

## Mixtures of Volatile Organic Compounds: Detection of Odor, Nasal Pungency, and Eye Irritation

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### INTRODUCTION

Human beings can typically sense, in sufficient concentration, almost any organic vapor via smell, though some substances have lower thresholds than others by as many as nine orders of magnitude (see Cain, 1988; Devos, Patte, et al., 1990). The reasons for this spread in potency remain surprisingly obscure. In part, it seems due to physicochemical properties, such as solubility. In aliphatic series, for example, more lipid-soluble members generally have greater potency (Cometto-Muñiz and Cain, 1994). This holds only up to a certain point in the series, where potency shows either no further increase or an actual decrease. Such an outcome suggests that, in addition to solubility or some correlated property, molecular size may also determine odor potency. That is, even with sufficient vapor pressure to become airborne in quantity, molecules can be too big to stimulate effectively.

At some concentration, typically above the odor threshold, organic vapors may also trigger chemesthesis, i.e., may be felt rather than smelled. The chemesthetic modality or common chemical sense (CCS) is mediated in the mucosae of the face by the trigeminal nerve. The difference in concentration between when a substance can be smelled and when that same substance can be felt depends in part upon its reactivity towards mucosal tissue. Aggressive vapors, such as formaldehyde, acrolein, ozone, and others, may damage tissue and thereby be felt. In these cases, the vapors often have chemesthetic thresholds in the rough vicinity of their odor thresholds. For formaldehyde, for example, nasal irritation occurs at a concentration only a few times higher than the odor threshold (Berglund and Shams Esfandabad, 1992).

Most organic vapors in the environment do not react readily with mucosal tissue, yet these too may have some ability to stimulate the chemesthetic sense. In such cases, the concentration required for detection via feel lies at least an order of magnitude, and in some instances many orders of magnitude, above that required for detection via odor. In recent investigations, we have charted the difference between olfactory and chemesthetic thresholds for various aliphatic series of chemicals (Cometto-Muñiz and Cain, 1990; Cometto-Muñiz and Cain, 1991; Cometto-Muñiz and Cain, 1993; Cometto-Muñiz and Cain, 1994). In general, the chemesthetic outcome of interest was nasal pungency, though

in some cases it was ocular pungency (eye irritation) (Cometto-Muñiz and Cain, 1995). As with odor potency, chemesthetic potency increased with chain length, though not in exactly the same way. Chemesthetic potency increased somewhat more predictably. For example, it increased with chain length in much the same way from one series to another. In this behavior, chemesthesis mimicked phenomena such as narcosis and anesthesia that these same organic vapors can induce under appropriate conditions.

Our results have also implied that, to a first approximation: a) chemesthetic detection via the eye occurs at about the same concentration as chemesthetic detection via the nose, and b) the threshold of detection via feel occurs at the same level of saturated vapor concentration irrespective of the substance or the series it comes from. Decades ago, Ferguson (Ferguson, 1939; Ferguson, 1951) articulated a principle that various phenomena, e.g., anesthesia, occurred at fixed percentages of saturated vapor, or, as he expressed it, at criterion levels of thermodynamic activity. This in turn suggested an equilibrium between concentration in the vapor phase and concentration in the biophase where biological activation occurred. Insofar as a biological effect takes place at a criterion percentage of saturated vapor only for members within a particular series, it is not sufficient to imply the veracity of the Ferguson principle. Insofar as the effect takes place at a criterion level across all series, it does. For chemesthesis, it seems to hold roughly (Abraham, Andonian-Haftvan, Cometto-Muñiz, and Cain, 1995).

Nielsen and associates (Nielsen and Alarie, 1982; Nielsen, 1991) argued for a protein receptor as the site of transduction for chemesthetic responses to nonreactive irritants, though they admitted that such compounds might exert their action through nonspecific physical adsorption. It is tempting to speculate that the rules of chemesthesis indicated in our various studies imply stimulation without need to invoke a receptor. Certainly, high quantities of organic chemicals can have direct effects on membranes. For example, high quantities can change fluidity and can allow ion flux across a membrane. Such phenomena can generally account for narcosis and anesthesia, two of the very biological properties seen as analogous to sensory irritation. Regarding olfaction, recent studies point towards the existence of many protein receptors (Buck and Axel, 1991). Olfaction distinguishes itself from chemesthesis in remarkable variation in sensory quality such as skunky, fishy, floral, minty, ethereal, and so on. The many receptors of olfaction presumably permit the discriminations upon which such qualitative variation is based. Chemesthesis exhibits only trivial qualitative variation in comparison.

