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Journal

Journal of applied toxicology : JAT, 22(1)

ISSN

0260-437X

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Publication Date

2002

Data Availability

The data associated with this publication are within the manuscript.

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Journal of Applied Toxicology **22**:25–30,2002

Psychometric Functions for the Olfactory and Trigeminal Detectability of Butyl Acetate and Toluene

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Running head: Olfactory and Trigeminal Psychometric Functions

Abstract

We measured psychometric (i.e., concentration–response) functions for the detection of odor, nasal pungency, and eye irritation from butyl acetate and toluene. Olfactory detection was measured in subjects with normal olfaction (i.e., normosmics) for whom nasal trigeminal detection does not interfere since it requires much higher concentrations. Nasal trigeminal detection, called nasal pungency, was measured only in subjects lacking olfaction (i.e., anosmics) to avoid odor interference. Ocular trigeminal detection, called eye irritation, was measured in both groups. The method employed entailed a two-alternative, forced choice procedure with presentation of increasing concentrations. The outcome showed, for both chemicals, similar ocular trigeminal chemosensitivity in normosmics and anosmics, and similar overall ocular and nasal trigeminal chemosensitivity. Olfactory sensitivity was much higher than both forms of trigeminal sensitivity by a concentration difference of 6 and 4 orders of magnitude for butyl acetate and toluene, respectively. Detectability plots (i.e., detection performance vs. log concentration) for the three sensory endpoints followed an S-shaped function with a middle range section that showed a robust linear fit ($r > 0.94$) on graphs of *z*-score vs. log concentration. These detectability functions allow calculation of olfactory and trigeminal thresholds at various levels of performance. At a point half-way between random and perfect detection, trigeminal and olfactory threshold concentrations were, respectively, 0.67 (±0.32) and 2.28 (±1.77) log units lower than those measured by us in the past for the same chemicals and using an analogous procedure but under just one, fixed, level of performance. The available data suggests that, although considerably laborious, detectability functions provide chemosensory thresholds of closer relevance to environmentally realistic conditions (e.g., whole-body exposures).

Key words: Odor - Nasal pungency - Eye irritation - Olfactory Nerve - Trigeminal nerve - Butyl acetate - Toluene - Chemosensory detectability functions

Introduction

Properly conducted and controlled whole-body exposures to airborne chemicals constitute the gold standard to assess the chemosensory impact of environmental pollutants. Nevertheless, work with such large-scale environmental chambers cannot proceed at the pace required for studies focused not just on one or a few compounds but looking at the broader and more fundamental issue of the physicochemical basis for human chemosensory detection of airborne chemicals [1,2]. Dilution techniques relying on static or dynamic principles (see [3]) and that restrict the delivery of the chemical stimulus to one or more face mucosae (i.e., nasal, ocular, oral) are typically more suitable tools for the basic knowledge sought in the fundamental-research approach. Another aspect of human chemosensory responses to chemicals, and certainly not a minor one, deals with the separation between a purely olfactory sensory response (i.e., odor) and one that also includes a trigeminal or chemesthetic (see [4,5]) nasal component (i.e., nasal pungency or irritation).

The chemicals selected for testing in this investigation include butyl acetate and toluene. The first one is a linear, aliphatic hydrocarbon, with an oxygen-containing chemical functionality (ester). The second one is a cyclic, aromatic hydrocarbon with no oxygen-containing chemical groups. Their selection followed the general idea of testing two compounds with substantial structural and physicochemical differences to mark a contrast with a previous study using identical methodology but employing the relatively similar compounds 1-butanol and 2-heptanone [6].

Butyl acetate can be considered a typical non reactive volatile organic compound (VOC), relevant to a number of occupational settings, and whose chemosensory and perceptual properties have been studied in the context of relatively long (20 min up to 4 hours), whole-body exposures [7,8]. The concentrations tested in these chamber studies

ranged from about 3 to up to 300 ppm. Toluene is another common solvent widely present in both domestic and industrial settings, particularly in the printing industry [9]. It has also been the target of a variety of investigations focusing on long exposures (up to 7 hours for up to 3 consecutive days) in environmental chambers [8,10–13]. The concentration of toluene used in these studies was as high as 100 ppm, and, in some experiments, it reached a peak of 300 ppm every 30 min.

An important aspect of our experimental approach to the study of the chemosenses aims at understanding the physicochemical determinants of olfactory and trigeminal potency for nonreactive VOCs. Our investigations entail very short exposures (1–2 sec), a simple but effective static delivery system: the squeeze bottles [14,15], and the consistent use of a standardized procedure (e.g., [16]). As described below, the method consists of a two-alternative, forced-choice procedure with presentation of increasing concentration steps until achieving a fixed criterion for threshold: five correct choices in a row (e.g., [17]). In the present study we have applied this very same methodology but, rather than fixing a criterion for threshold, we decided to build complete psychometric functions (also called detectability or stimulus-response functions) covering the overall range from chance detection to virtually perfect detection. Build-up of these functions entails a considerable amount of testing and subject-time but they provide comprehensive information on the characteristics of both the stimulus and the chemosensory system [6,18,19].

