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Concentration-detection functions for the odor of homologous n-acetate esters

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Running head: Concentration-detection Odor Functions

Abstract

Using air-dilution olfactometry, we measured concentration-response functions for the odor detection of the homologous esters ethyl, butyl, hexyl, and octyl acetate. Stimuli were delivered by means of an 8-station vapor delivery device (VDD-8) specifically designed to capture odor detection performance by humans under environmentally realistic conditions. Groups of 16-17 (half female) normosmic (i.e., having a normal olfaction) non-smokers (ages 18-38) were tested intensively. The method involved a three-alternative forced-choice procedure against carbon-filtered air, with an ascending concentration approach. Delivered concentrations were confirmed by gas chromatography before and during actual testing. A sigmoid (logistic) model provided an excellent fit to the odor detection functions both at the group and individual levels. Odor detection thresholds (ODTs) (defined as the half-way point between chance and perfect detection) decreased from ethyl (245 ppb by volume), to butyl (4.3 ppb), to hexyl acetate (2.9 ppb), and increased for octyl acetate (20 ppb). Interindividual threshold variability was near one and always below two orders of magnitude. The steepness of the functions increased slightly but significantly with carbon chain length. The outcome showed that the present thresholds lie at the very low end of those previously reported, but share with them a similar relative trend across n-acetates. On this basis, we suggest that a recent quantitative structure-activity relationship (QSAR) for ODTs can be applied to these and additional optimized data, and used to describe and predict not just ODTs but the complete underlying psychometric odor functions.

Keywords: Psychometric odor functions; Odor detection thresholds; Homologous n-acetates; Interindividual odor sensitivity; Olfactory structure-activity relationships

Introduction

A decisive event in the history of smell research occurred with the discovery of the large family of olfactory receptors (ORs) (1). Subsequent research led to important discoveries about the molecular, cellular, and anatomical features that characterize olfaction, see review in (2). For example, it was concluded that each olfactory sensory neuron (OSN) expresses one kind of olfactory receptor, and that a given odorant binds to an array of ORs. From this it followed that the detection and discrimination of an odorant is achieved by a combinatorial activation of ORs (3). The combinatorial coding also holds for odorant mixtures and could be present as well at higher levels of the olfactory pathway such as the olfactory cortex (4).

In humans there are approximately 800 olfactory genes but only about 400 of them code for functional proteins whereas the rest are pseudogenes (5). We do not know the ligand repertoire, i.e., the odorant binding range, for the immense majority of vertebrate ORs (6). In other words, most ORs are orphan. With the exception of Drosophila, very few ORs from any species have been linked to their respective odorant ligands (6), for example via structure-activity studies. Even fewer human ORs have been probed in this way (7-9). Among other factors, the combinatorial characteristics of smell, the large number of ORs, and the modulation and processing of olfactory information by the olfactory bulb (10-12) and higher brain structures (13-15) argue for the need to complement structure-activity insights gained at the molecular and cellular levels with those gained at the behavioral level, where we probe the integrated olfactory system. This is particularly true when investigating human olfactory sensitivity, i.e., odor detection thresholds (ODTs), a topic of high significance from both basic and applied perspectives involving neuroscience, environmental pollution, occupational exposures,

and food science, e.g. (16-19).

The present investigation is part of a project aiming at characterizing the absolute sensitivity of the human sense of smell towards volatile organic compounds (VOCs) in the form of concentration-response (i.e., psychometric) functions, and in a context of structure-activity relationships. Towards this goal we have employed a methodology that: a) aims to maximize the conditions for obtaining environmentally relevant odor detection measurements, and b) puts emphasis on the analytical quantification of each chemical vapor delivered. The strategy is complemented by selecting stimuli from homologous chemical series (in this study, n-acetates) where carbon chain length, within a series, and chemical functionality, across series, serve as practical "units of chemical change." Acetates, and esters in general, play an important role as odorants in foods and beverages, e.g. (20-23), and have been widely used in smell function tests, e.g. (24), assessment of safety and health from chemical exposures, e.g. (25-28), methodology comparisons in olfactometry, e.g. (29, 30), olfactory function in various physiological and pathological conditions, e.g. (31-38), and basic research, including structure-activity studies, e.g. (39-41).

The tens of thousands of odoriferous VOCs make it unfeasible to collect odor psychometric functions in humans for more than a sample. Hence, some method for modeling and, ultimately, predicting odor detection sensitivity for untested VOCs is needed. Within the framework of the results obtained here with homologous n-acetates and, recently, with homologous n-alcohols, we discuss below the merits of applying a solvation equation (42) to model odor potency of VOCs as reflected by concentration-detection functions.

Materials and Methods

Stimuli. The stimuli included the following acetates (purity in parenthesis, FCC stands for Food Chemical Codex quality): ethyl (99.9%), butyl (99.5%), hexyl (98+%, FCC), and octyl (98+%, FCC) acetate. They were selected as representative of the n-acetate homologous series.

Subjects. The subject pool included 36 participants (22 female), normosmics, nonsmokers, with an average age (±SD) of 25 (±5) years, ranging from 18 to 38 years. Normosmia was established via a clinical olfactory test (43). (One male tested mildly hyposmic; he participated in the threshold session for butyl acetate only. His exclusion leaves all conclusions unaltered.)

Not all subjects were tested with all stimuli. Nevertheless, a subgroup of 4 participants (males) was available for testing in common on **all** four acetates. Table 1 presents the characteristics of this subgroup and of those tested with each stimulus.

Insert Table 1 about here

Apparatus and Procedure. Stimuli were delivered via dynamic olfactometry (44) using an 8-station vapor delivery device (VDD-8) described in recent studies (18, 45, 46). The stimulus source was the neat chemical, stored in a syringe and introduced to the VDD-8 at a fixed flowrate via a syringe driver. The stimulus was vaporized in a heat block and carried by nitrogen (N_2). If necessary the stimulus flow can be pre-diluted with N_2 before entering a manifold where it splits into eight streams at rates forming a geometric progression by a selected factor (in this study, a factor of two). Each stream fed one of

eight stations, the smallest flow feeding station 8 (lowest concentration) and the largest flow feeding station 1 (highest concentration). A station consisted of three glass cones from where the subjects sampled (sniffed). Only one of the cones (active cone) delivered the stimulus diluted with carbon-filtered air to a total flowrate of 40 L/min. The other two cones (blanks) delivered just carbon-filtered air at 40 L/min. This flow was high enough to fully accommodate human sniffs (37, 47) while avoiding a sensation of draft since the flow exited the cones at a linear velocity of ≈13 cm/sec, similar to that found in mechanically ventilated spaces (48, 49). A toggle switch and solenoid valves determined which cone received the stimulus in each station. Local extraction of air above the cones and the overall ventilation of the room (30 ach, air changes per hour) maintained odorless conditions in the testing space.