A quantitative structure-activity relationship (QSAR) based upon a linear solvation energy relationship (LSER) has described the relative potency of organic vapors for a number of biological effects (e.g., anesthesia, respiratory depression) in animal models (Abraham, 1993). Abraham and colleagues have demonstrated that a five-parameter equation that describes the biophase as a solvent for impinging vapors can account for 97% or more of variance in potency (Abraham, Whiting, et al., 1990; Abraham, 1993; Abraham, Nielsen, et al., 1994). A roughly analogous four-parameter solution of an LSER for nasal irritation in humans has accounted for 96% of the variance (Abraham, Andonian-Haftvan, et al., 1995).

Our experimental strategy has entailed testing subjects diagnosed (Cain, 1989) as anosmic (i.e., lacking olfaction) to chart thresholds for nasal pungency independently from smell. We also test normosmic subjects (i.e., persons with normal olfaction) matched to the anosmics in age, gender, and smoking-status to map corresponding odor thresholds. In the present extension of our series of studies, we explored thresholds for odor, nasal pungency, and eye irritation for mixtures of the relatively nonreactive compounds studied previously individually.

Most studies of mixtures of odoriferous vapors have examined suprathreshold perception via ratings of intensity (e.g., Olsson, 1994). Such studies have found that the perceived intensity of mixtures falls short of the sum of the intensities of their (unmixed) components (e.g., Berglund and Olsson, 1993). Perceived intensity of suprathreshold mixtures also falls somewhat below predictions made from "addition" of concentration but

much closer to it than addition of sensory magnitude (Cain, Schiet, et al. 1995). Suprathreshold experiments with pungent odorants at concentrations that clearly appealed also to the nasal CCS yielded a concentration-dependent degree of addition of perceived intensity (Cometto-Muñiz, García-Medina, et al., 1989). Whereas suprathreshold odor in such mixtures displayed hypoadditivity of sensation, nasal pungency displayed additivity or even hyperadditivity (Cometto-Muñiz and Hernández, 1990).

Exploration of differences in "additivity" between odor and pungency at suprathreshold levels has had no counterpart at the threshold level. Few investigations have explored potency of odor in mixtures, and none has apparently explored potency of nasal pungency in mixtures. The outcomes for odor have uncovered simple stimulus agonism (Guadagni, Buttery, et al., 1963; Patterson, Stevens, et al., 1993) and some evidence of synergistic stimulus agonism, particularly for mixtures of three or more components (Rosen, Peter, et al., 1962; Baker, 1963; Laska and Hudson, 1991). Simple agonism in, for example, a balanced three-component mixture implies that when the substances are presented mixed, each needs to be at only 1/3 its individual threshold concentration for the mixture to be perceived. Synergistic agonism implies — following the same example of a three-component mixture — that individual substances need to be at a concentration even lower than 1/3 of their respective thresholds for the mixture to be perceived; whereas partial agonism implies that they need to be at a concentration higher than 1/3 of their respective thresholds but less than the threshold value itself. Independence implies that at least one of the components in the mixture needs to be at its individual threshold for the mixture to be perceived. Finally, antagonism implies that the components need to be at concentrations higher than their individual thresholds for the mixture to be perceived.

In view of earlier research in olfaction and in view of the success of an LSER to predict nasal pungency, we expected both olfaction and chemesthesis to show simple agonism with some possibility of an increase in agonism toward synergy as the mixtures increased in number of components.

## METHODS

### Stimuli

The stimuli included members of homologous series of alcohols (1-propanol, 1-butanol, 1-hexanol), esters (ethyl acetate, hexyl acetate, heptyl acetate), ketones (2-pentanone, 2-heptanone), and alkylbenzenes (toluene, ethyl benzene, and propyl benzene). All were analytical-grade reagents. Single chemicals tested for the three relevant sensory responses of odor, nasal pungency, and eye irritation were: 1-propanol, 1-hexanol, ethyl acetate, heptyl acetate, 2-pentanone, 2-heptanone, toluene, ethyl benzene, and propyl benzene. Mineral oil served as solvent to prepare three-fold dilution steps of the pure (100 %v/v) substance (i.e., 33, 11, 3.7, 1.1, etc., %v/v). Due to the limited solubility of 1-propanol in mineral oil, the first two members of its series, 33 and 11 %v/v, were prepared in deionized water.