Experimental

Subjects. Four normosmics (one male, three females) provided measures of odor and eye irritation detectability. The male was 56 years old, and the females were 25, 29, and 39 years old. All were nonsmokers.

Four congenital anosmics (one male, three females) provided measures of nasal pungency and eye irritation detectability. The male was 60 years old, and the females were 29, 41, and 44 years old. All were nonsmokers.

A standardized olfactory test [20] served to classify participants as normosmics or anosmics. The study protocol was approved by the Human Subjects Committee of the University of California, San Diego. All subjects gave written informed consent on forms approved by the Committee.

Stimuli and Equipment. Stimuli included butyl acetate (99+%) and toluene (99.8%). Mineral Oil (Light, Food Chemical Codex quality) served as solvent and blank. Duplicate dilution series made in two-fold dilution steps were prepared for each chemical. Each series started with undiluted chemical (100% v/v), labeled dilution step 0, and continued with 50, 25, 12.5, etc. % v/v, labeled dilution steps 1, 2, 3, etc., respectively. Stimuli were stored and delivered from cylindrical, squeezable, high-density polyethylene bottles (270-ml capacity) containing 25 ml of solution. For nasal testing, the bottles had a cap with a pop-up spout that fitted inside a nostril, allowing to test each nostril separately [17]. For ocular testing, the bottles had a cap of the sort used in variable volume dispensers leading to a 25-ml, roughly conical reservoir, the rim of which was placed around the eye, allowing to test each eye separately upon squeezing of the bottle [21].

Vapor concentrations in the headspace of every bottle were measured off-line by gas chromatography (flame ionization detector, FID) via direct sampling through a gas-tight syringe or a gas-sampling valve (1ml sampling loop). Measurements were done right after preparation of the stimuli, concomitantly with testing, and after all subjects were tested, to confirm stability. Figure 1 shows the average vapor-phase concentration (±SD) that corresponds to each liquid dilution step of butyl acetate and toluene. The

readings from all bottles were referred to those of the bottles containing undiluted stimulus, which were assumed to contain saturated vapor at room temperature (23°C). The coefficient of variation across dilution steps for the gas chromatographic measurements performed over time averaged (±SD) 6.9% (±2.3) for butyl acetate and 5.0% (±3.0) for toluene.

Insert Figure 1 about here

Procedure. To obtain stimulus-response (psychometric) functions for odor, nasal pungency, and eye irritation from the single chemicals we employed a two-alternative, forced choice procedure with presentation of ascending concentrations. Briefly, the method requires the subject to chose, on each trial, the stronger of two stimuli. Unknown to the participant, one stimulus is always a blank (mineral oil) and the other a dilution step of the chemical (starting with a step clearly below detection). Over the course of a session, and in ascending order of concentration, each step is presented paired with a blank a total of eight times (half with each nostril/eye). Testing for each nostril/eye ends when the subject chooses the chemical over the blank eight times in a row, four for each of two consecutive dilution steps. This performance was considered 100% detection. The ascending concentration order and an interstimulus interval of at least 45 sec to 1 min helped to minimize any potential changes in olfactory and/or trigeminal sensitivity [22].

Each subject participated in four sessions as the one described above. In each session, the subject provided two complete psychometric functions, one nasal and one ocular, for one chemical. The chemicals and sites (nose, eye) were tested in irregular order. The data from the two sessions per chemical were averaged within individuals and across individuals from the same group, i.e., normosmic or anosmic.

Data Analysis. Plots of detection probability as a function of stimulus concentration (in ppm by volume) summarize the outcome. Detection probability is corrected for chance [23] and ranges from 0.0, that is, chance detection, to 1.0, that is, perfect detection. These plots typically produce ogival psychometric functions that become linear when detection probabilities (*p*) are transformed into *z*–scores [24]. A normal distribution table is used to convert *p* values into *z*–scores.

Results

Psychometric functions of eye irritation for butyl acetate and toluene measured in normosmics fell into register with those measured in anosmics (Figure 2). In fact, for both chemicals, the average eye irritation function measured across all subjects (normosmics and anosmics) fell into register with the nasal pungency function measured in anosmics (Figure 3). Given this outcome, we averaged all functions representing trigeminal response, i.e., eye irritation (from both groups of subjects) and nasal pungency, into one sensory irritation function per chemical and compared it with the corresponding odor function (Figure 4).

Insert Figures 2, 3, and 4 about here

All functions show the typical ogival shape with an approximately linear component in the middle range. Relative position along the concentration axis (x) and slope over the linear range help to set apart olfactory from trigeminal responses for the two tested chemicals. In terms of relative position, odor functions, compared to sensory irritation functions, are displaced towards lower concentrations by an average 6 (butyl acetate) or 4 (toluene) orders of magnitude. In terms of slope along the linear range, odor functions show slopes around 0.36 (butyl acetate and toluene) whereas irritation functions show steeper slopes, around 0.82 (butyl acetate) or 1.7 (toluene). Thus, there

were differences in slope between the two compounds for sensory irritation but not for odor (Figure 5).

Insert Figure 5 about here

Discussion

The measurement of sensory thresholds in the chemical senses, particularly for olfaction (e.g., [18,19]), has been characterized by a large variability. Issues of psychophysical methodology and stimulus delivery system (see [1,3,25]), apart from true individual differences among subjects, certainly play an important role in the variability observed.