In a session, up to 6 subjects were simultaneously tested with one chemical during the course of the day (6 to 7 hours). The order in which chemicals were tested was randomized. The VDD-8 was started in the morning, about 1 hour before participants arrived to give it time to reach steady state conditions. Testing entailed a three-alternative forced-choice procedure with an ascending concentration approach as described in the following text. Subjects lined up, and a speaker system instructed the first subject to sample from cone 1, then cone 2, and finally cone 3 from station 8. At this point the participant decided which cone smelled different (i.e., stronger) from the other two, circled the response in a scoresheet, and assigned a confidence rating to the decision just made, using a scale from “1” (not certain at all, just guessing) to “5” (extremely confident). Then, this first subject moved to the next station (number 7) and repeated the process, while, at the same time, the second subject in line started from station number 8. In this way subjects moved in order, one by one, from the initial station (number 8) to the final station (number 1) in what can be called “a round.” Throughout

testing, participants were supervised by one, often two, experimenters who made sure they followed directions. Instructions heard through the speaker system guided participants to sniff from a cone in a 5-sec window and to wait 15 sec between stations. Each subject exited the room upon sampling the last station (number 1). After the last subject exited, the experimenter set a new randomly determined order of the active cones at each station and waited 5 min. A second round started, as described for the first.

During the course of the day, each subject went through a minimum of 35 rounds. It might seem that the regimen of testing could exhaust subjects, but in fact they spent most of a day resting between rounds. Most would read. Their total exposure to odorant equaled about 20 min spread out through their 6 to 7 hours of participation. The pace of testing ensured that subjects began each round of testing with reasonably fresh noses.

Gas chromatography (GC) (flame ionization detection, FID) served to quantify the vapors delivered by the VDD-8, via creation of a calibration curve for mass for each acetate (50). Stability of delivery of the odorants was established by GC both before and during actual testing in every experimental session. At least every hour, 1-ml vapor samples were taken from a sampling port in the stimulus line below the base of an active cone, before the odorized nitrogen mixed with dilution air. The samples were quantified by gas chromatography. Lines feeding different active cones from different stations were tested in irregular order throughout the testing day session. The average coefficient of variation of vapor samples (in ppb) across experimental sessions equaled 16% for ethyl, 12% for butyl, 15% for hexyl, and 12% for octyl acetate. The range of concentrations for each acetate delivered by the VDD-8 in seven binary steps from station 8 to station 1

was the following: For ethyl acetate, 16 to 1,995 ppb; for butyl acetate, 0.62 to 80 ppb; for hexyl acetate, 0.40 to 51 ppb; and for octyl acetate, 1.9 to 244 ppb.

Data analysis. The results were summarized as plots of detection probability (P), i.e., detectability, and confidence rating as a function of vapor concentration (in log ppb by volume). Detectability was corrected for chance to produce a value between P=0.0 (i.e., chance detection) and P=1.0 (i.e., perfect detection), according to:

$$P = (m \cdot p(c) - 1) / (m - 1) \qquad \text{Equation (1)}$$

where P = detection probability corrected for chance, m = number of choices per trial (in this case, three), and p(c) = proportion correct (i.e., number of correct trials / total number of trials) (51).

The concentration-detection, or psychometric, functions were modeled by a sigmoid (logistic) equation:

$$P = P_{max}/(1 + e^{(-(x-C)/D)}) \qquad \text{Equation (2)}$$

where P = detection probability ($0 \leq P \leq 1$), P_{max} = 1.0, x = vapor concentration (in log ppb by volume), and C and D are constants. C is the value of x when P=0.5, that is, when detection probability is half way between chance (P=0.0) and perfect (P=1.0) detection. This value was taken as the odor detection threshold (ODT). In turn, the constant D quantifies the steepness of the function. Statistical significance was established by analysis of variance (ANOVA) and Wilcoxon-Mann-Whitney Rank Sum tests (Kaleidagraph vs. 4.01, Synergy Software, Reading, PA).

Results

Figure 1 shows plots of detectability and confidence rating as a function of vapor concentration for the groups tested on each acetate. The functions for confidence rating follow the trend of those for detection. Table 2, upper section, presents the values (±SE) for constants C and D obtained from the group detectability function for each acetate, along with an estimate of goodness of fit (R^2). The fit of the sigmoid (Equation (2)) to the experimental data is quite adequate. In turn, Table 2, lower section, depicts data for the group of four subjects tested in common across all acetates. The results and trends from the larger and the smaller group are similar, providing support to the across-odorant comparability of data.

Insert Figure 1 and Table 2 about here

Figures 2 through 5 show the functions for individual subjects. Table 3 shows the corresponding values of C, D and R^2. In the figures and the table, each subject has been labeled with an univocal number that defines that participant. The performance of individuals tested on more than one chemical can therefore be followed across stimuli. The results show that the sigmoid also provides an adequate fit to individual data.

Insert Figures 2 to 5 and Table 3 about here

The outcome of a one-way ANOVA on the individual values of C for the factor acetates (four levels) showed a significant effect ($F(3,61)=78.71$, $p<0.0001$). Post-hoc comparisons revealed that C (i.e., the ODT in log ppb) was significantly different for every pair of acetates ($p<0.0001$) except butyl vs. hexyl acetate. A Wilcoxon-Mann-Whitney test revealed no significant differences between females and males in their odor sensitivity (measured as C) towards the acetates.

In analogy with dose-response relationships in pharmacology, and assuming that odor detection reflects, at least in part, ligand binding characteristics, the parameter D calculated form the average of individual D values reflects the interaction between odorant and olfactory receptors (52, 53). In turn, the value of D from the group reflects the mean response across subjects. The average of the individual values for the parameter D (Table 3) decreased with increasing carbon chain length of the homologs. A large value of D corresponds to a shallow function, whereas a small value corresponds to a steep function. A one-way ANOVA on the individual values of D for the factor acetates (four levels) showed a significant effect ($F(3,61)=4.70$, $p=0.005$). Post-hoc tests revealed that the function for ethyl acetate (the shallowest function) was significantly different from those for hexyl and octyl acetate (the steepest functions).

Discussion

Group thresholds

The present investigation strived to achieve measurements of olfactory sensitivity in a natural and environmentally relevant procedure, and to secure analytical quantification and stable delivery of the chemical stimuli. In a previous study we have measured odor thresholds for homologous acetates. There, we had also used gas chromatography to quantify the stimuli and had employed a forced-choice procedure with an ascending concentration approach. Nevertheless, stimuli were delivered via squeeze bottles, fewer subjects were tested (n=4), and, since the procedure involved measuring an odor threshold value using a fixed performance criterion (i.e., five correct choices in a row), the outcome produced a single point in the underlying psychometric

function instead of the complete function as obtained in the present case. Previous research indicated that squeeze bottles gave high absolute thresholds (54), at least in part because a subject would dilute the small bolus of puffed chemical with ambient air (55).

Figure 6 shows some similarity in how the present and the former thresholds vary with chain length. The gap between previous and present values decreased with carbon chain length, being 2.75 log units for ethyl and butyl acetate, 2.25 for hexyl, and 1.25 for octyl acetate. Such a decrease with carbon chain length has also been observed across homologous n-alcohols measured under previous and present methodologies (46). It has been suggested that the decreasing gap might reflect difficulties in achieving a stable and reliable delivery of stimulus for the highly volatile odorants, particularly under techniques using static headspace dilution.

