. Suppl.,* 16, 3, 2000.

238. **Roithmann, R., Chapnik, J. S., Zasmel, N., Barreto, S. M., and Cole, P.,** Acoustic rhinometric assessment of the nasal valve, *Am. J. Rhinol.,* 11, 379, 1997.

239. **Kesavanathan, J., Swift, D. L., Fitsgerald, T. K., Permutt, T., and Bascom, R.,** Evaluation of acoustic rhinometry and posterior rhinomanometry as tools for inhalation challenge studies, *J. Toxicol. Environ. Health*, 48, 295, 1996.

240. **Kane, L. E. and Alarie, Y.,** Sensory irritation to formaldehyde and acrolein during single and repeated exposures in mice, *Am. Ind. Hyg. Assoc. J.*, 38, 509, 1977.

241. **Gudziol, H. and Gramowski, K.-H.,** Respirations-Olfaktometrie - eine objektivierende Methode zur quantativen Bewertung einer Hyposmie, *Laryngorhinootologie*, 66, 570, 1987.

242. **Jalowayski, A. A., Cain, W. S., and Hong, G. K.,** A modified carbon dioxide Test (CO_2T) to evaluate nasal irritation, *Proceedings of the XIXth Annual Meeting of the Association for Chemoreception Sciences*, San Diego, 1997, (abstract).

243. **Lorig, T. S., Huffman, E., DeMartino, A., and DeMarco, J.,** The effects of low concentration odors on EEG activity and behavior, *J. Psychophysiol.*, *5*, 69, 1991.

244. **Walker, J. C., Reynolds, J. H., Warren, D. W., and Sidman, J. D.,** Responses of normal and anosmic subjects to odorants, in *Chemical Senses, Irritation,* Vol. 2, Green, B. G., Mason, J. R., and Kare, M. R., Eds., Marcel Dekker, New York, 1990, 95.

245. **Warren, D. W., Walker, J. C., Drake, A. F., and Lutz, R. W.,** Effects of odorants and irritants on respiratory behavior, *Laryngoscope*, 104, 623, 1994.

246. **Walker, J. C., Kendal-Reed, M., Hall, S. B., Morgan, W.T., Polyakov, V. V., and Lutz, R.W.,** Human responses to propionic acid. II. Quantification of breathing responses and their relationship to perception, *Chem. Senses,* 26, 351, 2001.

247. **Zajac, D. and Yates, C.,** Speech aerodynamics, in *Instrumental Clinical Phonetics*, Ball, M. and Code., C., Eds., Whurr Publishers, London, 1997, 87.

248. **Bloch, K. E., Barandun, J., and Sackner, M. A.,** Effect of mouthpiece breathing on cardiorespiratory response to intense exercise, *Am. J. Respir. Crit. Care Med.,* 151, 1087, 1995.

249. **Salah, B., Dinh Xuan, A. T., Fouilladieu, J. L., Lockhart, A., and Regnard, J.,** Nasal mucociliary transport in healthy subjects is slower when breathing dry air, *Eur. Respir. J.,* 1, 852, 1988.

250. **Wong, L., Park, C., and Yeates, D.,** Neuropeptide Y inhibits ciliary beat frequency in human ciliated cells via nPKC, independently of PKA, *Am. J. Physiol.,* 275, C440, 1998.

251. **Tay, H., Armoogum, N., and Tan, L.,** Nasal mucociliary clearance and salmeterol, *Clin. Otolaryngol.,* 22, 68, 1997.

252. **Bernstein, I. L.,** Is the use of benzalkonium chloride as a preservative for nasal formulations a safety concern? A cautionary note based on compromised mucociliary transport, *J. Allergy Clin. Immunol.,* 105, 39, 2000.

253. **Agius, A., Smallman, L., and Pahor, A.,** Age, smoking and nasal ciliary beat frequency, *Clin. Otolaryngol*., 23, 227, 1998.

254. **Kienast, K., Riechelmann, H., Knorst, M., Haffner, B., Muller-Quernheim, J., Schellenberg, J., and Ferlinz, R.,** Combined exposures of human ciliated cells to different concentrations of sulfur dioxide and nitrogen dioxide, *Eur. J. Med. Res.,* 20, 533, 1996.

255. **Andersen, I., Cammer, P., Jensen, P. L., Philipson, K., and Proctor, D. F.,** Nasal clearance in monozygotic twins, *Am. Rev. Resp. Dis.*, 110, 301, 1974.

256. **Mackay, I., Stanley, P., Greenstone, M., Holmes, P., and Cole, P.,** A nose clinic: initial results, *J. Laryngol. Otol.,* 97, 497, 1983.

257. **Kleinschmidt, E. and Witt, G.,** Evaluation of nasal mucociliary clearance with a modified saccharin test, *Laryngorhinootologie*, 74, 286, 1995.

258. **Armengot, M., Basterra, J., and Garain, L.,** Normal values of nasal mucociliary clearance. Comparison of various techniques and substance, *Acta Otorrinolaringol. Esp.*, 41, 336, 1990.

259. **Sisson, J., Yonkers, A., and Waldman, R.,** Effects of guaifenesin on nasal mucociliary clearance and ciliary beat frequency in healthy volunteers, *Chest*, 107, 747, 1995.

260. **Karnitzki, G., Mlynski, G., and Mlynski, B.,** Nasal mucociliary transport time and ciliary beat frequency in healthy probands and patients with sinusitis, *Laryngorhinootologie,* 72, 595, 1993.

261. **Lindberg, S. and Runer, T.,** Method for *in vivo* measurement of mucociliary activity in the human nose, *Ann. Otol. Rhinol. Laryngol.,* 103, 558, 1994.

262. **Paltieli, Y., Fradis, M., Ben-David, J., Podoshin, L., Shiti, H., and Kam, Z.,** *In vivo* measurement of human nasal mucociliary motility using a laser light scattering instrument, *Ann. Otol. Rhinol. Laryngol.,* 106,. 859, 1997.

263. **McMonnies, C. W. and Chapman-Davies, A.,** Assessment of conjunctival hyperemia in contact lens wearers. Part I, *Am. J. Optom. Physiol. Opt.,* 64, 246, 1987.

264. **Juto, J. E. and Lundberg, C.,** Human nasal mucosa reaction during chilling of the feet, *Rhinology,* 23, 131, 1985.

265. **Kolbeck, K. G., Ehnhage, A., and Juto, J. E.,** Nasal and bronchial histamine reactivity in patients with allergic rhinitis out of season, *Ann. Allergy Asthma Immunol*., 82, 55, 1999.

266. **Ohm, M. and Juto, J. E.,** Nasal hyperreactivity. A histamine provocation model, *Rhinology,* 31, 53, 1993.

267. **Andersen, I., Camner, P., Jensen, P. L., Philipson, K., and Proctor, D. F.,** Nasal clearance in monozygotic twins, *Am. Rev. Respir. Dis.,* 110, 301, 1974.

268. **Kumlein, J. and Perbeck, L.,** Fluorescein flowmetry in human nasal mucosa, *Acta Otolaryngol.,* 101, 286, 1986.