Our typical approach to measure odor, nasal pungency, and eye irritation thresholds across a wide variety of volatile organic compounds (VOCs) comprised the following standard features: 1) Use of a fairly simple stimulus delivery system, the often employed plastic "squeeze bottles" [20]. 2) Use of a double-blind, two-alternative, force choice procedure with presentation of ascending concentrations and a threshold criterion of five correct choices in a row (e.g., [17]). 3) Repeated vapor-phase measurements of concentration in the headspace of every bottle via gas chromatography (flame ionization detector or photoionization detector). 4) Separation of trigeminal from olfactory response in the nose through the use of anosmics. 5) Approximation to a "stimulus continuum" by testing homologous chemical series where carbon chain length serves as a convenient "unit of chemical change" to which the sensory results can be related.

Systematic application of this uniform methodology to the measurement of chemosensory thresholds for up to more than four dozen VOCs [2] in combination with a

solvation chemical model [26] allowed the build-up of robust quantitative structure-activity relationships for odor [27], nasal pungency [28], and eye irritation [29].

In the present study, we explored the issue of olfactory and trigeminal sensitivity to two common VOCs but resorted to measure complete psychometric functions rather than to measure a threshold according to a fixed performance criterion. Obtaining a psychometric function, particularly for the chemical senses, entails considerable more experimental work than just measuring a threshold based on a performance criterion, but psychometric functions provide more detailed information to help characterize the sensory modality. For example, psychometric functions were the outcome of choice when we probed into the rules of chemosensory agonism between 2-heptanone and 1-butanol [6]. Figure 6 illustrates how the outcome from the two approaches compares. In this figure, results from psychometric functions include those reported here for butyl acetate and toluene, and those reported previously for 2-heptanone and 1-butanol [6]; results from thresholds obtained under a fixed performance criterion for all four chemicals were taken from previous studies [17,21,28-32]. The technique employed to obtain all criterion thresholds (odor, nasal pungency, and eye irritation) was similar to the one employed here and also consisted of a two-alternative, forced-choice procedure with presentation of increasing concentrations. Unlike the present procedure, though, selection of the blank over the chemical automatically forced the presentation of the next higher concentration, also paired with the blank. Threshold was defined as the first concentration for which the chemical was selected over the blank five times in a row.

Insert Figure 6 about here

Overall, the outcome reveals that, for both chemosensory modalities and for all four chemicals, the concentration threshold obtained with the fixed criterion method is best approached by the value of the detectability function at $p = 1.00$, that is, close to

perfect (100%) detection. Nevertheless, in some cases (e.g., odor of butyl acetate and toluene, nasal pungency of the same chemicals, and eye irritation of toluene) the sensory threshold via fixed criterion is even higher than via detectability function at p = 1.00. This is most likely due to the fact that, under the fixed criterion procedure, selection of the blank by the subject, in the two-alternative forced choice, is followed by a step increase in concentration with no possibility of returning to a lower concentration. It is interesting to point out that, averaging across chemicals, trigeminal detection (including nasal pungency and eye irritation) measured via detectability function at p = 0.50 (i.e., half-way between random and perfect detection) produces concentration values that are 0.67 (±0.32, standard deviation) log units (in ppm by volume) lower than those produced via the fixed criterion method. In turn, the same trend is seen for olfactory detection but the difference between the two methods increases to 2.28 (±1.77, standard deviation) log units.

In a recent study we have shown that, compared to the squeeze bottles, an improved stimulus-delivery system based on glass vessels can drive nasal pungency thresholds uniformly down across chemicals by an average (±SD) of 0.57 (±0.23) log units, taking those values closer to what can be expected in environmentally realistic conditions (i.e., whole-body exposures) [1]. This same study also showed that, although the absolute values for nasal pungency thresholds were lower when obtained via the glass vessels (compared to the squeeze bottles), their relative position and trend across chemicals remained the same under both delivery systems. The improvements brought by the glass vessels included a tight nose-nosepiece connection to avoid stimulus dilution and a larger volume of the vapor-source to accommodate whole sniffs. In the present investigation we show that by building psychometric or detectability functions, instead of measuring a single threshold based on a fixed criterion of performance, we can also arrive at lower concentration values for trigeminal as well as for olfactory

detection (even when employing the simple squeeze bottles), bringing them to what could be obtained under realistic exposures.

The present results represent acute sensory responses to the compounds investigated, and, though the approach produces valuable information as discussed above, it does not address the influence of time of exposure or of the simultaneous presence of many chemicals. In environmentally realistic conditions people can be exposed for long periods (from hours or days up to months or even years) to low-levels of dozens of airborne chemicals. A comprehensive understanding of chemosensory detection needs to elucidate the effects of stimulation time, e.g., olfactory adaptation [33] and chemesthetic sensitization/adaptation [34], as well as the effects of mixtures of chemicals [35].

Acknowledgments

The work described in this article was supported by research grant numbers R29 DC 02741 and R01 DC 02741 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health, and by the Center for Indoor Air Research. Thanks are due to René Loya and Regina Meijninger for excellent technical assistance.

