UNIVERSITY OF CALIFORNIA, SAN DIEGO

Determining Potency of Odorants: Concentration-Detection Functions and Recognition Point of Fragrance Chemicals

A Thesis submitted in partial satisfaction of the requirements for the degree

Master of Science

in

Biology

by

Sang Mun Park

Committee in charge:

Professor William S Cain, Chair
Professor Takaki Komiyama, Co-Chair
Professor Jing Wang

2012

The Thesis of Sang Mun Park is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

_____

_____

Co-Chair

_____

Chair

University of California, San Diego

2012

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT OF THE THESIS

Determining Potency of Odorants: Concentration-Detection Functions and Recognition Point of Fragrance Chemicals

by

Sang Mun Park

Master of Science in Biology

University of California, San Diego, 2012

Professor William S Cain, Chair

Professor Takaki Komiyama, Co-Chair

Recent studies by Abraham et al have shown that quantitative structure-activity relationship (QSAR) can be used for prediction of odor detection thresholds. Using the vapor delivery device 8 (VDD8), an 8-station device designed to measure odor detection performance of humans, the concentration-response functions for odor detection of various fragrance materials of high potency were measured. Subjects with a normal sense of smell were tested in small groups until data were accumulated. Samples of odorant in the vapor phase were taken before and during testing to confirm that the

delivered concentrations were stable. The method used was a three-alternative forced-choice procedure at each station, in ascending order of concentration. The functions generally conformed to fitted ogive curves. The chemical with the lowest odor detection threshold was Patchouli Alcohol at 0.44 ppt, and the highest was Herbane at 4.1 ppb. Using the solvation equation erected by Abraham et al, we compared and found that some of the observed values of ODTs were very close to the experimental value. While gathering data on detection, we also investigated the recognition threshold to understand any relationship between detection and recognition point. The recognition thresholds were found to be generally within a magnitude 2 to 4 times above the detection threshold.