In our previous data, the ODTs declined to a plateau for octyl acetate and beyond, whereas the present data shows an increase for octyl acetate (Figure 6). Decreasing thresholds often ending in a plateau or an increase at about the level of octyl acetate have also been observed for ODTs across acetates in other mammalian species, including non-human primates (56, 57) and rats (58).

Insert Figure 6 about here

Two comprehensive literature compilations of human data are available. They are those by Devos et al. and by van Gemert (59, 60). From the second source, only odor detection (not recognition) thresholds in air were considered. Figure 7 shows how the present ODTs compare with those listed in both compilations. The present values lie

at the very low end of the range. Assuming accurate chemical quantification, and on the expectation that subjects cannot perform better than their physiological limits, but can always perform worse, low values have more credibility than high values. Insofar as methodological shortcomings will interfere with performance, poor measurements will reflect themselves in higher values.

Insert Figure 7 about here

Interindividual variability

Another relevant criterion for the adequacy of threshold measurements concerns individual differences in performance. Methodological adequacy should reflect itself in a reduction of individual differences. The subjects studied here were relatively young adults of both genders (18 to 38 years old), normosmics, and non-smokers. Their olfactory sensitivity was tapped seconds at a time during the course of a whole day in relatively adaptation-free circumstances. The interface between subject and device had environmental realism, and the participants gave enough judgments to permit construction of adequate psychometric functions. Figures 2 to 5 show and Table 3 quantifies individual functions for each acetate. The ODT ratio between the least and the most sensitive subject equaled 15 for ethyl acetate, 22 for butyl acetate, 69 for hexyl acetate, and 27 for octyl acetate, that is, between one and two log units (often closer to one). Measured as the interquartile range of individual thresholds expressed in log ppb, interindividual variability equaled 0.35 for ethyl and butyl acetate, 0.52 for hexyl, and 0.42 for octyl acetate. These values lie well below the common outcome (61, 62). In a recent study of ODTs along homologous n-alcohols that employed the same apparatus used here (46), the ODT ratios for ethanol (n=14 subjects), 1-butanol (n=14), 1-hexanol

(n=17) and 1-octanol (n=14) equaled 152, 13, 162, and 59, respectively, higher than here though still low by historical standards. The higher interindividual variability for the alcohols presumably arose from the broader age range of the participants (18 to 59 years old) and the inclusion of two smokers (all normosmics). In addition, the individual data for the alcohols was based on 21 trials per concentration compared to the 35 trials recorded here for the acetates. For both data sets, the measured variability of 1 to 2 orders of magnitude is within the low range previously reported (63) but it is much smaller than the 3 to 5 (64, 65) and up to 16 (66) orders of magnitude also reported.

A quantitative structure-activity relationship (QSAR) for odor thresholds

Previous studies have established that human ODTs can be described by a QSAR based on a solvation equation model (42, 67). The QSAR is based on up to five physicochemical properties or "descriptors" (see below) of the odorous VOCs. The model works best when applied to biological responses that depend upon "selective" effects, viz., those that control transfer of the VOC from the air into the nasal mucus, until reaching the olfactory receptors in the cilia of the neurons that form the olfactory epithelium (2). In such transfer-driven effects, small structural changes in a VOC evoke predictable, often small and gradual, changes in biological activity. In contrast, the model is less prepared to account for what can be called "specific" effects, viz., those that depend heavily on the odorant possessing a narrowly-defined structure, conformation, functional group, or position of the functional group. In these kind of effects, small structural changes in the VOC evoke less predictable, often large and sudden, changes in biological activity. The outcome of applying the solvation QSAR to ODTs from up to 60 VOCs revealed that selective transfer could account for about 77% of the total effect, and that the reminder was due to a size effect and to a specific effect for aldehydes and

carboxylic acids (42). (The size effect has also been observed here for octyl acetate as noted further ahead.)

The data obtained here for acetates and recently for alcohols (46) reveals ODTs considerably lower than those originally used to developed the QSAR (68, 69) but with a relatively similar pattern along the homologous series (cf. Figure 6). This supports the notion that the same solvation-based QSAR can be applied to the recent, more environmentally relevant ODTs and produce an equally successful description of the odor potency of VOCs. Furthermore, the new data obtained for n-acetates and n-alcohols entail the whole psychometric function. This opens the opportunity to explore whether the same (or other) QSAR descriptors could account for the value of D, the parameter quantifying the steepness of the function.

To assess whether predicting a psychometric function using the solvation-based QSAR might be feasible, we have made a preliminary analysis of such functions for ten VOCs, in terms of the defining parameters C and D. The five physicochemical properties or "descriptors" of VOCs that we have previously used (42, 67) are **E** the excess molar refraction of a VOC, **S** the VOC dipolarity/polarizability, **A** the VOC hydrogen bond acidity, **B** the VOC hydrogen bond basicity, and **L** the logarithm of the gas to hexadecane partition coefficient at 25^{o}C, a measure of the lipophilicity and size of the VOC. A detailed explanation of these descriptors, and how they are obtained from experimental data has been given (70). It is technically incorrect to attempt to fit values of C or D for only 10 VOCs with five variables, and so we used the best combination of two descriptors out of the five descriptors that we usually employ. In both cases, the combination of **S** and **L** yielded the best equations, as shown in Equation (3) and Equation (4). The value of C for octyl acetate was out of line compared with other values

of C, and so we did not use C and D for octyl acetate to construct Equations (3) and (4) which are then based on 9 VOCs, see Table 4. There are a number of reasons why a VOC might be regarded as an outlier: the model used could be inadequate, the experimental value might be in error, or there might be some specific effect that the model does not take into account. We note that the departure of the predicted C value for octyl acetate could rest on a molecular size effect that, in addition to selective transfer, has been shown to play a role in odor detection (42).

$C = 3.058 + 0.821\ \mathbf{S} - 0.708\ \mathbf{L}$ Equation (3)

$D = 0.623 - 0.437\ \mathbf{S} - 0.0172\ \mathbf{L}$ Equation (4)

Once C and D can be predicted through equations such as Equation (3) and Equation (4), they can be used to predict the shape of the entire psychometric plots, through Equation (2). These two equations are new QSARs. Of course, data for more than 9 or 10 VOCs will be needed before values of C and D and hence the psychometric plots can be predicted with certainty. However, as shown from the observed and calculated psychometric plots in Figure 8, this is a viable future aim.

Insert Table 4 and Figure 8 about here

To achieve the goal of predicting psychometric functions for a wide range of VOCs, odor detection data from additional homologous series, e.g., ketones, alkylbenzenes, carboxylic acids, and aldehydes, to name a few, need to be measured under the same conditions employed here. Ongoing work at our lab is addressing this need.

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Table 1. Characteristics of the groups of participants.

Subject groups	Number of subjects	Average Age (±SD) (years)	Age range (years)	Number of males	Number of females
Ethyl acetate	16	25 (±5)	18-32	8	8
Butyl acetate	17	25 (±5)	18-38	8	9
Hexyl acetate	16	26 (±5)	19-35	8	8
Octyl acetate	16	26 (±4)	19-35	8	8
Common subjects	4	25 (±2)	23-27	4	0

Table 2. Upper section. Showing for each acetate the ODT (in ppb), and the average value (±SE) for constants C (i.e., ODT in log ppb) and D from Equation (2) applied to the group psychometric odor function (n: number of subjects). Lower section. Same data but from the group of four subjects tested in common for all acetates.