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Figure Legends

Figure 1. Average and variability (standard deviation, SD) of vapor-phase concentrations of all dilutions of butyl acetate and toluene, as measured by gas chromatography (FID detector). Bars indicating SD are sometimes hidden by the symbol.

Figure 2. Showing how the functions for detectability of eye irritation in normosmics (filled diamonds) and in anosmics (empty diamonds) display close agreement, both in the case of butyl acetate (continuous lines) and of toluene (broken lines). Each point represents the outcome of 64 judgments per concentration (half with each eye) made by 4 subjects.

Figure 3. Showing how the functions for detectability of eye irritation in normosmics and anosmics (diamonds) and for detectability of nasal pungency in anosmics (squares) display close agreement, both in the case of butyl acetate (continuous lines) and of toluene (broken lines). For eye irritation, each point represents the outcome of 128 judgments per concentration (half with each eye) made by 8 subjects (4 normosmics and 4 anosmics); for nasal pungency, each point represents the outcome of 64 judgments per concentration (half with each nostril) made by 4 anosmics.

Figure 4. Functions for detectability of odor (circles) and of sensory irritation (i.e., eye irritation and nasal pungency combined) (squares) of butyl acetate (continuous lines) and of toluene (broken lines). For odor, each point represents the outcome of 64 judgments per concentration (half with each nostril) made by 4 normosmics; for sensory irritation, each point represent the outcome of 192 judgments per concentration (half with each nostril/eye) made by 8 subjects (4 normosmics and 4 anosmics). Bars, sometimes hidden by the symbol, indicate standard errors (SE).

Figure 5. Upper part. Detectability functions for the odor (circles) and sensory irritation (i.e., nasal pungency and eye irritation combined) (squares) of butyl acetate (continuous lines) and toluene (broken lines). Data along the middle of the detectability range (filled circles and squares) were approximated by a linear function whose parameters, for each sensory modality and chemical, are presented at the right side of the figure. Lower part. Same linear range as depicted above but with the y-axis converted into *z*-scores, the formally proper unit for linearization of psychometric functions.

Figure 6. Comparison of detection threshold concentration for four chemicals measured via two approaches (see text): 1) build-up of a complete psychometric (i.e., detectability) function with selection of three levels of detectability: p=0.50 (i.e., half-way between random and perfect detection), p=0.75 (i.e., three quarters of the way), and p=1.00 (i.e., perfect detection), and 2) measurement of a threshold based on a fixed performance criterion (5 out of 5 trials correct). Upper graph. Trigeminal chemosensory detection (nasal pungency and eye irritation). Lower graph. Olfactory chemosensory detection (odor). P.F.: psychometric function; F.P.C.: fixed performance criterion.