269. **Nitzan, M., Brama, I., and Mahler, Y.,** Measurements of nasal mucosal blood flow by a thermal clearance method, *IEEE Trans. Biomed. Eng.,* 32, 1063, 1985.

270. **Abe, Y. and Jackson, R. T.,** The use of labeled microspheres to determine blood flow in the dog's nasal mucosa, *Ann. Otol. Rhinol. Laryngol.*, 81, 82, 1972.

271. **Malm, L.,** Responses of resistance and capacitance vessels in the feline nasal mucosa to vasoactive agents, *Acta Otolaryngol.,* 78, 90, 1974.

272. **Grudemo, H. and Juto, J. E.,** Rhinostereometry and laser doppler flowmetry in human nasal mucosa: Changes in congestion and microcirculation during intranasal histamine challenge, *ORL J. Otorhinolaryngol. Relat. Spec.,* 59, 50, 1997.

273. **Grudemo, H. and Juto, J. E.,** The impact of the measuring distance on laser-doppler measurements of the microcirculation in human nasal mucosa, *ORL J. Otorhinolaryngol. Relat. Spec.,* 59, 280, 1997.

274. **Druce, H. M., Bonner, R. F., Patow, C., Choo, P., Summers, R. J., and Kaliner, M. A.,** Response of nasal blood flow to neurohormones as measured by laser-Doppler velocimetry, *J. Appl. Psychol.,* 57, 1276, 1984.

275. **Mevio, E., Perano, D., and Bulzomi, A. G.,** Correlations between the olfacto-respiratory reflex and nasal mucosa blood flow: Comparative evaluation through rhinomanometry and laser-doppler flowmeter testing, *Acta Otorhinolaryngol. Belg.,* 48, 23, 1994.

276. **Thürauf, N., Hummel, T., Kettenman, B., and Kobal, G.,** Nociceptive and reflexive responses recorded from the human nasal mucosa, *Brain Res.,* 629, 293, 1993.

277. **Peitl, B., Petho, G., Porszasz, R., Nemeth, J., and Szolcsanyi, J.,** Capsaicin-insensitive sensory-efferent meningeal vasodilatation evoked by electrical stimulation of trigeminal nerve fibres in the rat, *Br. J. Pharmacol.,* 127, 457, 1999.

278. **Olsson, P.,** A comparison between the ^{133}Xe washout and laser doppler techniques for estimation of nasal mucosal blood flow in humans, *Acta Otolaryngol*., 102, 106, 1986.

279. **Miller, R. E., Paradise, J. L., Friday, G. A., Fireman, P., and Voith, D.,** The nasal smear for eosinophils, *Am. J. Dis. Child.*, 136, 1009, 1982.

280. **Farr, B., Hackett, S. F., Winther, B., and Hendley, J. O.,** A method for measuring polymorphonuclear leukocyte concentration in nasal mucus, *Acta Otolaryngol. Suppl. (Stockh),* 413, 15, 1984.

281. **Pelikan, Z. and Pelikan-Filipek, M.,** Cytologic changes in the nasal secretions during the late nasal response, *J. Allergy Clin. Immunol.,* 83, 1068, 1989.

282. **Naclerio, R. M., Meir, H. L., Kagey-Sobotka, A., Adkinson, N. F., Meyers, D. A., Norman, P. S., and Lichtenstein, L. M.,** Mediator release after nasal airway challenge with allergen, *Am. Rev. Resp. Dis.,* 128, 597, 1983.

283. **Greiff, L., Pipkorn, U., Alkner, U., and Persson, C.,** The nasal pool device applies controlled concentrations of solutes on human airway mucosa and samples its surface exudations/secretions, *Clin. Exp. Allergy*, 20, 253, 1990.

284. **Wålinder, R., Norbäck, D., Wieslander, G., Smedje, G., Erwall, C., and Venge, P.,** Nasal patency and biomarkers in nasal lavage – the significance of air exchange rate and type of ventilation in schools, *Int. Arch. Occup. Environ. Health*, 71, 479, 1998.

285. **Noah, T. L., Henderson, F. W., Henry, M. M., Peden, D. B., and Devlin, R. B.,** Nasal lavage cytokines in normal, allergic, and asthmatic school-age children, *Am. J. Respir. Crit. Care Med.,* 152, 1290, 1995.

286. **Raphael, G. D., Druce, H. M., Baranuik, J. N., and Kaliner, M. A.**, Pathophysiology of rhinitis. 1. Assessment of the sources of protein in methacholine induced nasal secretions, *Am. Rev. Respir. Dis.,* 138, 413, 1988.

287. **Restrick, L. J., Sampson, A. P., Piper, P. J., and Costello, J. F.,** Inulin as a marker of dilution of bronchoalveolar lavage in asthmatic and normal children, *Am. J. Respir. Crit. Care Med.,* 151, 1211, 1995.

288. **Bisgaard, H., Krogsgaard, O. W., and Mygind, N.,** Measurement of secretions in nasal lavage, *Clin. Sci.,* 73, 217, 1987.

289. **Biewenga, J., Stoop, A. E., Baker, H. E., Swart, S. J., Nauta, J. J. P., Van Kamp, G. J., and Van der Baan, S.,** Nasal secretions from patients with polyps and healthy individuals collected with a new aspiration system. Evaluation of total protein and immunoglobin concentration, *Ann. Clin. Biochem.,* 28, 260, 1991.

290. **Okuda, M. and Otsuka, H.,** Basophilic cells in allergic nasal secretions, *Arch. Otorhinolaryngol.,* 214, 283, 1977.

291. **Bernheim, N., Wang, D., and Clement, P. A.,** The kinetics of the cytologic and rhinomanometric changes in the nose after challenge with allergen, *Acta Otorhinolaryngol. Belg.,* 49, 229, 1995.

292. **Knowles, G. K., Townsend, P., and Turner-Warwick, M. A.,** standardized filter paper technique for assessing nasal secretory activity, *Clin. Allergy*, 11, 287, 1981.

293. **Baroody, F. M., Wageman, M., and Naclerio, R. M.,** Comparison of the secretory response of the nasal mucosa to methacholine and histamine, *J. Appl. Physiol*., 74, 2661, 1993.

294. **Barrody, F. M., Ford, S., Lichtenstein, L. M., Kagey-Sobotka, A., and Naclerio, R. M.,** Physiologic responses and histamine after nasal allergen challenge: Effect of atropine, *Am. J. Respir. Crit. Care Med*., 149, 1457, 1994.

295. **Klimek, L. and Rasp, G.,** Normal values for eosinophil cationic protein in nasal secretions: Influence of specimen collection, *Clin. Exp. Allergy*, 29, 367, 1999.

296. **Bryan, W. and Bryan, M. P.,** Cytologic diagnosis in otolaryngology, *Trans. Am. Acad. Ophthalmol. Otolayngol*., 63, 597, 1959.

297. **Lin, R. Y., Nahal, A., Lee, M., and Menikoff, H.,** Cytologic distinction between clinical groups using curette-probe compared to cytology brush, *Ann. Allergy Asthma Immunol.,* 86, 226, 2001.