Five mixtures were prepared using mineral oil as solvent: two 3-component mixtures (labeled A and B, below), two 6-component mixtures (labeled C and D), and one 9-component mixture (labeled E). Mixture A comprised: 1-propanol, ethyl acetate, and 2-pentanone. Mixture B comprised: 1-hexanol, heptyl acetate, and 2-heptanone. Mixture C comprised: 1-propanol, 1-butanol, ethyl acetate, 2-pentanone, toluene, and ethyl benzene. Mixture D comprised: 1-hexanol, 1-heptanol, hexyl acetate, heptyl acetate, 2-heptanone, and propyl benzene. Mixture E comprised: 1-propanol, 1-hexanol, ethyl acetate, heptyl acetate, 2-pentanone, 2-heptanone, toluene, ethyl benzene, and propyl benzene. These particular mixtures were chosen to give one 3-component (i.e., A) and one 6-component (i.e., C) mixture of relatively low molecular weight, high vapor pressure chemicals; one 3-

component (i.e., B) and one 6-component (i.e., D) mixture of higher molecular weight, lower vapor pressure chemicals; and one 9-component mixture (i.e., E) having both kinds of chemicals.

Substances in each liquid mixture were present in proportions that reflected their odor thresholds measured in our previous studies (Cometto-Muñiz and Cain, 1990; Cometto-Muñiz and Cain, 1991; Cometto-Muñiz and Cain, 1993; Cometto-Muñiz and Cain, 1994). This was done by using a "reference" that contained, for each liquid mixture, components at their individual odor thresholds as found previously. For example, the odor thresholds for 1-propanol, ethyl acetate, and 2-pentanone equaled 0.0051, 0.0017, and 0.0017%v/v, respectively, and the reference for this three-component mixture therefore had these concentrations. Based on such a reference, threefold steps in concentration were prepared for each mixture both above and below the reference. Accordingly, the first step for mixture A above the reference was: 0.015 (0.0051 X 3), 0.0051 (0.0017 X 3), and 0.0051 (0.0017 X 3)%v/v, respectively, for 1-propanol, ethyl acetate, and 2-pentanone. The first step below the reference was: 0.0017 (0.0051 ÷ 3), 0.00056 (0.0017 ÷ 3), 0.00056 (0.0017 ÷ 3)%v/v, respectively, for the same chemicals in the same order. Steps above the reference continued until they reached the maximum value that could be presented in a mixture of the specified proportions (e.g., for mixture A: 33, 11, and 11%v/v of 1-propanol, ethyl acetate, and 2-pentanone, respectively). Steps below the threshold continued until they fell definitively below the odor threshold for even the most sensitive subject.

We prepared duplicate series for each of the nine single chemicals and each of the five mixtures. Stimuli were delivered from cylindrical, squeezable polyethylene bottles (270 ml capacity) (Cain, 1989) containing 30 ml of solution. For measurements of odor and nasal pungency, the bottle closure had a pop-up spout that fit into the nostril being tested. Each nostril was tested separately. For measurements of eye irritation, the bottle caps held a tube that led to a 25-ml measuring chamber (of the type used in variable volume dispensers), the rim of which was placed around the eye. Each eye was tested separately. The tube that fed the chamber was connected to the headspace of the bottle. A squeeze of the bottle delivered a puff of vapor into the measuring chamber where the eye was exposed. A polyethylene dust cover closed the open end of the measuring chamber when the bottle was not in use.

The concentration of each compound in the headspace of every bottle was measured by a gas chromatograph (photoionization detector) equipped with a gas sampling valve, allowing direct sampling of the headspace. For every single or mixed stimulus, repeated chromatographic readings were taken at each dilution step. Readings were also taken from bottles containing pure chemicals (100 %v/v). The headspace of a bottle with a pure chemical contains vapor saturated with the chemical at room temperature (23 °C). The concentration of the saturated vapor from each substance was retrieved from the literature. Knowledge of saturated vapor concentration (at 23°C) and its associated average chromatographic reading allowed conversion of the readings from the other bottles into concentration units (ppm by volume), and derivation of a calibration curve.

## Subjects

Eight subjects, four males and four females, provided values for odor and eye irritation thresholds. The subjects covered a wide range of age (21 to 60 years) in order to match the group of anosmics available for testing nasal pungency thresholds. Participants included a male and a female in each of the following categories: early twenties, early thirties, early forties, and late fifties/sixty. A 21 year-old male became unavailable after being tested for odor thresholds. In order to complete the group, he was replaced by a 22 year-old male tested only for eye irritation thresholds.