All subjects

	n	ODT (ppb)	C (log ppb)	SE (C)	D	SE (D)	R^2
Ethyl Acetate	16	245	2.39	± 0.025	0.32	± 0.023	0.99
Butyl Acetate	17	4.3	0.63	± 0.021	0.29	± 0.018	0.99
Hexyl Acetate	16	2.9	0.46	± 0.022	0.35	± 0.020	0.99
Octyl Acetate	16	20	1.31	± 0.016	0.27	± 0.014	1.00

Common subjects

	n	ODT (ppb)	C (log ppb)	SE (C)	D	SE (D)	R^2
Ethyl Acetate	4	275	2.44	± 0.045	0.38	± 0.042	0.98
Butyl Acetate	4	7.2	0.86	± 0.051	0.39	± 0.047	0.97
Hexyl Acetate	4	6.9	0.84	± 0.039	0.36	± 0.036	0.98
Octyl Acetate	4	25	1.40	± 0.031	0.17	± 0.027	0.99

Table 3. Showing for each acetate the value of constants C (i.e., individual ODT in log ppb) and D from Equation (2) applied to individual psychometric odor functions. Also shown is the R^2 for each function, as an estimate of goodness of fit.

Ethyl Acetate (n=16)				**Butyl Acetate (n=17)**				**Hexyl Acetate (n=16)**				**Octyl Acetate (n=16)**			
Subject	**C (log ppb)**	**D**	**R^2**	**Subject**	**C (log ppb)**	**D**	**R^2**	**Subject**	**C (log ppb)**	**D**	**R^2**	**Subject**	**C (log ppb)**	**D**	**R^2**
3	2.33	0.26	0.93	1	0.23	0.18	0.99	4	-0.01	0.11	1.00	2	1.43	0.26	0.97
5	2.06	0.27	0.98	3	0.62	0.28	0.89	11	0.13	0.23	0.93	4	0.91	0.14	0.98
9	2.28	0.20	0.99	7	0.65	0.38	0.95	12	0.36	0.20	0.94	6	1.29	0.28	0.91
10	2.27	0.16	0.99	10	0.76	0.23	0.90	15	0.16	0.16	0.99	8	1.08	0.14	0.97
14	2.80	0.35	0.85	14	0.69	0.29	0.99	16	1.14	0.14	0.97	11	0.63	0.18	0.97
16	2.21	0.41	0.95	16	1.09	0.21	0.96	17	0.00	0.43	0.90	13	0.98	0.16	0.99
19	2.44	0.18	0.97	20	0.38	0.015	0.99	18	0.58	0.10	0.97	16	1.15	0.095	0.96
20	2.18	0.40	0.96	21	0.19	0.22	0.94	19	0.39	0.073	0.91	18	1.37	0.14	0.97
23	2.18	0.26	0.95	22	0.41	0.21	0.98	20	0.29	0.21	0.96	20	1.62	0.19	0.96
25	2.37	0.15	0.95	25	0.59	0.21	0.96	23	0.60	0.11	0.98	23	1.91	0.20	0.96
26	2.40	0.16	0.99	28	0.19	0.14	1.00	24	-0.39	0.095	1.00	25	1.36	0.11	0.98
30	1.99	0.20	0.96	29	0.93	0.16	0.95	25	0.53	0.20	0.97	26	1.18	0.063	0.99
31	2.15	0.27	0.95	31	0.61	0.14	0.99	26	0.39	0.19	0.99	29	1.00	0.20	0.98
33	2.85	0.34	0.89	32	0.72	0.17	0.97	32	0.90	0.20	0.93	33	1.59	0.25	0.96
34	2.94	0.23	0.83	34	1.54	0.31	0.93	33	1.27	0.18	0.93	34	1.45	0.10	0.96
36	3.18	0.51	0.88	35	0.52	0.21	0.96	34	1.45	0.28	0.80	36	2.06	0.16	0.92
				36	1.04	0.32	0.95								
Average	**2.41**	**0.27**			**0.66**	**0.21**			**0.49**	**0.18**			**1.31**	**0.17**	
SE	0.09	0.03			0.09	0.02			0.12	0.02			0.09	0.02	

Table 4. Experimental (i.e., observed) values of C (i.e., ODT in log ppb) and D from psychometric plots for alcohols (46), acetates (this paper) and ketones (data unpublished) used to construct Equation (3) and Equation (4).

Compound	**C**	**D**
Ethanol	2.52	0.43
1-Butanol	0.90	0.41
1-Hexanol	0.91	0.36
1-Octanol	0.64	0.33
Ethyl acetate	2.39	0.32
Butyl acetate	0.63	0.29
Hexyl acetate	0.46	0.35
Pentan-2-one	2.00	0.23
Heptan-2-one	0.68	0.27

Figure Legends

Figure 1. Group psychometric odor functions (left) and confidence ratings as a function of concentration (right) for the four acetates. For ethyl, hexyl, and octyl acetate, each point represents the outcome of 560 trials made by 16 subjects. For butyl acetate, each point represents the outcome of 595 trials made by 17 subjects. In both graphs, bars depict standard error (SE).

Figure 2. Individual psychometric odor functions for ethyl acetate fitted by the sigmoid Equation (2). Each point represents the outcome of 35 trials made by that subject.

Figure 3. Same as in Figure 2 but for butyl acetate.

Figure 4. Same as in Figure 2 but for hexyl acetate.

Figure 5. Same as in Figure 2 but for octyl acetate.

Figure 6. Comparison between the odor detection thresholds for acetates from Cometto-Muñiz and Cain, 1991 [69] and those from the present study. Carbon chain length refers to the number of carbon atoms in the variable section of the molecules (e.g., 2 = ethyl acetate, 4 = butyl acetate, etc.). Bars depict standard error (in the case of the present data they are mostly hidden by the symbol).

Figure 7. Representing the odor detection thresholds reported for the acetates in each of the studies compiled by Devos et al., 1990 (empty symbols) and by van Gemert, 1999 (filled symbols). (Values from the different studies in each compilation are spread out

along the x-axis for clarity.) The crosses represent the ODTs measured in the present investigation.

Figure 8. Comparison between observed (experimental) and calculated (from Equations (3) and (4)) odor psychometric plots for acetates, alcohols, and ketones. To facilitate visual comparison of relative potency across odorants, the vapor concentration range (x-axis) is the same in all plots.