298. **Jalowayski, A. A. and Zeiger, R. S.,** Examination of nasal or conjunctival epithelium specimens, in *Manual of Allergy and Immunology: Diagnosis and Therapy*, 2nd ed., Lawlor, G. and Fische, T. J., Eds., Little Brown & Company, Boston, 1988, 238.

299. **Galindo, G., Jalowayski, A. A., and Meltzer, E. O.,** Correlation between nasal cytogram and blown technique for the diagnosis of allergic rhinitis, *Ann. Allergy,* 66, 86, 1991.

300. **Welch, M. J., Meltzer, E. O., Kemp, J. P., Orgel, H. A., Ostrom, N. K., and Jalowayski, A. A.,** Comparison of two different techniques for obtaining specimens for nasal cytology. Nose blowing vs. nasal mucosal scraping, *J. Allergy Clin. Immunol.,* 87, 144, 1991 (abstract).

301. **Lin, R. Y., Clarin, E., Lee, M., Menikoff, H., and Nahal, A.,** Patterns of nasal eosinophilia in allergy clinic patients as determined by swab and curette sampling, *Allergy Asthma Proc.,* 18, 221, 1997.

302. **Walker, J. C., Kendal-Reed, M., Utell, M. J., and Cain, W. S.,** Breathing and eye blink rate responses of humans to airborne chemicals, *Environ. Health Perspect. Suppl.,* 109, 507, 2001.

303. **Cho, P. and Yap, M.**, Schirmer test. I. A review, *Optom. Vis. Sci.,* 70, 152, 1993.

304. **Norn, M. S.,** Diagnosis of dry eye, in *The dry eye. A comprehensive guide*, Lemp, M. A. and Marquardt, R., Eds., Springer-Verlag, Berlin, 1992, 134.

305. **Proud, D., Sweet, J., Stein, P., Settipane, R. A., Kagey-Sobotka, A., Friedlaender, M. H., and Lichtenstein, L. M.,** Inflammatory mediator release on conjunctival provocation of allergic subjects with allergen, *J. Allergy Clin. Immunol.,* 85, 896, 1990.

306. **Bacon, A. S., Ahluwalia, P., Irani, A. M., Schwartz, L. B., Holgate, S. T., Church, M. K., and McGill, J. I.,** Tear and conjunctival changes during the allergen-induced early- and late-phase responses, *J. Allergy Clin. Immunol.,* 106, 948, 2000.

307. **Norn, M. S.,** External eye--methods of examination. Scriptor, Copenhagem, 1983, 112.

308. **Sørensen, T. and Jensen, F. T.,** Tear flow in normal human eyes. Determination by means of radioisotope and gamma camera, *Acta. Ophthalmol.,* 57, 564, 1979.

309. **Hansel, F. K.,** Observations on the cytology of the secretions in allergy of the nose and paranasal sinuses, *J. Allergy Clin. Immunol.,* 5, 357, 1934.

310. **Pipkorn, U. and Karlsson, C.,** Methods for obtaining specimens from the nasal mucosa for morphological and biochemical analysis, *Eur. Respir. J.,* 1, 856, 1988.

311. **McKenna, E. L.,** Nasal mastocytosis, *Laryngoscope,* 84, 112, 1974.

312. **Meltzer, E. O., Orgel, A. H., and Jalowayski, A. A.,** Cytology, in *Allergic and Non-Allergic Rhinitis. Clinical Aspects*, Myglind, N., and Naclerio, R.M., Eds., Munksgaard, Copenhagen, 1993, 66.

313. **Baroody, F. M.,** Mucosal cytology, in *Rhinologic Diagnosis and Treatment*, McCaffrey, T. V., Ed., Thieme Medical Publishers, New York. 1997, 175.

314. **Connell, J. T.,** Nasal disease: Mechanisms and classification, *Ann. Allergy*, 50, 227, 1983.

315. **Keiger, C. J. H., Case, D., Kendal-Reed, M., Jones, K. R., Drake, A. F. and Walker, J. C.,** Nicotinic cholinergic receptor expression in the human nasal mucosa, *Ann. Otol. Rhinol. Laryngol.,* 2002 (in press).

316. **Steerenberg, P. A., Fischer, P. H., Gmelig Meyling, F., Willighagen, J., Geerse, E., van de Vliet, H., Ameling, C., Boink, A. B., Dormans, J. A., van Bree, L., and Van Loveren, H.,** Nasal lavage as tool for health effect assessment of photochemical air pollution, *Hum. Exp. Toxicol.,* 15, 111, 1996.

317. **Svensson, C., Andersson, M., Persson, C., Venge, P., Alkner, U., and Pipkorn, U.,** Albumin, bradykinins, and eosinophilic cationic protein on the nasal mucosal surface in patients with hay fever during natural allergen exposure, *J. Allergy Clin. Immunol.,* 85, 828, 1990.

318. **Naclerio, R. M., Proud, D., Togias, A. G., Adkinson, N. F., Meyers, D. A., Kagey-Sobotka, A., Plaut, M., Norman, P. S., and Lichtenstein, L. M.,** Inflammatory mediators in late-induced rhinitis, *N. Engl. J. Med.,* 313, 65, 1985.

319. **Ciprandi, G., Pronzato, C., Passalacqua, G., Ricca, V., Grogen, J., Mela, G. S., Varese, P., Bertolini, C., Bagnasco, M., and Canonica, G. W.,** Topical azelastine reduces eosinopohil activation and intercellular adhesion molecule-1 expression on nasal epithelial cells: An antiallergic activity, *J. Allergy Clin. Immunol.,* 98, 1088, 1996.

320. **Calderon, M. A., Devalia, J. L., Prior, A. J., Sapsford, R. J., and Davies, R. A.,** Comparison of cytokine release from epithelial cells cultured from biopsy specimens of atopic patients with and without rhinitis and nonatopic subjects without rhinitis, *J. Allergy Clin. Immunol.,* 99, 65, 1997.

321. **Bottcher, R.,** An environmental nuisance: Odor concentrated and transported by dust, *Chem. Senses*, 26, 327, 2001.

322. **Nadel, J. A. and Widdicombe, J. G.,** Reflex effects of upper airway irritation on total lung resistance and blood pressure, *Appl. Physiol*., 17, 861, 1962.

323. **Tucker, D.,** Nonolfactory responses from the nasal cavity: Jacobson's organ and the trigeminal system, in *Handbook of Sensory Physiology, Chemical Senses,* Vol. IV, Beidler, L. M., Ed., Springer, Berlin, 1971, 151.

324. **Smith, C. J., Scott, S. M., and Ryan, B. A.,** Cardiovascular effects of odors, *Toxicol. Ind. Health,* 15, 595, 1999.

325. **Gotshall, R. W., Gootman, J., Byrnes, W. C., Fleck, S. J., and Valovich, T. C.,** Noninvasive characterization of the blood pressure response to the double-leg press exercise, *J. Exerc. Physiol. Online*, 2, 4, 1999.

326. **Atkinson, P. and Woodcock, J. P.,** *Doppler Ultrasound and Its Use in Clinical Measurement.* Academic Press, New York, 1982.