Four anosmic subjects, two males and two females, provided values for nasal pungency thresholds. One participant was a head-trauma anosmic (male, 66 years old), the

other three were congenital anosmics (a male, 43 years old, and two females, 20 and 62 years old, respectively).

### Procedure

On odor and nasal pungency trials, participants delivered the stimulus by inserting the pop-out probe inside the specified nostril and squeezing the bottle as they sniffed. On eye irritation trials, they squeezed the bottle while the eye was exposed in the measuring chamber. Subjects quickly learned to squeeze and sniff with equal vigor on every trial.

We used a forced-choice ascending method of limits to measure threshold. The subject had to choose the stronger smelling or stronger feeling of two stimuli. One was a blank of mineral oil and, at the start, the other was a high dilution-step, low concentration of the stimulus, either single chemical or mixture. If the choice was correct, testing continued with the same step from the duplicate set, also paired with a blank. If the choice was incorrect, testing continued with the next step — a liquid-phase concentration three times higher — paired with a blank. In this way, correct choices entailed the presentation of the same concentration, whereas errors triggered step-wise increments in concentration. The procedure continued until five correct choices were made in a row, in which case that step was taken as threshold. Once the threshold was measured for one nostril or eye, the other nostril or eye was tested. After this, testing began again with another stimulus. Both the ascending-concentration approach to the threshold and the separate testing of each nostril helped to minimize effects of adaptation, frequently encountered in olfactory investigations (see Cometto-Muñiz and Cain, 1995).

Sessions typically lasted between one and two hours and were repeated until eight thresholds per subject, four for each nostril or eye, were obtained for each single compound and each mixture. This equaled a total of 64 odor or eye irritation thresholds per stimulus and 32 nasal pungency thresholds per stimulus.

### Data analysis

Geometric means served to summarize threshold concentrations within and among subjects. The geometric mean acknowledges that threshold data for chemosensory stimuli conform to log normal distributions (Brown, Mac Lean, et al., 1968; Amoore, 1986; Cain and Gent, 1991).

For analysis of agonism in mixtures, consider the case of a three-component mixture with a formulation that reflects perfectly a subject's relative sensitivity to its three components unmixed. Such a mixture would be perfectly balanced. If the components were simply to add their individual effects, detection would occur when each component fell at one-third the concentration of its unmixed threshold. Hence, if component A had a threshold of 3 ppm, component B of 9 ppm, and component C of 27 ppm, and if the mixture were made up in the proportions 1:3:9, respectively, for balance, then we would expect detection for the mixture to occur with component A at 1 ppm, component B at 3 ppm, and component C at 9 ppm.

The relative contribution of components in a just-detectable, three-component mixture can be reflected in the formula:  $WS = a(R_A) + b(R_B) + c(R_C)$ , where WS stands for weighted sum;  $R_A$  = concentration of A in mixture ÷ threshold A;  $R_B$  = concentration of B in mixture ÷ threshold B;  $R_C$  = concentration of C in mixture ÷ threshold C; and with a, b, and c as weighting coefficients to reflect the degree of balance in the mixture. These weighting coefficients in our three-component mixture are defined as follows:  $a = [R_A / (R_A + R_B + R_C)] * 3$ ;  $b = [R_B / (R_A + R_B + R_C)] * 3$ ;  $c = [R_C / (R_A + R_B + R_C)] * 3$ . The second factor (3 in our case) of each coefficient represents the number of components in the mixture.

In our example,  $WS = 1(1/3) + 1(1/3) + 1(1/3) = 1$ , which implies complete agonism of stimulating power. In an unbalanced mixture, which occurs more commonly than not because no single subject has exactly the same sensitivity as the reference group used to formulate the mixture, a, b, and c will take on different values, but will always add up to 3 for a three-component mixture, to 6 for a six-component mixture, and to 9 for a nine-component mixture. Irrespective of the relative weights of the components, whenever WS lies at 1.0 simple agonism will have held. Whenever WS lies significantly above or below 1.0, then departure from simple agonism will have held, as follows: 1) WS above 1.0 implies that the components do not add their sensory potency completely when mixed (partial agonism or even antagonism) and 2) WS below 1.0 implies that the components have gained sensory potency when mixed (synergistic agonism).