FIGURE 1

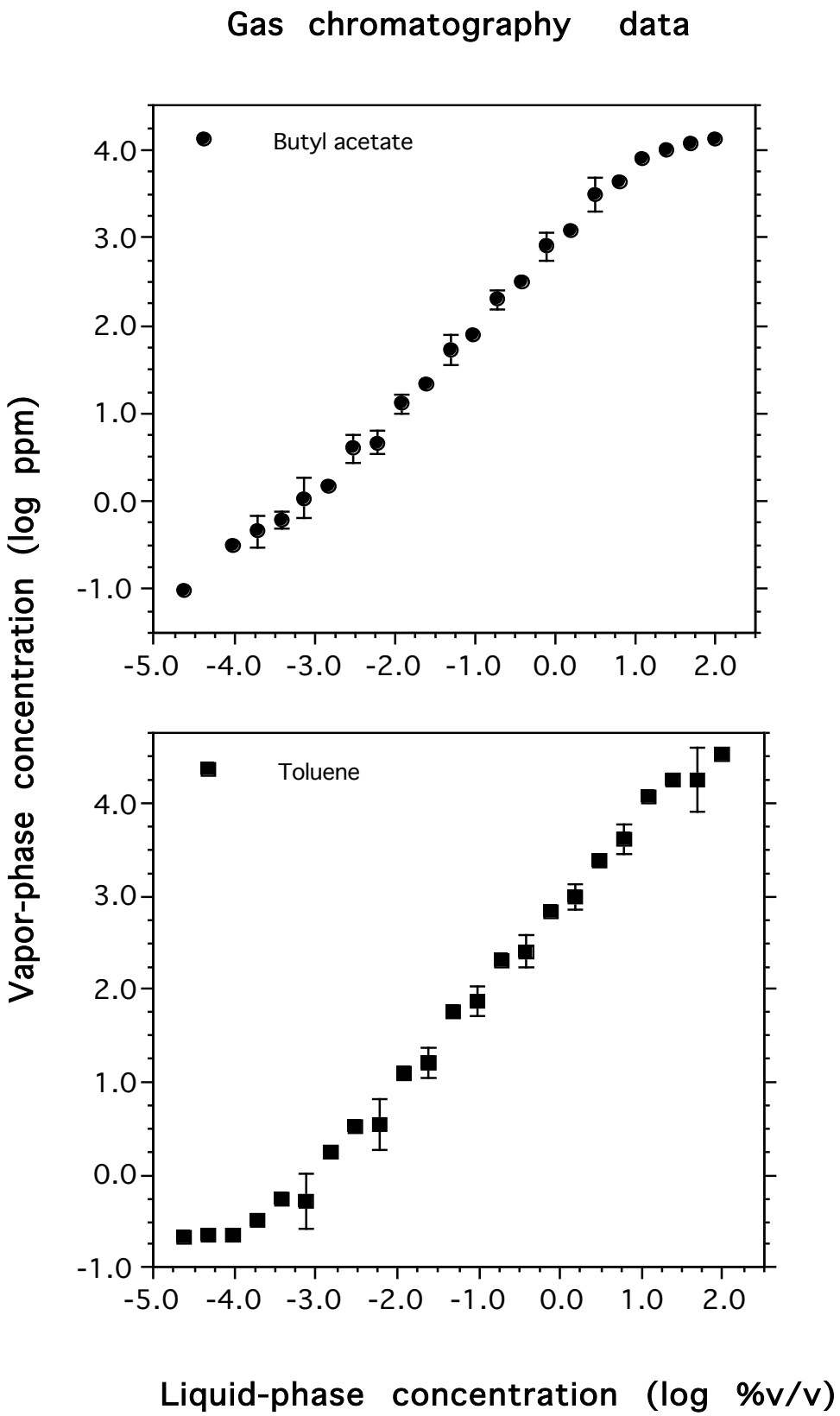

Gas chromatography data
Butyl acetate
Toluene
Vapor-phase concentration (log ppm)
Liquid-phase concentration (log %v/v)
4.0
3.0
2.0
1.0
0.0
-1.0
-5.0
-4.0
-3.0
-2.0
-1.0
0.0
1.0
2.0

FIGURE 2

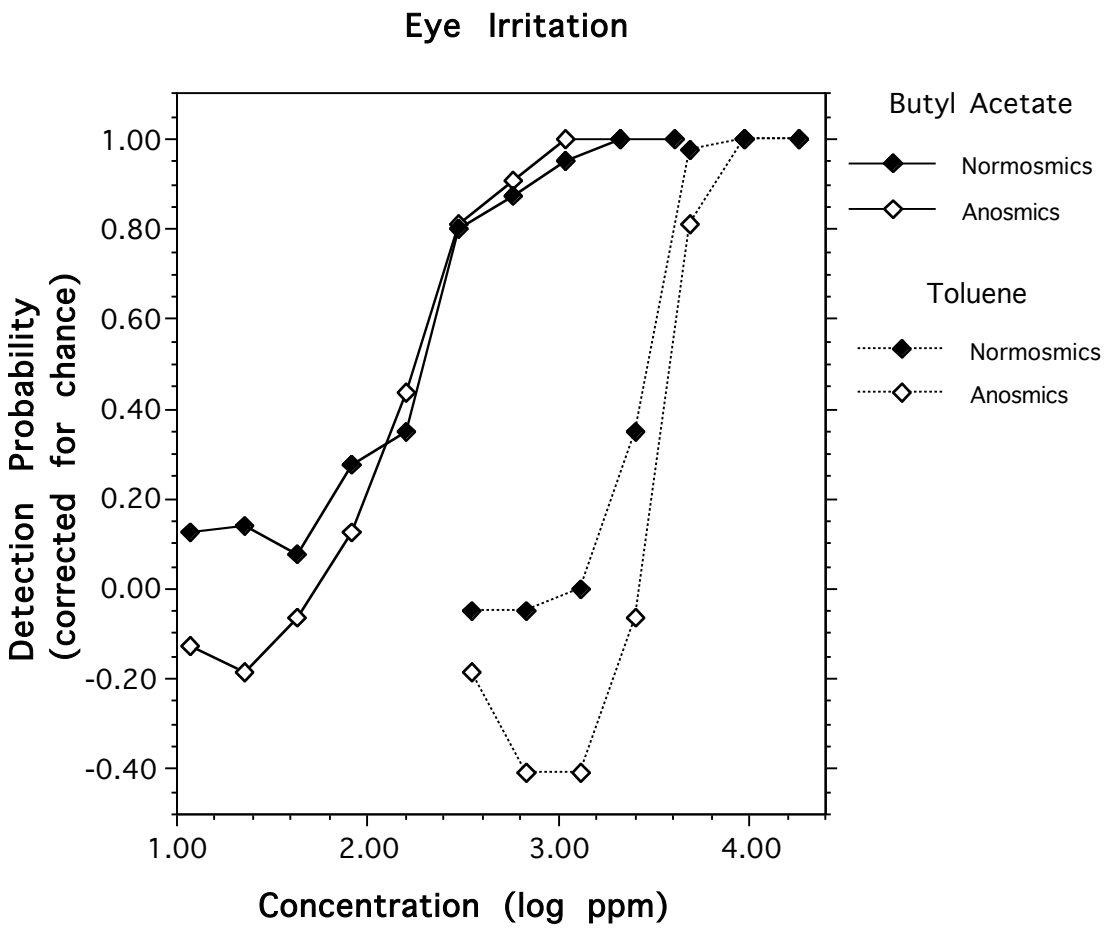

Eye Irritation
Detection Probability (corrected for chance)
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
-0.40
1.00
2.00
3.00
4.00
Concentration (log ppm)
Butyl Acetate
Normosmics
Anosmics
Toluene
Normosmics
Anosmics