327. **Van Toller, S. and Reed, M. K.,** Brain electrical activity topographical maps produced in response to olfactory and chemosensory stimulation, *Psychiatry Res.,* 29, 429, 1989.

328. **Danuser, B.,** Candidate physiological measures of annoyance from airborne chemicals, *Chem. Senses*, 26, 333, 2001.

329. **Walker, J. C., Payne, V. M., Stancill, M. W., Bombick, D. W., Green, C. R., Hege, R. B., Conrad, F. W., Pritchard, W. S., Smith, C. J., and Doolittle, D. J.,** Assessment of possible perceptual, cognitive and affective effects of side stream smoke on non-smokers, in *Proceedings of the Smoke & Technology Meeting*, Yokohama, Japan, 1997, 37.

330. **Gevins, A. and Remond, R.,** *Handbook of Electroencephalography and Clinical Neurophysiology: Methods of Analysis of Brain Electrical and Magnetic Signals*, Elsevier Biomedical Press, Amsterdam, 1987.

331. **Loveless, N. E. and Brunia, C. H. M.,** Effects of rise-time on late components of the auditory evoked potential, *J. Psychophysiol.,* 4, 369, 1990.

332. **Kobal, G. and Hummel, C.,** Cerebral chemosensory evoked potentials elicited by chemical stimulation of the human olfactory and respiratory nasal mucosa, *Electroencephalogr. Clin. Neurophysiol.,* 71, 241, 1988.

333. **Kobal, G.,** Electrophysiological measurement of olfactory function, in *Handbook of Olfaction and Gustation*, 2nd ed., Doty, R. L., Ed, Marcel Dekker, New York, 2002, (in press).

334. **Kendal-Reed, M.,** Approaches to understanding chemosensory responses: New directions and new caveats, *Am. Ind. Hyg. Assoc. J.*, 62, 717, 2001.

335. **Geisler, M. W. and Murphy, C.,** Event-related brain potentials to attended and ignored olfactory and trigeminal stimuli, *Int. J. Psychophysiol.,* 37, 309, 2000.

336. **Kobal, G.,** Testing the analgesic activity of dipyrone in human subjects using an experimental pain model with tonic and phasic pain stimuli, in *New Pharmaco-logical and Epidemiological Data in Analgesics Research*, Brune, K., Ed., Birkhäuser, Basel, 1990, 29.

337. **Kobal, G., Hummel, C., Gruber, M., Geisslinger, G., and Hummel, T.,** Dose-related effects of ibuprofen on pain-related potentials, *Br. J. Clin. Pharmacol.,* 37, 445, 1994.

338. **Li, C., Yousem, D. M., Doty, R. L., and Kennedy, D. W.,** Evaluation of olfactory deficits by medical imaging, in *Handbook of Olfaction and Gustation*, Doty, R.L., Ed., Marcel Dekker, New York, 1995, 395.

339. **Paling, M. R., Black, W. C., Levine, P. A., and Cantrell, R. W.,** Tumor invasion of the anterior skull base: a comparison of MR and CT studies, *J. Comput. Assist. Tomogr.,* 11, 824, 1987.

340. **Sobel, N. and Yousem, D.,** Functional neuroimaging of human olfaction. in *Handbook of Olfaction and Gustation*, 2nd ed., Doty, R. L., Ed, Marcel Dekker, New York, 2002, (in press).

341. **May, A., Kaube, H., Buchel, C., Eichten, C., Rjintjes, M., Juptner, M., Weiller, C., and Diener, H. C.,** Experimental cranial pain elicited by capsaicin: a PET study, *Pain,* 74, 61, 1998.

342. **Yousem, D. M., Williams, S. C. R., Howard, R. O., Andrew, C., Simmons, A., Allin, M., Geckle, R. J., Suskin, D., Bullmore, E. T., Brammer, M.J., and Doty, R. L.,** Functional MRI imaging during odor stimulation: Preliminary data, *Neuroradiology,* 204, 833, 1997.

343. **Leondes, C. T.,** *Medical Imaging Systems Techniques and Applications*, Gordon and Breach Science Publishers, Amsterdam, 1997.

344. **Frackowiak, R. S. J., Friston, K. J., Frith, C. D., Dolan, R. J., and Mazziotta, J. C.,** *Human Brain Function*, Academic Press, New York, 1997.

345. **Ogawa, S., Lee, T. M., Kay, A. R. and Tank, D. W.,** Brain magnetic resonance imaging with contrast dependent on blood oxygenation, *Proc. Nat. Acad. Sci. USA,* 87, 9868, 1990.

346. **Raichle, M. E.,** Circulatory and metabolic correlates of brain function in normal humans, in *The Handbook of Physiology: The Nervous System*, *Higher Functions of the Brain*, Plum F. and Mountcastle, V., Eds., Vol. 5, American Physiological Association, Bethesda, 1987, 643.

347. **Roland, P. E., Kawashima, R., Gulyás, B., and O'Sullivan, B.,** Positron emission tomography in cognitive neuroscience: Methodological constraints, strategies, and examples from learning and memory, in *The Cognitive Neurosciences*, Gazzaniga, M. S., Ed., MIT Press, Cambridge, 1996, 781.

348. **Di Nardo, W., Di Girolamo, S., Galli, A., Meduri, G., Paludetti, G., and De Rossi, G.,** Olfactory function evaluated by SPECT, *Am. J. Rhinol.,* 14, 57, 2000.

349. **Ware, J. H., Spengler, J. D., Neas, L. M., Samet, J. M., Wagner, G. R., Coultas, D., Ozkaynak, H., and Schwab, M.,** Respiratory and irritant health effects of ambient volatile organic compounds: The Kanawha Valley Health Study, *Am. J. Epidemiol.,* 137, 1287, 1993.

350. **Brooks, S. M., Weiss, M. A., and Bernstein, I. L.,** Reactive airways dysfunction syndrome (RADS) persistent asthma syndrome after high level irritant exposures, *Chest,* 88, 376, 1985.

351. **Shusterman, D., Lipscomb, J., Neutra, R., and Satin, K.,** Symptom prevalence and odor-worry interaction near hazardous waste sites, *Environ. Health Perspect.,* 94, 25, 1991.

352. **First, M. W.,** Public-health aspects: management of environmental odors, in *Odors from Stationary and Mobile Sources*, National Research Council, Academy of Sciences, Washington, 1979, 441.

353. **Sparks, P. J.,** *Multiple Chemical Sensitivity/Idiopathic Environmental Intolerance*, State of the Art Reviews Occupational Medicine, Hanley & Belfus, Philadelphia, 2000.

354. **Samet, J. M. and Speizer, F.E.,** Assessment of health effects in epidemiologic studies of air pollution, *Environ. Health Perspect.,* 101, 149, 1993.

355. **Samet, J., Buist, S., Bascom, R., Garcia, J., Lipsett, M., Mauderly, J., Mannino, D., Rand, C., Romieu, I., Utell, M., and Wagner, G.,** What constitutes an adverse health effect of air pollution?, *Am. J. Respir. Crit. Care Med.,* 161, 665, 2000.