FIGURE 1

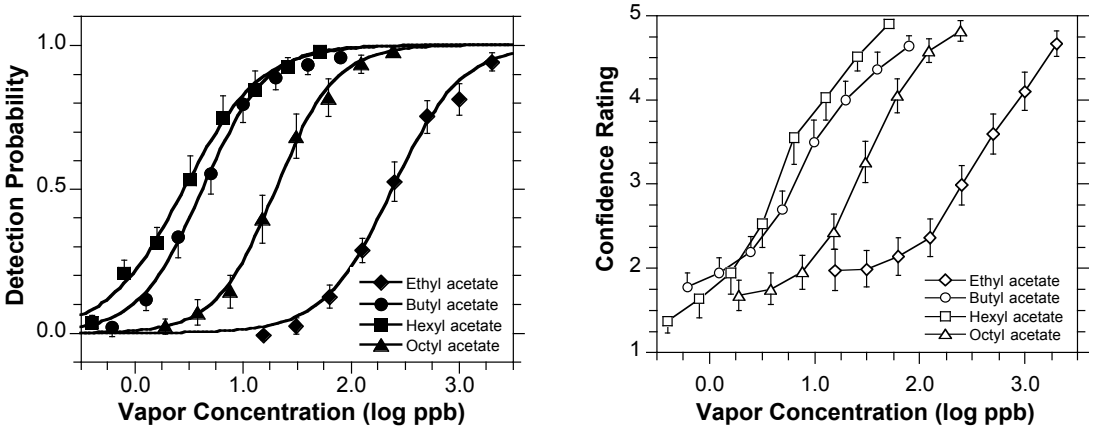

Detection Probability
1.0
0.5
0.0
0.0
1.0
2.0
3.0
Vapor Concentration (log ppb)
Ethyl acetate
Butyl acetate
Hexyl acetate
Octyl acetate
Confidence Rating
5
4
3
2
1
0.0
1.0
2.0
3.0
Vapor Concentration (log ppb)
Ethyl acetate
Butyl acetate
Hexyl acetate
Octyl acetate

FIGURE 2

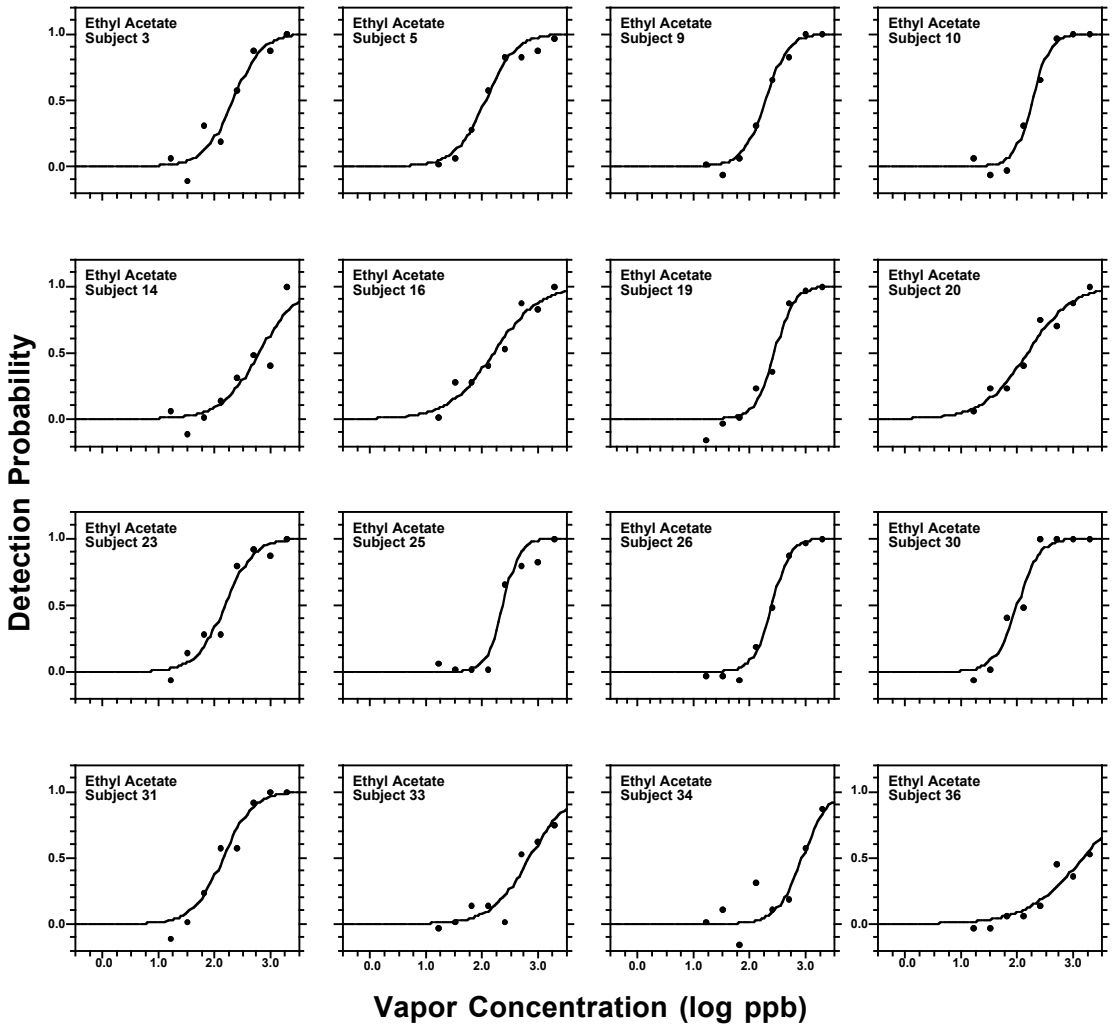

Ethyl Acetate
Subject 3
Ethyl Acetate
Subject 5
Ethyl Acetate
Subject 9
Ethyl Acetate
Subject 10
Ethyl Acetate
Subject 14
Ethyl Acetate
Subject 16
Ethyl Acetate
Subject 19
Ethyl Acetate
Subject 20
Ethyl Acetate
Subject 23
Ethyl Acetate
Subject 25
Ethyl Acetate
Subject 26
Ethyl Acetate
Subject 30
Ethyl Acetate
Subject 31
Ethyl Acetate
Subject 33
Ethyl Acetate
Subject 34
Ethyl Acetate
Subject 36
Detection Probability
1.0
0.5
0.0
0.0
1.0
2.0
3.0
Vapor Concentration (log ppb)