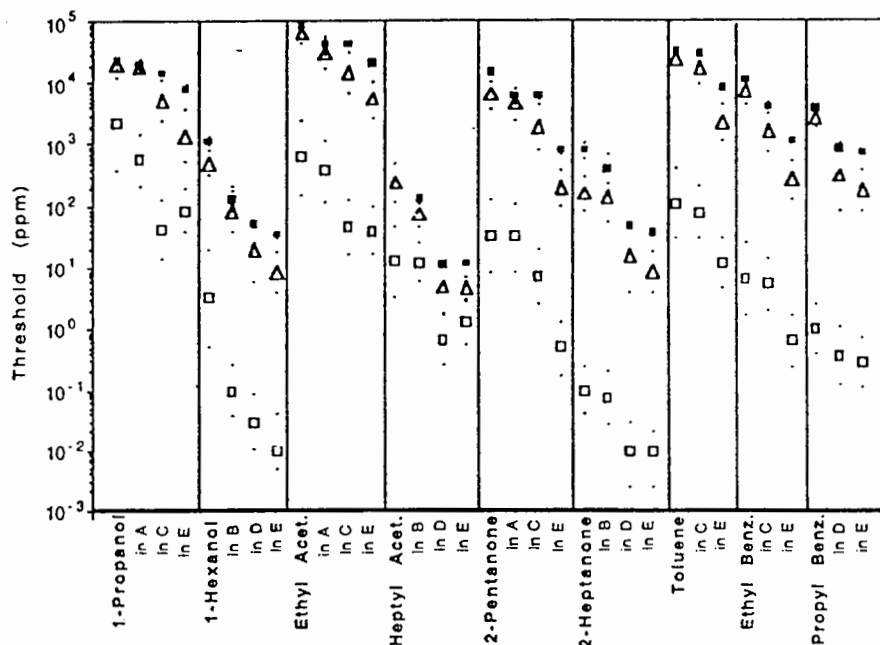


Figure 1. Thresholds (ppm  $\pm$  SD) for nasal pungency (filled squares), eye irritation (triangles), and odor (empty squares). The SD is indicated by the dots. The nine sections of the graph correspond to nine substances. Each section lists, first, the threshold for the substance by itself (e.g., 1-propanol), then, consecutively, the level at which that substance was present when the threshold was achieved for mixtures of increasing complexity, e.g., 1-propanol in A (3 components) when A achieved threshold, 1-propanol in C (6 components) when C achieved threshold, 1-propanol in E (9 components) when E achieved threshold.

## RESULTS

Figure 1 presents thresholds (ppm by volume) for all three sensory responses plotted for each of the nine substances. The graph is divided in nine sections one per substance. Within a section, the first value corresponds to the threshold (odor, eye irritation, and nasal pungency) of the substance when presented alone. Subsequent thresholds correspond to the concentration at which the substance was present when that particular

mixture reached threshold (e.g., 1-propanol in A corresponds to the level of 1-propanol found in mixture A when the mixture achieved threshold). A common trend in the three sensory responses and all chemicals is for thresholds to decline with increasing number of components in the mixture. This indicates that some degree of stimulus agonism is taking place in the mixtures to help precipitate odor, eye irritation, and nasal pungency when each component is below its individual sensory threshold. It should be pointed out that pungency thresholds for heptyl acetate and propyl benzene presented alone could only be measured in two to four of eight repetitions per subject (depending on the anosmic). Probably as a result of this, the pungency threshold for mixture D could only be measured in two to five of the eight repetitions. Hence, values given for the pungency of these two chemicals and of mixture D do not represent the average of all anosmics on all repetitions, but do represent the average of those cases where a threshold was obtained.

Table 1. Weighted sum (WS) of ratios of mixed thresholds/unmixed thresholds.