356. **Cain, W. S., Samet, J. M., and Hodgson, M.,** The quest for negligible health risk from indoor air, *ASHRAE J.,* July, 38, 1995.

357. **Burge, S., Hedge, A., Wilson, S., Bass, J. H., and Robertson, A.,** Sick building syndrome: A study of 4373 office workers, *Ann. Occup. Hyg.,* 31, 492, 1987.

358. **Andersson, K., Fagerlund, I., Bodin, L., and Ydreborg, B.,** Questionnaire as an instrument when evaluating indoor climate, in *Proceedings of the 3rd International Conference on Healthy Buildings*, *Healthy Buildings '88*, 1, 139, 1988.

359. **Malkin, R., Wilcox, T., and Sieber, W.K.,** The National Institute for Occupational Safety and Health indoor environmental evaluation experience. Part two: Symptom prevalence, *Appl. Occup. Environ. Hyg.,* 11, 540, 1996.

360. **Brightman, H. S., Wallace, L. A., Sieber, W. K., McCarthy, J. F., and Spengler, J. D.,** Comparing symptoms in United States office buildings,), in *Proceedings of the 8th International Conference on Indoor Air Quality and Climate, Indoor Air '99*, 1, 847, 1999.

361. **Mendell, M. J., Sieber, W. K., Dong, M. X., Malkin, R., and Wilcox, T.,** Symptom revalence distributions in US Office buildings investigated by NIOSH for indoor environmental quality complaints, in *Proceedings of the 7th International Conference on Indoor Air Quality and Climate, Indoor Air '96*, 2, 877, 1996.

362. **Crandall, M. S. and Sieber, W. K.,** The National Institute for Occupational Safety and Health indoor environmental evaluation experience. Part one: Building environmental evaluations, *Appl. Occup. Environ. Hyg.*, 11, 533, 1996.

363. **Robertson, A. S., Roberts, K. T., Burge, P. S., and Raw, G.,** The effect of change in building ventilation category on sickness absence rates and the prevalence of sick building syndrome, in *Proceedings of the 5th International Conference on Indoor Air Quality and Climate, Indoor Air '90*, 1, 237, 1990.

364. **Burge, S., Robertson, A. S., and Hedge, A. H.,** The development of a questionnaire suitable for the surveillance of office buildings to assess the building symptom index: A measure of the sick building syndrome, in *Proceedings of the 6th International Conference on Indoor Air Quality and Climate, Indoor Air '93*, 1, 731, 1993.

365. **Guilford, J. P.,** The correlation of an item with a composite of the remaining items in a test, *Educ. Psychol. Measur.*, 13, 87, 1953.

366. **Seffelaar, A. M., van der Zalm, C. J. A., Daamen, D. D. L., Dijksterhuis, G. G., and Punter, P. H.,** A comparison of odor annoyance survey results, *Staub – Reinhaltung der Luft,* 52, 1, 1992.

367. **Winneke, G. and Kaska, J.,** Odour pollution and odour annoyance reaction in industrial areas of the Rhine-Ruhr-region, in *Proceedings of the 6th International Symposium on Olfaction and Taste,* LeMagnen, J. and McLeod, P., Eds., Information Retrieval Services, London, 1977, 471.

368. **Winneke, G. and Kaska, J.,** Comparison of odour annoyance data from different industrial sources: Problems and implications, in *Environmental Annoyance: Characterization, Measurement and Control,* Koelega, H. S., Ed., Elsevier Science Publishers, Amsterdam, 1987, 129.

369. **Cronbach, L. J.,** Coefficient alpha and the internal structure of tests, *Psychometrika*, 16, 297, 1951.

370. **Doty, R. L.,** Diagnostic tests and assessment, *J. Head Trauma Rehabil.,* 7, 47, 1992.

371. **Andersen, I., Seedorff, L., and Skov, A.,** A strategy for reduction of toxic indoor emissions, *Environ. Int.,* 8, 11, 1982.

372. **Wolkoff, P., Johnsen, C. R., Franck, C., Wilhardt, P., and Albrechtsen, O.,** A study of human reactions to office machines in a climatic chamber*, J. Exp. Anal. Environ. Epidemiol. Suppl.,* 1, 71, 1992.

373. **Otto, D., Hudnell, H. K., House, D., Mølhave, L., and Counts, W.,** Exposure of humans to a volatile organic mixture. III. Behavioral Assessment, *Arch. Environ. Health*, 47, 23, 1992.

374. **Koren, H. S., Graham, D. E., and Devlin, R. B.,** Exposure of humans to a volatile organic mixture. III. Inflammatory response, *Arch. Environ. Health,* 47, 39, 1992.

375. **Hodgson, M. J., Frohliger, J., Permar, E., Tidwell, C., Traven, N. D., Olenchock, S. A., and Karpf, M.,** Symptoms and microenvironmental measures in non-problem buildings, *J. Occup. Med.,* 33, 527, 1991.

376. **Ten Brinke, J., Selvin, S., Hodgson, A. T., Fisk, W. J., Mendell, M. J., Koshland, C. P., and Daisey, J. M.,** Development of new VOC exposure metrics and their relationship to sick building syndrome symptoms, *Indoor Air,* 8, 140, 1998.

377. **Sundell, J., Lindvall, T., and Stenberg, B.,** Associations between type of ventilation and airflow rates in office buildings and the risk of SBS-symptoms among occupants. *Environ. Int.,* 20, 239, 1994.

378. **Weschler, C. J.,** Ozone in indoor environments: Concentration and chemistry, *Indoor Air,* 10, 269, 2000.

379. **Kjaergaard, S. K. and Pedersen, O. F.,** Dust exposure, eye redness, eye cytology and mucous membrane irritation in a tobacco industry, *Int. Arch. Occup. Environ. Health*, 61, 519, 1989.

380. **Jaakkola, J. J. K., Miettinen, O. S., Komulainen, K., Toumaala, P., and Seppänen O.,** The effect of air recirculation on symptoms and environmental complaints in office workers. A double-blind, four period cross-over study, in *Proceedings of the 5th International Conference on Indoor Air Quality and Climate, Indoor Air '90*, 1, 281, 1990.

381. **Pennebaker, J. W. and Skelton, J. A.,** Psychological parameters of physical symptoms, *Personal. Social Psychol. Bull.,* 4, 524, 1978.

382. **Mendell, M. J.,** Non-specific symptoms in office workers: A review and summary of the epidemiologic literature, *Indoor Air*, 3, 227, 1993.

383. **Bullinger, M., von Mackensen, S., and Patjens, S.,** Psycho-social determinants of the sick-building-syndrome, in *Proceedings of the 7th International Conference on Indoor Air Quality and Climate, Indoor Air '93*, 1, 459, 1996.

384. **Stenberg, B. and Wall, S.,** Why do women report 'sick building symptoms' more often than men?, *Soc. Sci. Med.,* 40, 491, 1995.

385. **Cederlöf, R., Friberg, L., Jonsson, E., Kaij, L., and Lindvall, T.,** Studies of annoyance connected with offensive smell from a sulphate cellulose factory, *Nord. Hyg. Tidskr.,* 45, 39, 1964.