FIGURE 3

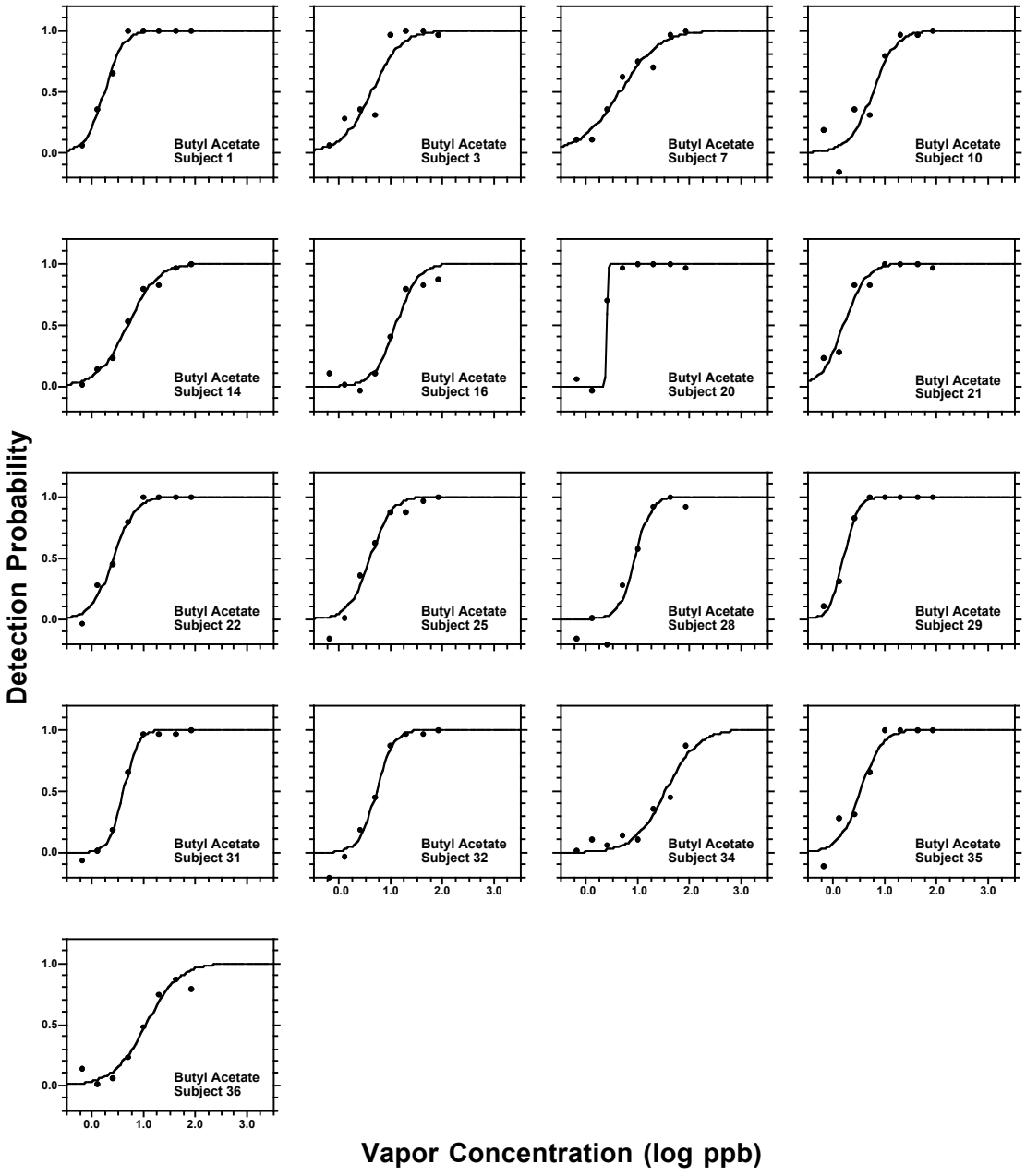

Detection Probability
Vapor Concentration (log ppb)
Butyl Acetate Subject 1
Butyl Acetate Subject 3
Butyl Acetate Subject 7
Butyl Acetate Subject 10
Butyl Acetate Subject 14
Butyl Acetate Subject 16
Butyl Acetate Subject 20
Butyl Acetate Subject 21
Butyl Acetate Subject 22
Butyl Acetate Subject 25
Butyl Acetate Subject 28
Butyl Acetate Subject 29
Butyl Acetate Subject 31
Butyl Acetate Subject 32
Butyl Acetate Subject 34
Butyl Acetate Subject 35
Butyl Acetate Subject 36
0.0
0.5
1.0
0.0
1.0
2.0
3.0

FIGURE 4

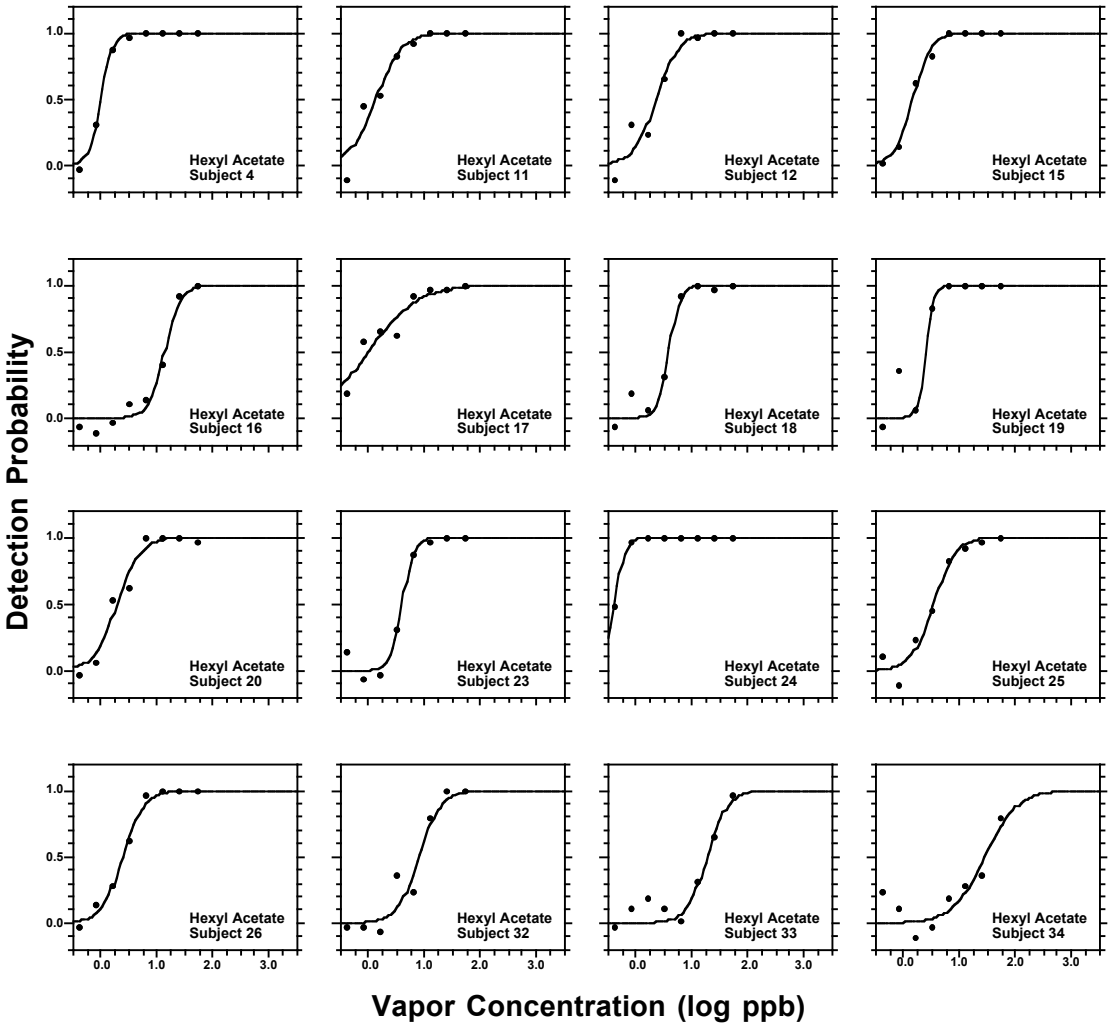

Detection Probability
Vapor Concentration (log ppb)
Hexyl Acetate Subject 4
Hexyl Acetate Subject 11
Hexyl Acetate Subject 12
Hexyl Acetate Subject 15
Hexyl Acetate Subject 16
Hexyl Acetate Subject 17
Hexyl Acetate Subject 18
Hexyl Acetate Subject 19
Hexyl Acetate Subject 20
Hexyl Acetate Subject 23
Hexyl Acetate Subject 24
Hexyl Acetate Subject 25
Hexyl Acetate Subject 26
Hexyl Acetate Subject 32
Hexyl Acetate Subject 33
Hexyl Acetate Subject 34
0.0
0.5
1.0
0.0
1.0
2.0
3.0