INTRODUCTION

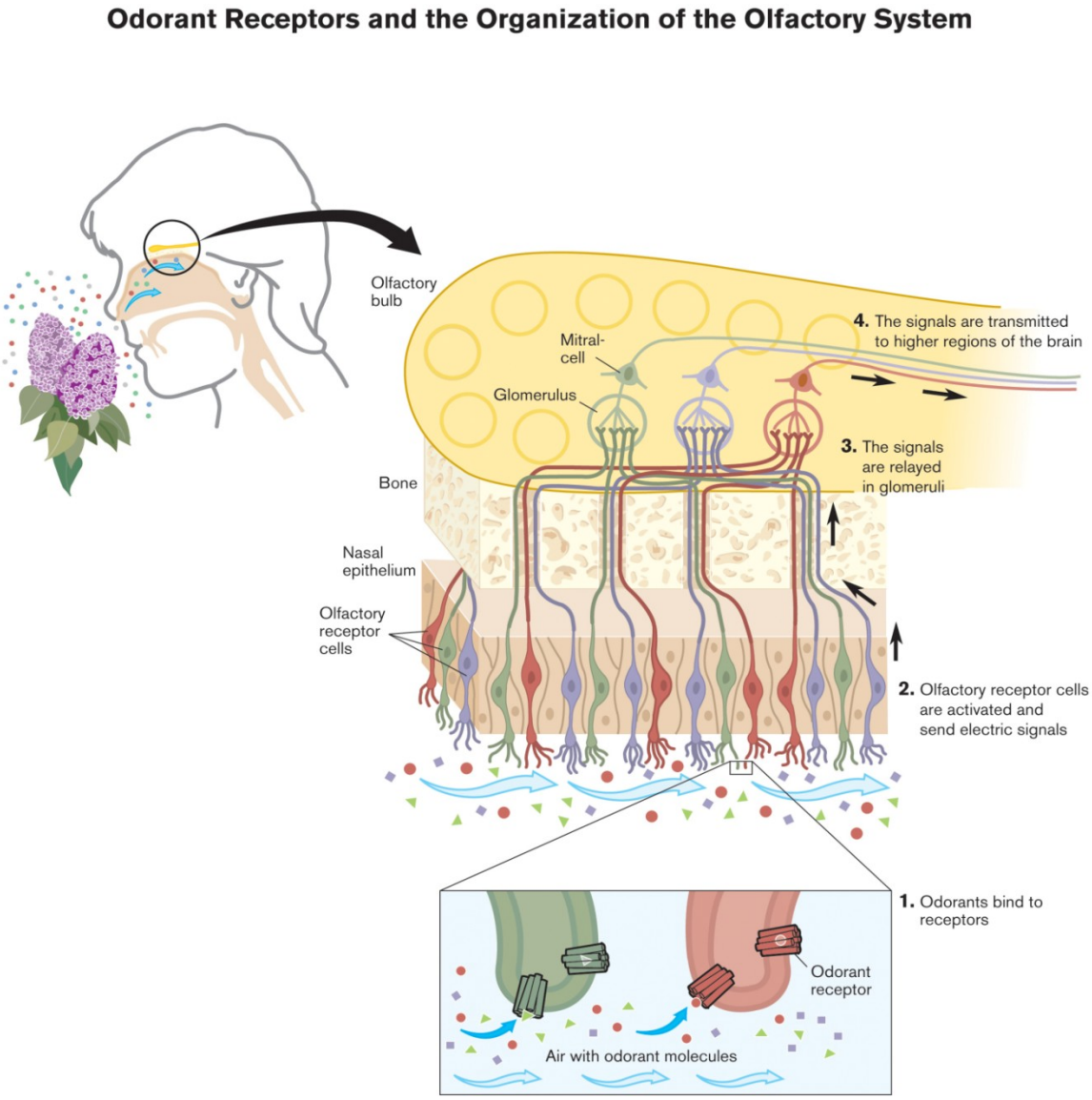


Figure 1. Close up of the Nasal Epithelium and Olfactory Receptor Cells.

Organization of the Olfactory System

Humans are able to detect very low concentrations of odorants. How are we able to perceive airborne chemicals at such low concentrations? Located high in the nasal cavity is the olfactory mucosa, which contains olfactory sensory neurons (Figure 1).

The neurons have cilia which contain protein strings called olfactory receptors. Buck and Axel (2001) used molecular biology techniques to discover the variety of olfactory receptors in mammalian species, and further research indicated that each neuron contains only one type of receptor. When odorants bind to the receptors, a conformational change occurs resulting in transduction, creating an electrical signal to the glomeruli in the olfactory bulb. Initially, each receptor activates a G protein which in turn stimulates the production of cAMP to activate ion channels. Each glomerulus then combines signals from sensory neurons to relay signals to the mitral cells in olfactory bulb, and information is further sent to higher areas. How we are able to recognize odor is the formation of an "odorant pattern", a combinatorial code resulting from an odorant molecule able to activate many receptors.

Psychophysical Testing

We generally understand how the neural process of olfaction works, but it does not tell us about the odor sensitivity of an organism. We do not understand the potency of an odorant, and how wide the range of potency is. To learn about the potency of odorants, we need to study the intact organism, and from the data gathered from the organism's behavior, we may be able to understand factors affecting sensitivity.

Psychophysics is the scientific study of the relationship between stimulus and sensation. A stimulus is presented to an observer, and they are asked to report whether or not they perceive it through multiple trials. The proportion of correct responses are then collected and plotted into a psychometric function, which is an S-shaped or ogive function. This function is also the integral of an underlying log-normal distribution curve.

Each point on the ogive specifies the proportion of the area under normal distribution (Gescheider 1997).

Psychophysical testing helps us gain insight in measuring the potency of an odorant and the sensitivity of the organism by obtaining the odor detection threshold (ODT). To determine the ODT, the point at which the lowest concentration of the odor can be detected, an experiment can be set up with different concentrations to create a psychometric function. At this threshold, psychophysicists define as the smallest intensity required for the stimulus to be reported 50% of the time (Gescheider 1997). There many factors such as "noise" in our physiological system that can lead to perceive the same stimulus differently on more than one occasion. As a result, we perform multiple trials to correctly estimate the probability. However, at this threshold one can only just detect the presence of the odor, not the quality or characteristics of it. We can acknowledge that there is another olfactory threshold: the recognition point. As our knowledge of olfaction broadens, it is relevant to study the recognition threshold, where quality of an odor can be recognized or can be distinguished from other odorants.