386. **Friberg, L., Jonsson, E., and Cederlöf, R.,** Studies on sanitary nuisance of smoke from a sulphate cellulose factory. Parts I and II, *Nord. Hyg. Tidskr.,* 41, 41, 1960.

387. **Jonsson, E.,** Annoyance reactions to environmental odors, in *Human Responses to Environmental Odors*, Turk, A., and Johnston, Jr., J. W., Eds., Academic Press, New York, 1974, 329.

388. **Smith, W. S., Schuenman, J. J., and Zeidberg, L. D.,** Public reaction to air pollution in Nashville, Tennessee, *J. Air Pollut. Control Assoc.,* 14, 418, 1964.

Newest references:

Abraham, M.H., Zhao, Y.H., Le, J., Hersey, A., Luscombe, C.N., Reynolds, D.P., Beck, G., Sherborne, B. and Cooper, I. (2002) On the mechanism of human intestinal absorption. Eur. J. Med. Chem., **37**(7), 595-605.

Cometto-Muñiz, J.E. and Cain, W.S. (1995) Relative sensitivity of the ocular trigeminal, nasal trigeminal, and olfactory systems to airborne chemicals. Chem. Senses, **20**, 191-198.

Cometto-Muñiz, J.E. and Cain, W.S. (1998) Trigeminal and olfactory sensitivity: comparison of modalities and methods of measurement. Int. Arch. Occup. Environ. Health, **71**, 105-110.

Kasanen, J.P., Pasanen, A.L., Pasanen, P., Liesivuori, J., Kosma, V.M. and Alarie, Y. (1998) Stereospecificity of the sensory irritation receptor for nonreactive chemicals illustrated by pinene enantiomers. Arch. Toxicol., **72**(8), 514-23.

Table 1. Characteristics of the term "Chemical Sensory Irritation" as used in this review.*

Chemical Sensory Irritation				
Stimuli	Receptive structures	Sensory correlates (examples of sensations)		Other physiological correlates
Airborne chemicals	Free nerve endings, particularly those on readily exposed mucosae: Ocular, nasal, oral, and upper respiratory tract	Stinging Piquancy Burning Tingling Freshness Prickling Irritation Itching Cooling	Most of these sensations, if unwanted within a certain context, can be labeled irritative	Nasal airflow changes Breathing pattern changes Changes in secretions Changes in ciliary beat frequency Changes in blood flow Inflammation Release of mediators Electrophysiological Responses

*It should be noted that the threshold for eliciting sensory or physiological response to a volatile irritant can vary depending on the type of response being examined and the duration of exposure. For example, among non-occupationally exposed individuals, acetone is capable of eliciting cooling sensations in the nasal and ocular mucosa at approximately 500 ppm, while tingling or stinging sensations are not reported until concentrations are typically much higher (e.g., ~2500 ppm).[5] In contrast, physiological correlates can provide quite different results. Electrophysiological responses (such as the nasal evoked potential that is elicited by brief duration pulses of 2000 ppm acetone) occur when peripheral nerve endings are stimulated and signify the threshold for a purely trigeminal response that may be accompanied by subtle changes in breathing patterns.[6] Other *changes*, such as *nasal congestion or alterations in regional* blood *flow are not observed at exposure concentrations up to 6000 ppm for 5 minutes*, *whereas the* release of *secretory biomarkers such as albumin or mucin are variably altered by acetone exposures ranging from 1500 to 6000 ppm*, *depending on the exposure duration.*[7] *In fact, some studies indicate that acetone exposures as low as 500 ppm can affect inflammatory biomarkers such as peripheral white blood cell counts if repetitive daily 6 hour exposures are* experienced.[8] Notably most sensory and many of the physiological responses show marked abatement immediately or within minutes following removal from *the* exposure.

Table 2: Divisions and sub-divisions of the Trigeminal nerve (Cranial Nerve V)

Major divisions	**Sub-divisions**	**Innervations**
Ophthalmic nerve (sensory only)	Lachrymal	Conjunctivae, lachrymal glands, skin of part of upper eyelid
	Frontal (further sub-divides into *supraorbital* & *supratrochlear* nerves)	*Supraorbital*: forehead and scalp *Supratrochlear*: part of forehead and upper eyelid
	Nasociliary	Nasal septum, mucus membrane, areas of dermis near tip of nose
Maxillary nerve (mixed sensory and motor)	Infraorbital	Skin and conjunctivae of lower eyelid, skin on side of nose, cheek and upper lip
	Anterior and posterior *superior alveolar* branches	Gums, teeth and mucus membrane of maxillary sinus
Mandibular nerve (mixed sensory and motor)	Sensory fibers become *auriculotemporal*, *lingual* and *inferior alveolar* nerves	*Auriculotemporal*: parotid gland, tempero-mandibular joint, auricle, skin & fascia of scalp and temple *Lingual*: mucosa of part of tongue, floor of mouth, sublingual salivary gland *Inferior alveolar*: lower teeth

FIGURE CAPTIONS

Figure 1. An artist's representation of the regions within the nasal and oral cavities innervated by each of several cranial nerves. CN I = olfactory nerve; CN V = trigeminal nerve; CN IX = glossopharyngeal nerve; CN X = vagal nerve. CN VII (facial nerve) innervates the taste buds in the anterior tongue and is not shown in this diagram. The cross hatched area represents regions of overlap between CN IX and X. CN I may extend farther down onto the middle turbinate that depicted here.

Figure 2. Schematic diagram of the branches of the trigeminal nerve that innervate the nasal, oral and ocular epithelia. From B. Bryant and W.L. Silver.[37]

Figure 3. Low-power electron micrograph (x 670) of a longitudinal section through a biopsy specimen of human olfactory mucosa taken from the nasal septum. Four cell types are indicated: ciliated olfactory receptors (c), microvillar cells (m), supporting or sustentacular cells (s), and basal cells (b). The arrows point to ciliated olfactory knobs of the bipolar receptor cells. d = degenerating cells; bs = base of the supporting cells; lp = lamina propria; n = nerve bundle; bg = Bowman's gland. Photograph courtesy of David T. Moran.

Figure 4. The Burghard OM4/B air-dilution olfactometer, a devise that presents odorants or irritants to the nasal chamber at well-defined quantities and durations. Left: Subject being presented with nasal stimulants and performing a computerized visual attention task. Right: Data collection module. Center: olfactometer body. Photo courtesy of the University of Pennsylvania Smell and Taste Center, Philadelphia, PA.

Figure 5. Illustration of the the similarity of nasal pungency (squares) and eye irritation (triangles) thresholds in humans towards a variety of vapor compounds. Bars, sometimes hidden by the symbol, indicate standard deviations.

Figure 6. Examples of four rating scales. From left to right: (a) A standard category scale in which the subject provides answers in discrete categories; (b) a visual analog or graphic scale with anchors (descriptors) at each end; (c) a category scale with logarithmic visual density referents to denote non-linear increasing magnitudes of sensation, with verbal anchors at each end; (d) a labeled magnitude scale with labels or anchors positioned in logarithmic fashion. In these examples the scales are oriented in a vertical position; in many cases, such scales are presented in a horizontal (left:right) configuration.