FIGURE 5

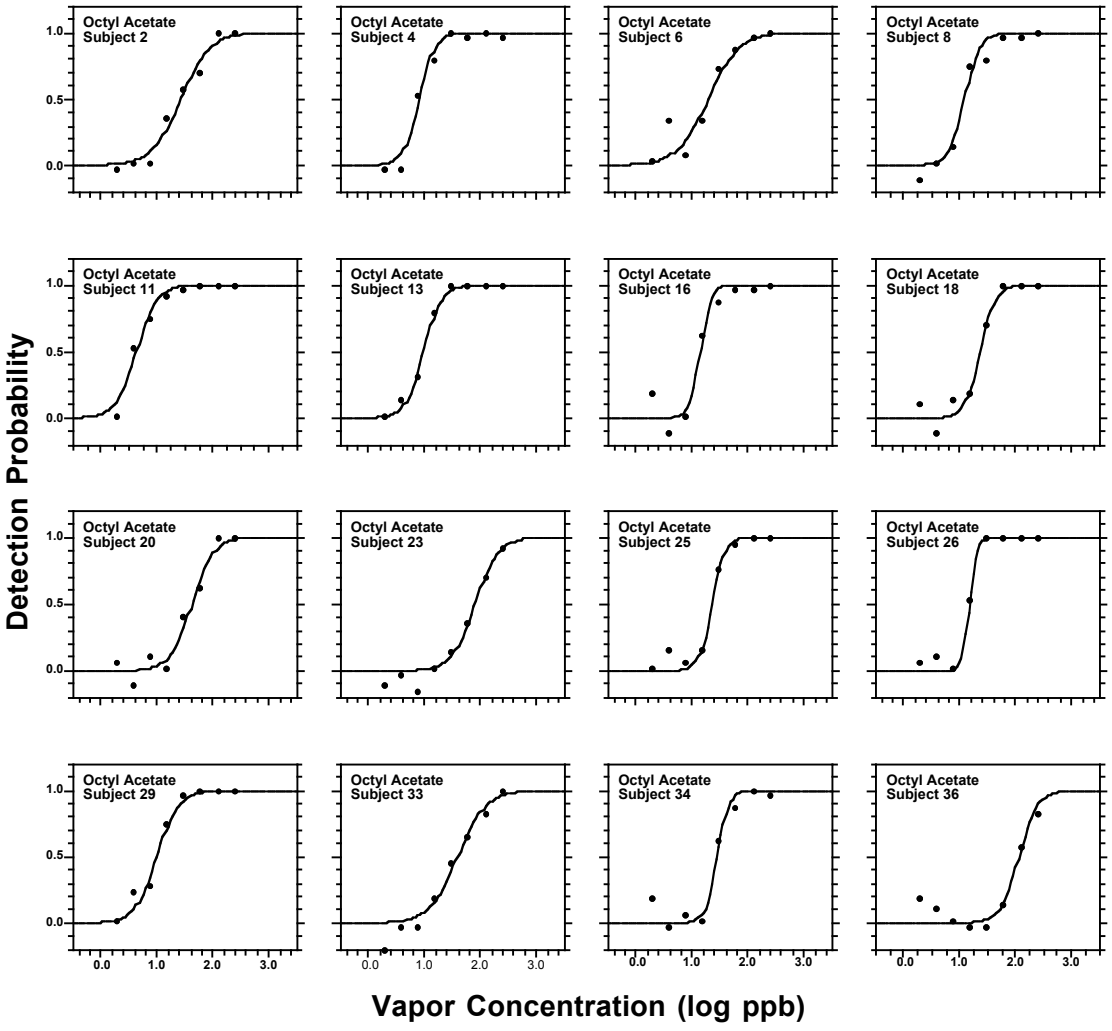

Octyl Acetate
Subject 2
Octyl Acetate
Subject 4
Octyl Acetate
Subject 6
Octyl Acetate
Subject 8
Octyl Acetate
Subject 11
Octyl Acetate
Subject 13
Octyl Acetate
Subject 16
Octyl Acetate
Subject 18
Octyl Acetate
Subject 20
Octyl Acetate
Subject 23
Octyl Acetate
Subject 25
Octyl Acetate
Subject 26
Octyl Acetate
Subject 29
Octyl Acetate
Subject 33
Octyl Acetate
Subject 34
Octyl Acetate
Subject 36
Detection Probability
0.0
0.5
1.0
0.0
1.0
2.0
3.0
Vapor Concentration (log ppb)

FIGURE 6

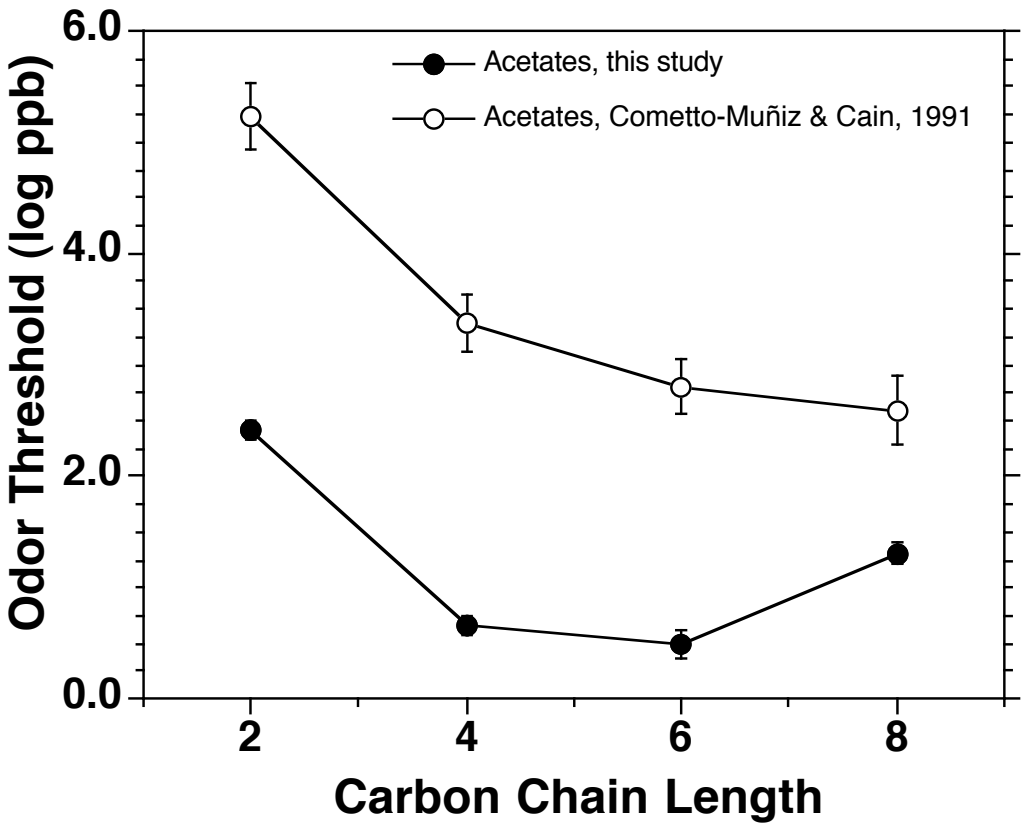

Acetates, this study
Acetates, Cometto-Muñiz & Cain, 1991
Odor Threshold (log ppb)
6.0
4.0
2.0
0.0
2
4
6
8
Carbon Chain Length