|                       | Three Components |           | Six Components |           | Nine Components |
|-----------------------|------------------|-----------|----------------|-----------|-----------------|
|                       | Mixture A        | Mixture B | Mixture C      | Mixture D | Mixture E       |
| <b>Odor</b>           |                  |           |                |           |                 |
| Subject 1             | 1.0              | 6.6       | 0.9            | 1.8       | 1.4             |
| 2                     | 4.9              | 2.8       | 6.3            | 1.6       | 1.3             |
| 3                     | 6.4              | 1.9       | 3.7            | 0.4       | 1.2             |
| 4                     | 3.7              | 3.0       | 2.3            | 3.2       | 2.0             |
| 5                     | 2.6              | 6.3       | 6.5            | 1.4       | 1.8             |
| 6                     | 5.9              | 1.4       | 2.0            | 0.6       | 1.0             |
| 7                     | 5.0              | 0.3       | 7.2            | 2.3       | 2.0             |
| 8                     | 2.8              | 4.7       | 4.2            | 1.1       | 3.0             |
| Geo. Mean             | 3.6              | 2.5       | 3.4            | 1.3       | 1.6             |
| <b>Eye Irritation</b> |                  |           |                |           |                 |
| Subject 1             | 2.2              | 6.0       | 3.1            | 0.6       | 0.8             |
| 2                     | 1.6              | 0.9       | 2.0            | 0.4       | 0.5             |
| 3                     | 1.9              | 1.4       | 3.0            | 0.2       | 0.6             |
| 4                     | 1.9              | 1.6       | 2.6            | 0.6       | 0.6             |
| 5                     | 1.0              | 0.6       | 2.9            | 0.4       | 0.7             |
| 6                     | 2.1              | 1.1       | 1.4            | 0.03      | 0.3             |
| 7                     | 3.3              | 2.2       | 2.6            | 0.2       | 0.9             |
| 8                     | 2.0              | 4.6       | 1.1            | 0.1       | 0.3             |
| Geo. Mean             | 1.9              | 1.7       | 2.2            | 0.2       | 0.6             |
| <b>Nasal Pungency</b> |                  |           |                |           |                 |
| Subject 1             | 2.1              | 0.7       | 5.8            | 0.8       | 1.7             |
| 2                     | 2.2              | 2.3       | 7.0            | 0.5       | 1.5             |
| 3                     | 1.3              | 2.0       | 6.6            | 1.0       | 1.5             |
| 4                     | 2.4              | 0.9       | 6.7            | 1.1       | 1.8             |
| Geo. Mean             | 1.9              | 1.3       | 6.5            | 0.8       | 1.6             |

Table 1 shows, subject-by-subject, values of WS from the formula given in the section on data analysis. For averages across subjects, 12 out of 15 cases yielded WS's above 1.0 which implies a general tendency for components to act, at most, as partial

agonists in mixtures. This varied, however, both with sense modality and with complexity of a mixture.

To compare odor with eye irritation, an analysis of variance (ANOVA) was performed on the logarithm of the values in Table 1 with the two variables modality (two levels: odor vs eye irritation) and mixtures (five levels) in a repeated-measures design (recall that seven subjects were common to all measures, with the eighth subject in each group matched to a counterpart in the other). The results revealed a significant difference between modalities ( $F[1,7]=25.86$ ,  $p=0.001$ ), with eye irritation showing higher stimulus agonism; a significant difference among mixtures ( $F[4,28]=14.01$ ,  $p<0.00005$ ), with the more complex mixtures tending to show greater agonism; and a significant interaction of modality by mixture ( $F[4,28]=4.37$ ,  $p=0.007$ ) which reflected the tendency for complexity to have more leverage for eye irritation.

ANOVA on the same values, but excluding mixture E, which contained substances of both low and high lipophilicity gave a view of the variables modality, complexity of mixture, and lipophilicity. The results again revealed significant differences for modality ( $F[1,7]=18.98$ ,  $p=0.003$ ), as well as for three vs six components ( $F[1,7]=9.38$ ,  $p=0.02$ ), with six showing greater agonism, and for lipophilicity ( $F[1,7]=11.18$ ,  $p=0.01$ ), with the mixtures (B and D) of the more lipophilic substances showing greater agonism. The two-way interaction of number of components by lipophilicity and the three-way interaction were also significant ( $F[1,7]=23.97$ ,  $p=0.002$  and  $F[1,7]=8.71$ ,  $p=0.02$ , respectively).

**Table 2.** Results of 95% confidence intervals on log WS. Expected value for agonism = 0, i.e., log of 1.0.

| Modality       | Mixture | n | Mean   | S.D.  | Result              |
|----------------|---------|---|--------|-------|---------------------|
| Odor           | A       | 8 | 0.551  | 0.263 | Partial agonism     |
|                | B       | 8 | 0.390  | 0.436 | Partial agonism     |
|                | C       | 8 | 0.532  | 0.317 | Partial agonism     |
|                | D       | 8 | 0.115  | 0.296 | Agonism             |
|                | E       | 8 | 0.207  | 0.151 | Partial agonism     |
| Eye Irritation | A       | 8 | 0.281  | 0.145 | Partial agonism     |
|                | B       | 8 | 0.238  | 0.343 | Agonism             |
|                | C       | 8 | 0.344  | 0.167 | Partial agonism     |
|                | D       | 8 | -0.645 | 0.444 | Synergistic agonism |
|                | E       | 8 | -0.261 | 0.180 | Synergistic agonism |
| Nasal Pungency | A       | 4 | 0.285  | 0.124 | Partial agonism     |
|                | B       | 4 | 0.113  | 0.246 | Agonism             |
|                | C       | 4 | 0.813  | 0.035 | Partial agonism     |
|                | D       | 4 | -0.093 | 0.157 | Agonism             |
|                | E       | 4 | 0.203  | 0.046 | Partial agonism     |