Figure 7. A typical series of breathing waveforms produced during a brief exposure to a single odorant or irritant stimulus, which is generated by a computerized olfactometer. In the "Pre" period, the experimental participant receives only warmed and humidified pure air, during which the breathing pattern is recorded as a baseline. An exhalation ("A") controls the onset ("B") of recording the breathing pattern responses to the stimulus, to which the participant is exposed throughout the "During" period. Typically, many of the responses during breathing pattern analysis are seen during the first inhalation after stimulus onset ("C"). Reproduced, with permission, from Walker *et al.*[302].

Figure 1:

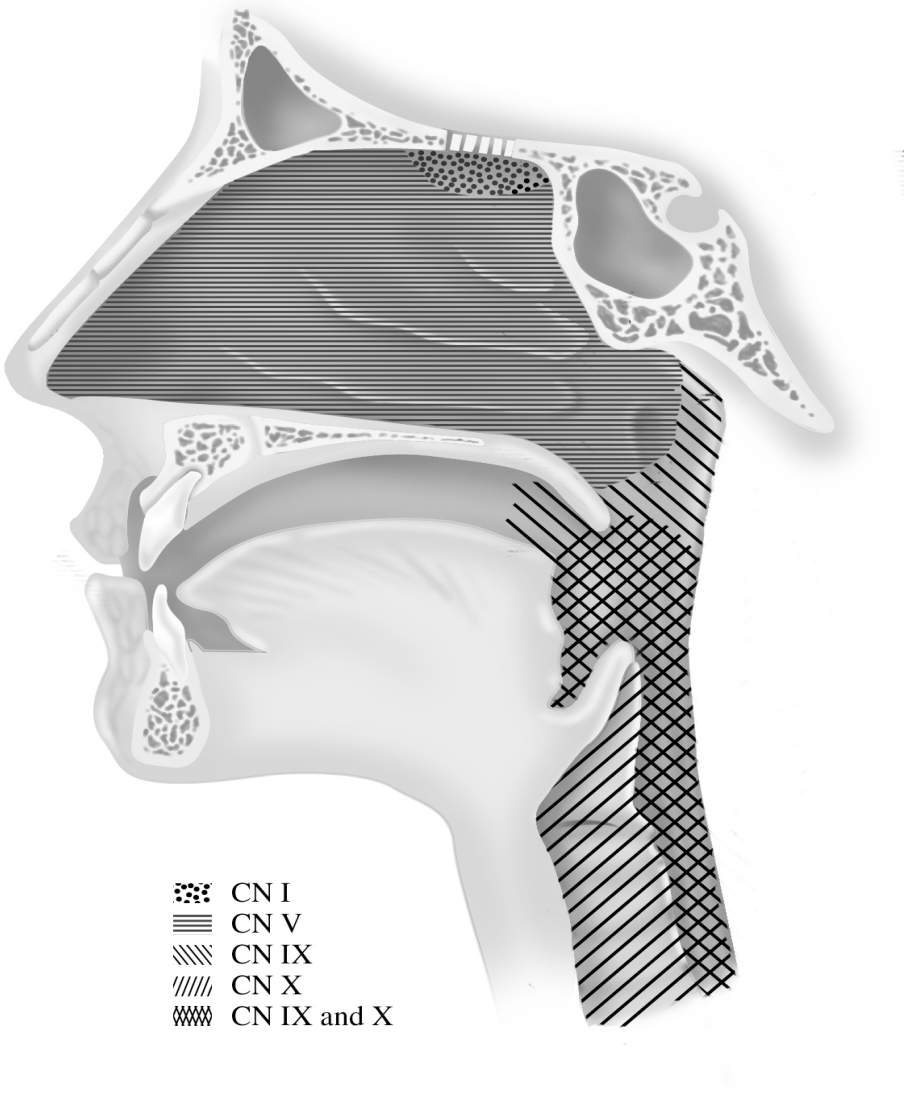


Figure 2:

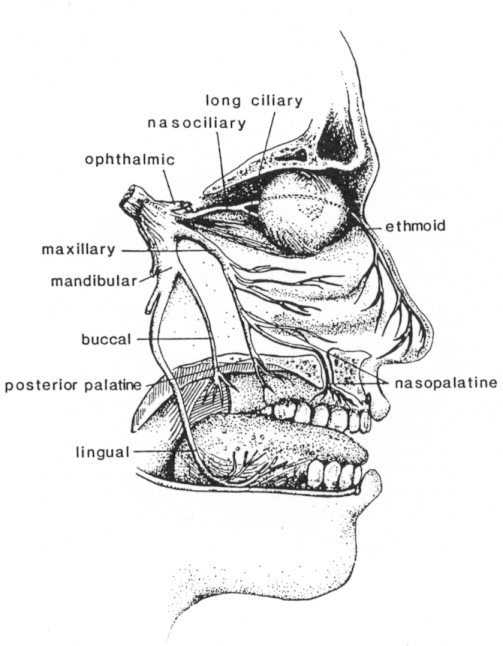

long ciliary
nasociliary
ophthalmic
ethmoid
maxillary
mandibular
buccal
posterior palatine
nasopalatine
lingual

Figure 3.

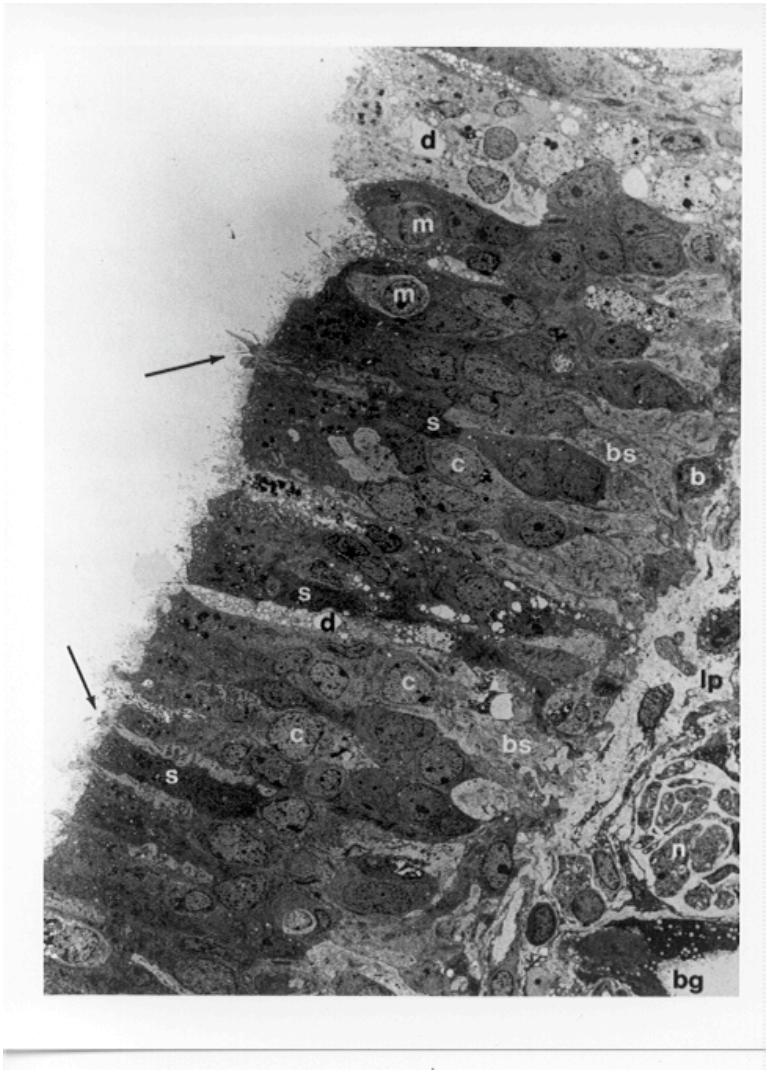


Figure 4: Burghardt Olfactometer

Figure 5:

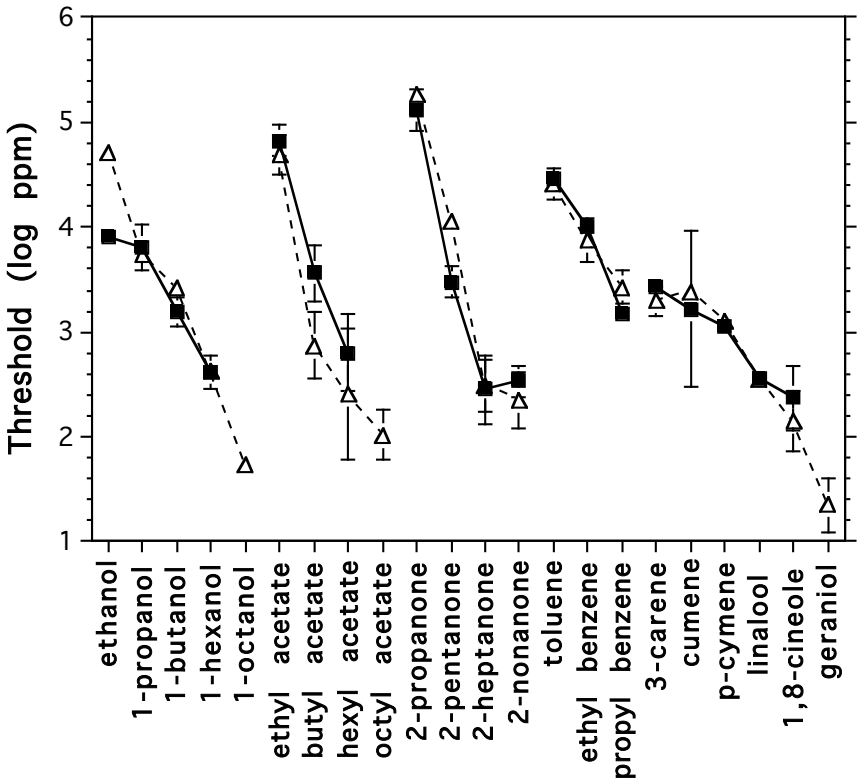

Threshold (log ppm)
6
5
4
3
2
1
ethanol
1-propanol
1-butanol
1-hexanol
1-octanol
ethyl acetate
butyl acetate
hexyl acetate
octyl acetate
2-propanone
2-pentanone
2-heptanone
2-nonanone
toluene
ethyl benzene
propyl benzene
3-carene
cumene
p-cymene
linalool
1,8-cineole
geraniol

Figure 6

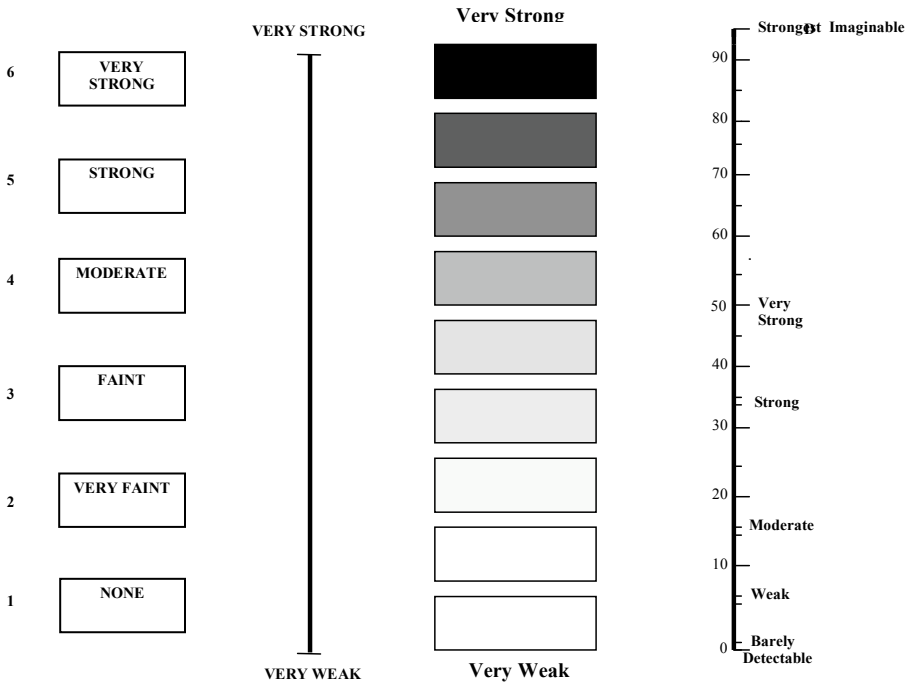
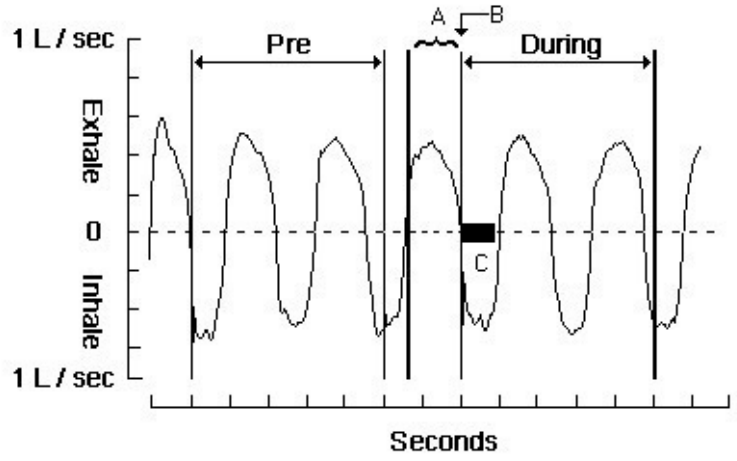

6
VERY STRONG
5
STRONG
4
MODERATE
3
FAINT
2
VERY FAINT
1
NONE
VERY STRONG
VERY WEAK
Very Strong
Very Weak
Strongest Imaginable
90
80
70
60
50
Very Strong
40
Strong
30
20
Moderate
10
Weak
0
Barely Detectable

Figure 7:



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