FIGURE 7

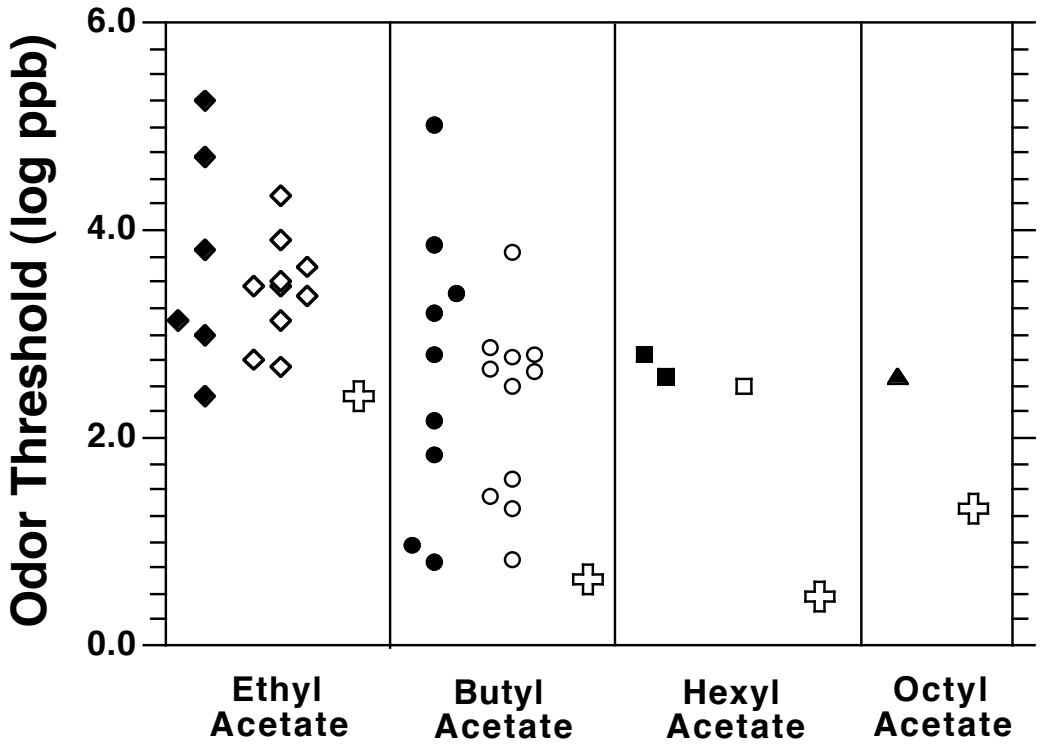
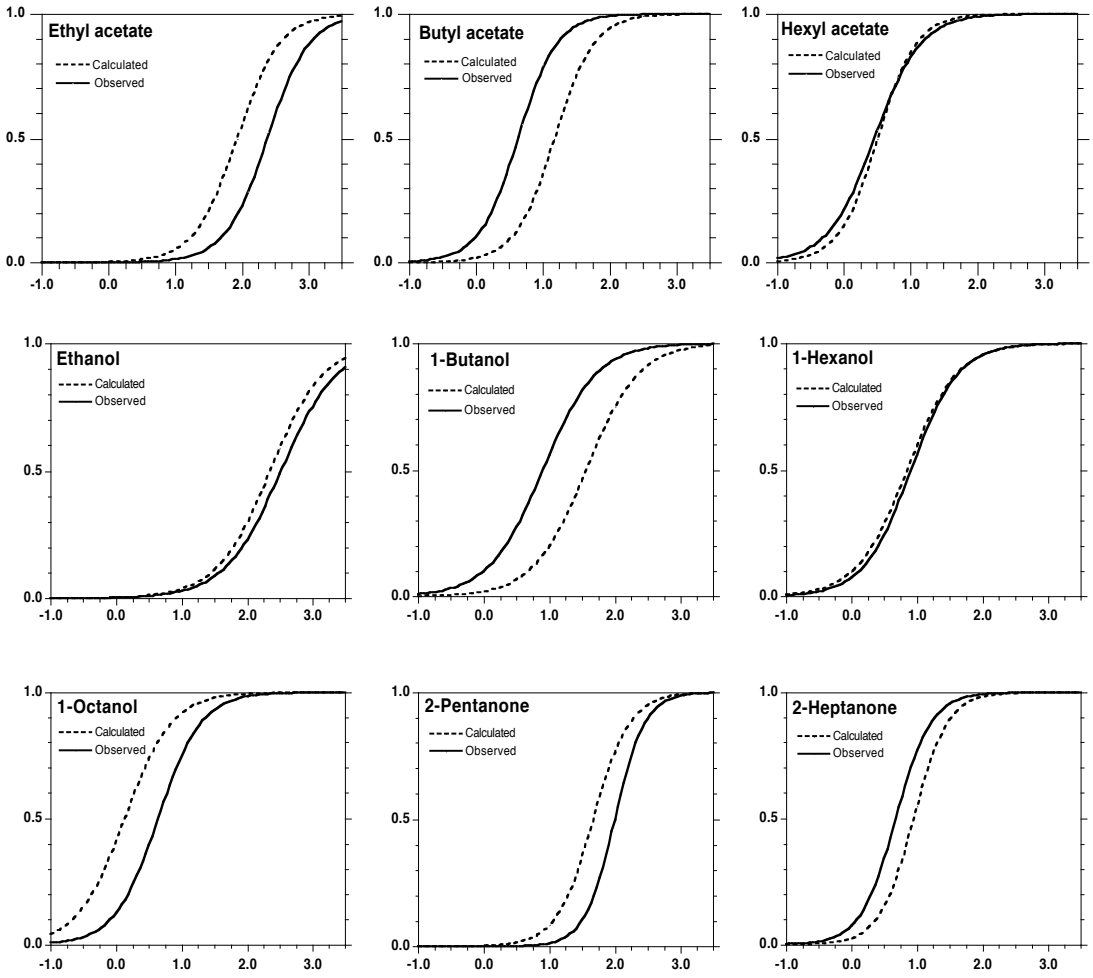

Odor Threshold (log ppb)
6.0
4.0
2.0
0.0
Ethyl Acetate
Butyl Acetate
Hexyl Acetate
Octyl Acetate

FIGURE 8


Ethyl acetate
Butyl acetate
Hexyl acetate
Ethanol
1-Butanol
1-Hexanol
1-Octanol
2-Pentanone
2-Heptanone
Calculated
Observed
1.0
0.5
0.0
-1.0
0.0
1.0
2.0
3.0

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