FIGURE 3

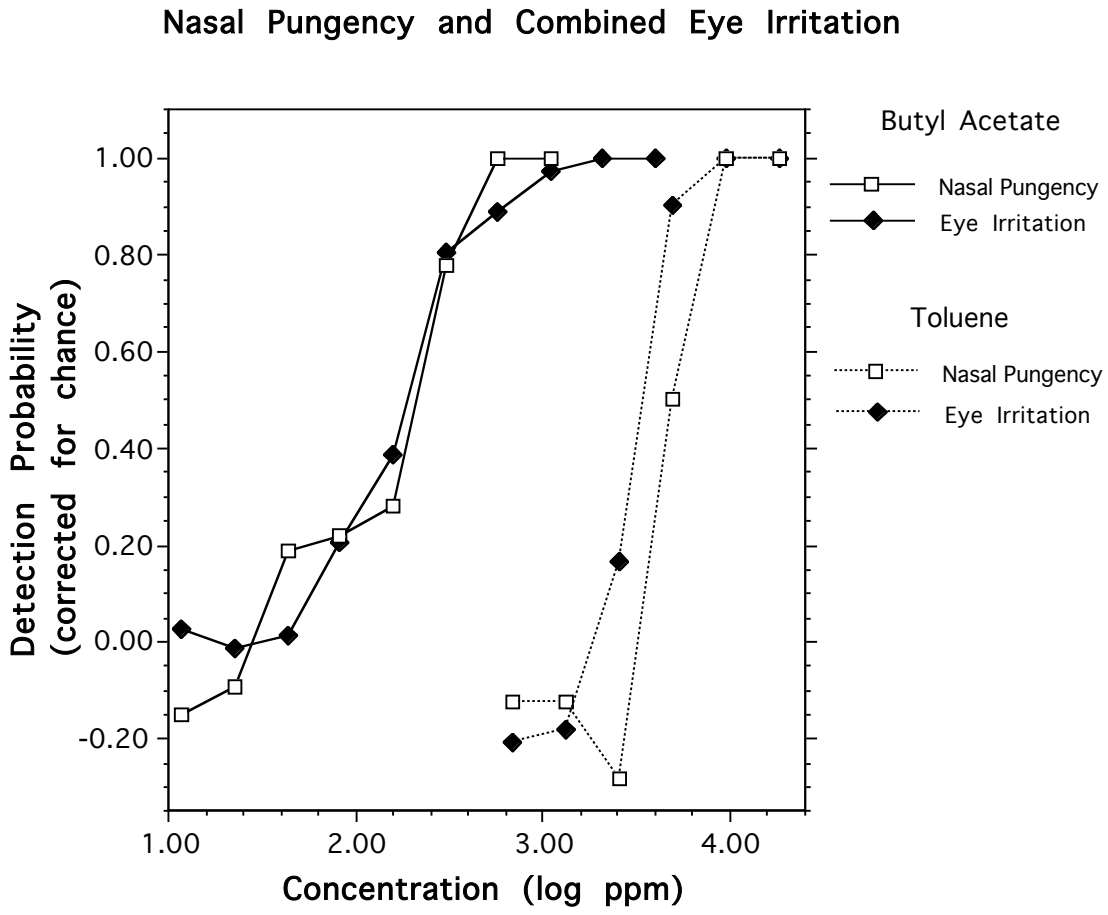

Nasal Pungency and Combined Eye Irritation
Detection Probability (corrected for chance)
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
1.00
2.00
3.00
4.00
Concentration (log ppm)
Butyl Acetate
Nasal Pungency
Eye Irritation
Toluene
Nasal Pungency
Eye Irritation