Two ANOVAs were analogously performed on log WS for nasal pungency. The first was a one-way ANOVA that showed significant differences among mixtures ( $F[4,12]=18.40$ ,  $p<0.00005$ ). The second was a two-way ANOVA that again excluded mixture E and showed significant differences for lipophilicity ( $F[1,3]=50.26$ ,  $p=0.006$ ), with the more lipophilic substances showing greater agonism, and for the interaction



number-of-components by lipophilicity ( $F[1,3]=12.10$ ,  $p=0.04$ ), but not for number of components.

Ninety-five percent confidence intervals around the log WSs allowed us to determine if the values for each mixture and modality differed significantly from 0 (simple agonism), i.e., if WS differed significantly from 1.0. The results, shown in Table 2, reinforce the picture that mixtures increase their degree of stimulus agonism with increasing number of components and increasing lipophilicity of components. Eye irritation even reached synergistic agonism for the most lipophilic (D) and the most complex (E) mixtures.

## DISCUSSION

Studies of odor mixtures at threshold levels have been very few and have generally entailed no vapor-phase calibration of the stimuli, typically liquid solutions of odorant presented in open containers. Without such calibration, the data could in any instance reflect departures from Raoult's law (proportionality between the vapor-pressure of a solute and its mole fraction in solution) as readily as they do the biological rules of how humans detect mixtures.

A study of odor thresholds for three chemicals (1-butanol, p-cresol, and pyridine) relevant to off-flavors in drinking water yielded simple agonism most commonly in binary mixtures and synergistic agonism in the ternary mixture (Rosen, Peter, et al., 1962). A study of food-related chemicals by Guadagni et al. (Guadagni, Buttery, et al., 1963) also yielded simple agonism among components in all but one of 20 mixtures composed of two to 10 components. The components included various saturated and unsaturated aliphatic aldehydes, an alcohol, a carboxylic acid, a sulfide, and an amine. Another study motivated by the flavor of drinking water yielded synergistic agonism for many binary mixtures of eight starting materials (m-cresol, pyridine, 1-butanol, acrylonitrile, n-amyl acetate, 2,4-dichlorophenol, acetophenone, n-butyl mercaptan) and for the eight-component mixture (Baker, 1963). The investigation yielded antagonism for the pair m-cresol—acetophenone.

More recently, Laska and Hudson (Laska and Hudson, 1991) measured odor thresholds for various single chemicals (isoamyl acetate, alpha-pinene, cyclohexanone, cineole, linalool, (-)-carvone, t-butylcyclohexyl acetate, methylpropylketone, and decyl acetate) and for three-component mixtures. The general outcome suggested simple-to-synergistic agonism.

The trend of the data therefore lay towards simple-to-synergistic agonism, with greater synergism for more complex mixtures. When, in a recent investigation, we studied a psychophysically balanced, rigorously-calibrated ternary mixture of 1-butanol, 2-pentanone, and n-butyl acetate, we also obtained simple agonism (Patterson, Stevens, Cain, and Cometto-Muñiz, 1993). Hence, when the mixture achieved odor threshold, the headspace concentration of each component was approximately one third that of the individual odor threshold of the component. This outcome lent credence to earlier results with non-calibrated stimuli insofar as it indicated that the olfactory system could display simple agonism.

The present data argue for partial as opposed to simple agonism, though degree of agonism tended toward simple as the number of components increased. Differences among stimuli and among people (see Table 1) both need further attention in the quest for generalities. For at least the present stimuli, it seems reasonably certain that irritation exhibits more agonism than odor and that eye irritation exhibits the most. Lipophilicity seems important in mixtures for both modalities.