FIGURE 4

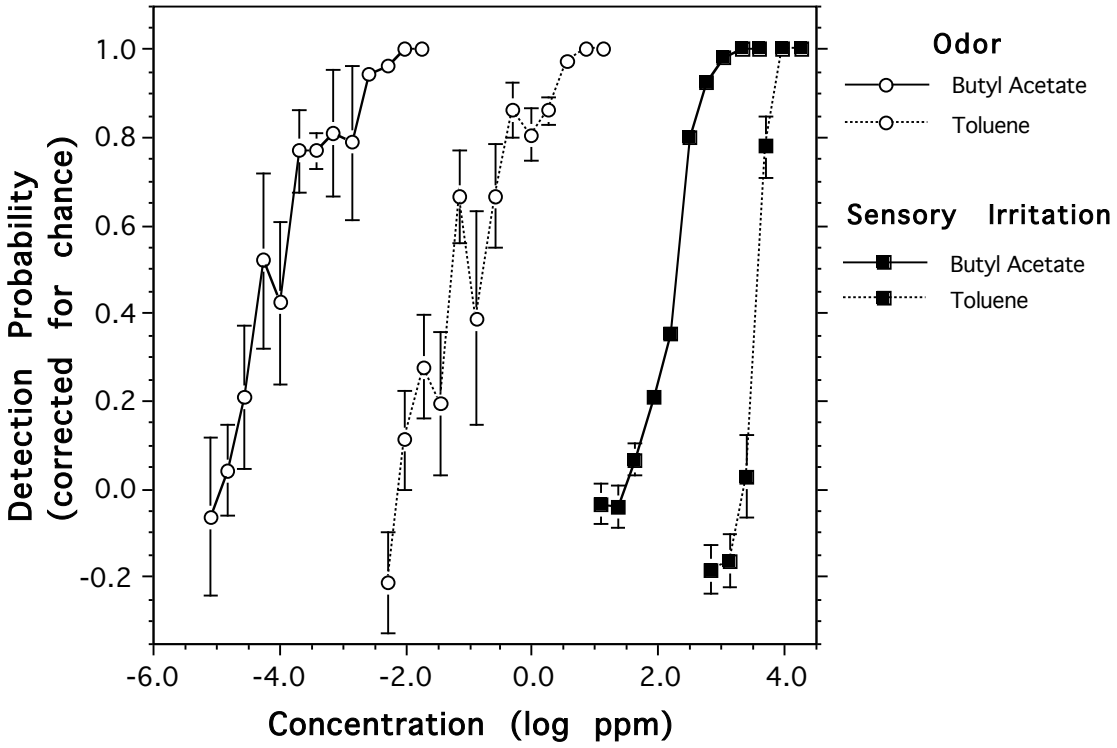

Detection Probability (corrected for chance)
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-6.0
-4.0
-2.0
0.0
2.0
4.0
Concentration (log ppm)
Odor
Butyl Acetate
Toluene
Sensory Irritation
Butyl Acetate
Toluene

FIGURE 5

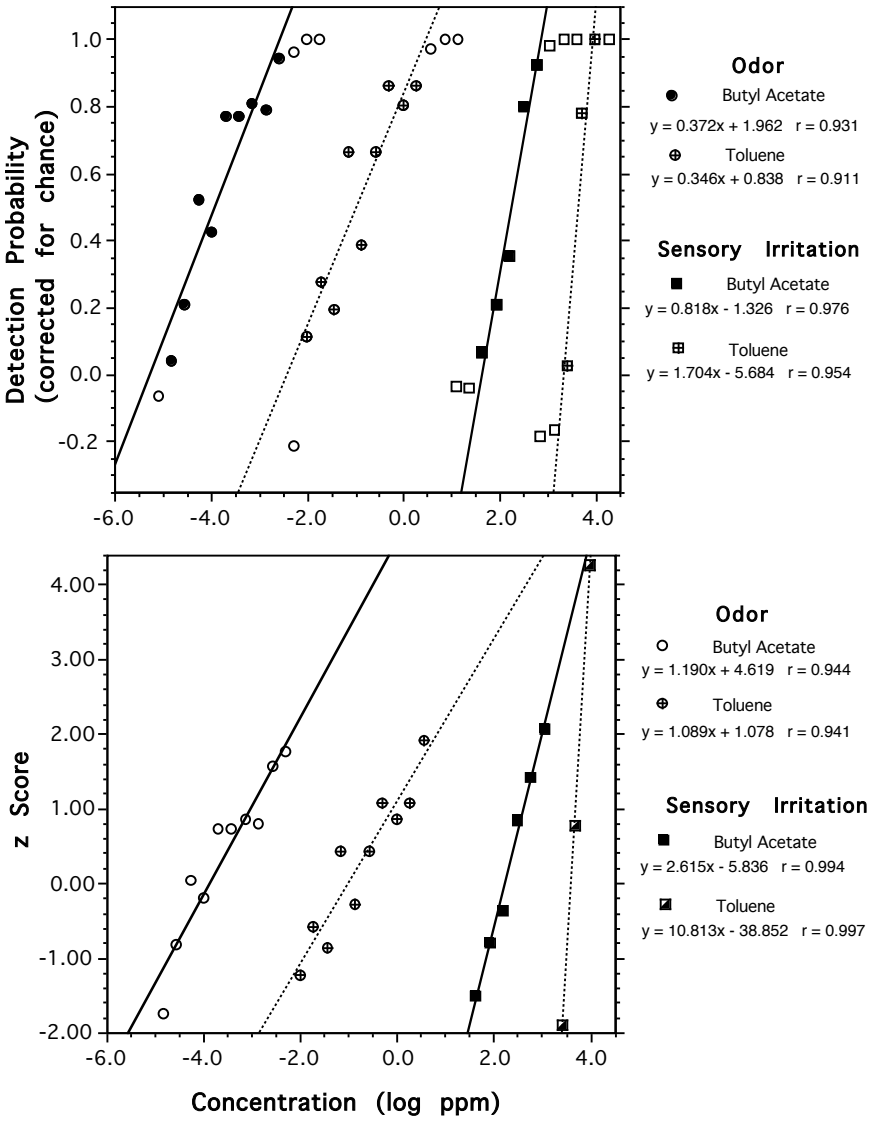
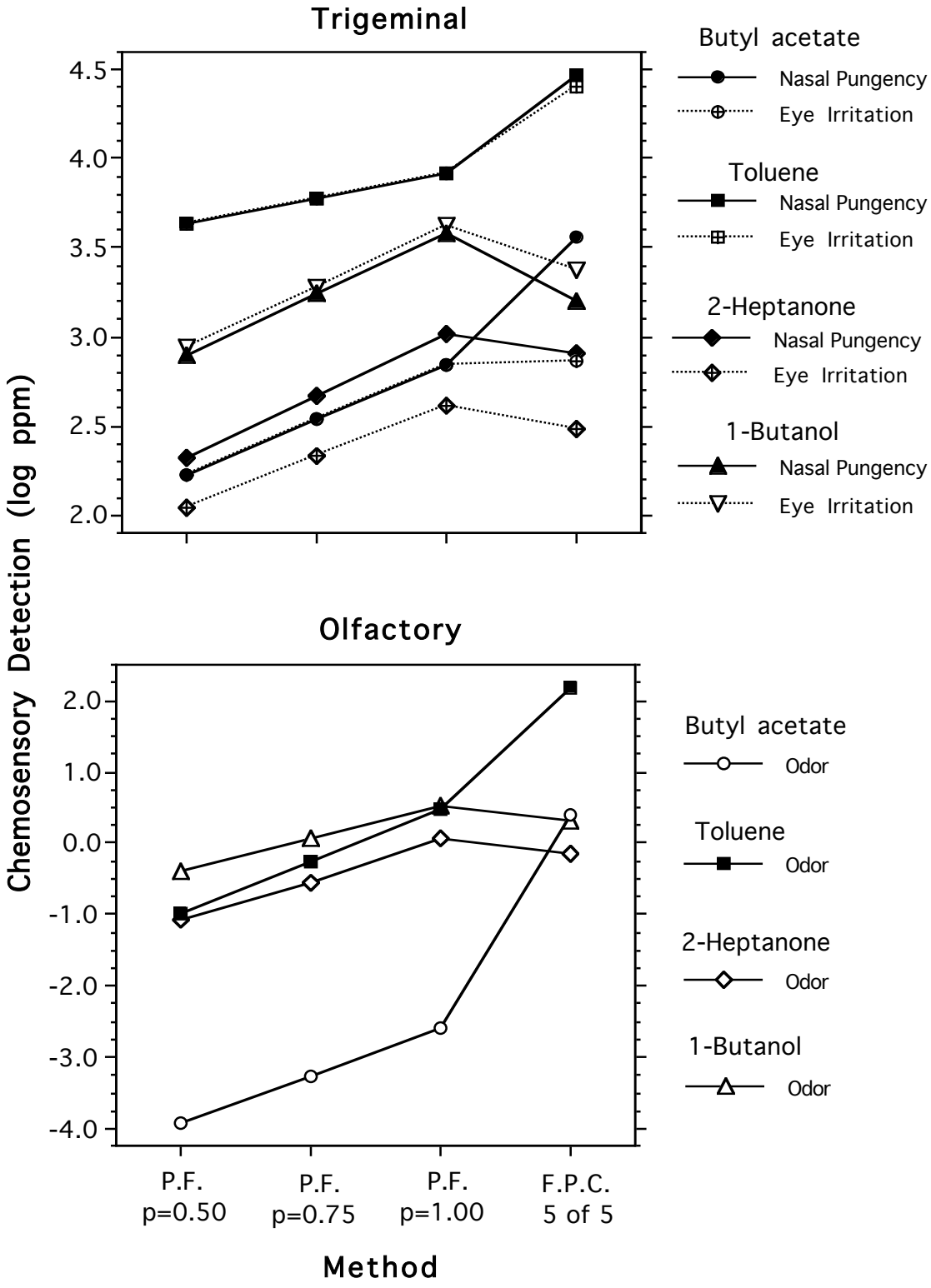

Detection Probability (corrected for chance)
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-6.0
-4.0
-2.0
0.0
2.0
4.0
Odor
Butyl Acetate
y = 0.372x + 1.962 r = 0.931
Toluene
y = 0.346x + 0.838 r = 0.911
Sensory Irritation
Butyl Acetate
y = 0.818x - 1.326 r = 0.976
Toluene
y = 1.704x - 5.684 r = 0.954
z Score
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
Concentration (log ppm)
Odor
Butyl Acetate
y = 1.190x + 4.619 r = 0.944
Toluene
y = 1.089x + 1.078 r = 0.941
Sensory Irritation
Butyl Acetate
y = 2.615x - 5.836 r = 0.994
Toluene
y = 10.813x - 38.852 r = 0.997

FIGURE 6


Trigeminal
Butyl acetate
Nasal Pungency
Eye Irritation
Toluene
Nasal Pungency
Eye Irritation
2-Heptanone
Nasal Pungency
Eye Irritation
1-Butanol
Nasal Pungency
Eye Irritation
4.5
4.0
3.5
3.0
2.5
2.0
Olfactory
Butyl acetate
Odor
Toluene
Odor
2-Heptanone
Odor
1-Butanol
Odor
2.0
1.0
0.0
-1.0
-2.0
-3.0
-4.0
Chemosensory Detection (log ppm)
P.F.
p=0.50
P.F.
p=0.75
P.F.
p=1.00
F.P.C.
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Method

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