We have yet to develop any theory to explain why agonism would increase with complexity. Theory might need to await definitive dismissal of the possibility that the tendency for agonism to increase with complexity results from a complexity-dependent vulnerability towards imbalance in mixtures, whether for odor or irritation. For simple mixtures (e.g., two or three components), small errors of measurement could mimic just

about any outcome, from synergistic agonism to antagonism. Scrutiny of our data revealed no significant correlation between degree of imbalance, measured as root-mean-squared deviation from perfect balance, and degree of agonism. Nevertheless, the matter merits continued attention in the choice of conditions in future investigations. Despite the penalty of additional work, complex mixtures will reveal more about agonism than will simple mixtures.

As if to add insult to injury, the proper study of agonism should also include variation in the proportions of components in mixtures. Measurements should therefore include psychometric (stimulus-response) functions for each component unmixed and against the many backgrounds of various proportions of the components. A ten-component mixture, studied in a few subjects, could consequently entail millions of sensory and analytical measurements. Would it be worth the effort of a few person-years of work to make such measurements in a strategically-chosen mixture. In our view, it would, both for theoretical and practical understanding. We see our present work and our work on single chemicals chosen from homologous chemical series as relevant to the choice of that strategic mixture.

What are the practical implications of what we know now for human detection of VOCs in indoor air, where scores may be present simultaneously? In any such mixture, the more lipophilic substances will have much more weight as sensory stimuli. The LSER developed by Abraham, Andonian-Haftvan, Cometto-Muñiz, and Cain (1995) gives quantitative predictions of exactly how much. In mixtures, lipophilicity takes on even more importance, for it may increase the "gain" in detection, but agonism would in any case reduce the amount by which any single substance needed to be present to contribute to detection of irritation or odor. Conservatively predicted, the "gain" from complexity would be just proportional to the level of complexity. In that case, a 100-component mixture, balanced for the effect of its components, would require 1/100th of the threshold of each component to cause a sensory effect. The possibility that complexity-dependent agonism may actually lead to synergistic agonism, no matter what the sensory channel, would make the prediction of a 100-fold gain conservative possibly by orders of magnitude. In reality, though, mixtures may be dominated by just a few components and until we know just how agonism operates in such unbalanced cases via the study of varying proportions we will be left with an uncomfortable degree of uncertainty.

## Summary

Thresholds for detection of odor, nasal pungency (irritation), and eye irritation were measured for single volatile organic compounds (VOCs) (1-propanol, 1-hexanol, ethyl acetate, heptyl acetate, 2-pentanone, 2-heptanone, toluene, ethyl benzene, and propyl benzene) and certain mixtures of them (two three-component mixtures, two six-component mixtures and one nine-component mixture). Nasal pungency was measured in persons lacking a functional sense of smell to avoid interference from olfaction. The results showed the existence of various degrees of stimulus agonism (additive effects) in the three sensory channels. Such agonism increased with the complexity of the mixtures and with the lipophilicity of their components. Eye irritation even showed synergistic stimulus agonism for the most lipophilic (one of the six-component) and the most complex (the nine-component) mixtures. The results indicate that complex chemical environments may enable chemosensory, and particularly irritative, detection when single VOCs lie far below their individual thresholds. Even the present rather rudimentary state of knowledge of how lipophilicity influences detection of single VOCs and how it and complexity (number of components) influence detection of mixtures would presumably allow refinement of a measure such as total mass (or concentration) of volatile organic compounds (TVOC) into a much more meaningful index that we might call the perceptually weighted level of VOCs.

or PWVOC. Such an index, like the index dB(A) for sound, could make an otherwise strictly physical measurement at least somewhat predictive of human responses.

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| 16. ABSTRACT<br>The paper gives results of the measurement of threshold responses of odor, nasal pungency (irritation), and eye irritation for single chemicals (1-propanol, 1-hexanol, ethyl acetate, heptyl acetate, 2-pentanone, toluene, ethyl benzene, and propyl benzene), and their mixtures (two three-component mixtures, two six-component mixtures, and one nine-component mixture). Nasal pungency was measured in subjects lacking a functional sense of smell (i. e., anosmics) to avoid interference from olfaction. Various degrees of stimulus agonism (additive effects) were observed for each of the three sensory channels when testing mixtures. As the number of components and the lipophilicity of such components in the mixtures increased, so did the degree of agonism. Synergistic stimulus agonism characterized the eye irritation response for the most complex (the nine-component) and the most lipophilic (one of the six-component) mixtures. Physicochemical properties play a large role in the determination of sensitivity to airborne chemicals, particularly to their ability to evoke irritation. Whereas this has revealed itself previously with respect to single chemicals, it seems to have relevance to mixtures as well. |  |  |                       |
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