Odor Detection Threshold Testing

Controlling the amount of vapor delivery of the odorant, or volatile organic compound (VOC), is necessary for the scientific study of olfaction. The control of the chemical may occur via static or dynamic means (Cain 1992). In the case of static control, the vapor is presented from the headspace of a closed container. In dynamic control, the vapor flows continuously in a carrier gas (Cometto-Muniz et al 2003).

There are four variables that must be taken into consideration. One is control of the vapor via static or dynamic means, second is validation for delivery, third is the interface between vapor and subject, and finally, the psychophysical method. Many measurements of olfactory sensitivity suffer from unreliability, with variation shown from study to study of about 4 to 5 orders of magnitude for most odorants (van Germert 2003). Sniffing from an enclosed container can allow the vapor to mix with the surrounding air. Subjects are susceptible to adaptation and response bias, and these problems can be avoided by presenting an ascending series of concentration and using a forced-choice procedure (Cain 1989).

Quantitative structure-activity relationship

Early studies of biological potency of gases were performed by K.H. Meyer et al who investigated anesthesia in mice and salamanders. The relationship between potency and solubility of the gases in organic solvents was of interest. With new quantitative methods to study physicochemical properties of gases, quantitative structure-activity relationships(QSAR) have been recently used to study odor potency and thresholds in organisms (Abraham 1996).

QSARs represent a quantitative correlation between chemical structure of compounds and a well defined response, such as anesthesia, which can be expressed by the concentration of odorant required to elicit the response. An equation can be formed from physicochemical descriptors, which are determined empirically or by computational methods. These may include molar refraction, dipolarity, and other properties. The equation becomes useful for predicting potency of gases and for understanding of

biological processes. Predicting odor potency becomes necessary when thousands of experiments would be required for the of thousands of chemicals that evoke odor. Some are toxic for human testing and, even with animal studies, it is impractical to test every chemical.

This present study has two aims. The major aim of this study is to understand and analyze human sensitivity to various fragrance materials of high potency in the form of psychometric functions and in structure-activity relationships. The first step is to gather odor thresholds from psychophysical testing, and then attempt to analyze and predict ODT values from the equations previously devised by Abraham et al. We want to test how well the equations can estimate odor levels of highly potent chemicals.

The second aim is to attempt to begin an initial phase to explore and understand the recognition threshold of odors. Although in a preliminary phase, this study began to assess the recognition point during our performance-based study of the detection point. With these data, we will seek the presence of any general relationship between detection and recognition.

MATERIALS AND METHODS

Abraham et al have devised a general solvation equation for correlation of a variety of processes in which VOCs, volatile organic compounds, are transferred from a gas to a condensed phase:

$$SP = c + e\mathbf{E} + s\mathbf{S} + a\mathbf{A} + b\mathbf{B} + l\mathbf{L} \quad (1)$$

The dependent variable SP, will be log (1/ODT) in parts per million by volume. The larger the value of log (1/ODT), the more potent the chemical. The QSAR is based on five physicochemical properties, also called descriptors or parameters. E is the solute excess molar refractivity, S is the solute dipolarity/polarizability, A and B are the overall hydrogen bond acidity and basicity, and L is the logarithm of the gas to hexadecane partition coefficient at 25 degrees C. The coefficients *c,e,s,a,b* and *l* are found by multiple linear regression analysis, which reflect the complementary properties of the receptor phase (Abraham et al 2011). The solvation equation has parameters that sum up the solubility of the odorant. To predict the potency of odorants, our strategy is to apply the general equation to odor threshold values.

Subjects

Healthy human subjects participated in this study, with age ranges between 18 and 40. They were recruited from the San Diego community, and their ethnicities comprised of: 45% White (non-Hispanic), 9% Hispanic, 27% Asian, 14% White & Asian, and 5% African American. Females comprised 59% of the pool, and subjects gave informed consent by a protocol approved by Human Research Protections Program at the University of California, San Diego. Each participant completed a health history form

regarding their age, gender, allergies, and smoking habits as part of the screening process, as these criteria are known to affect establishing thresholds. They were then tested by the Connecticut Chemosensory Clinical Research Center (CCCRC) threshold test. They sniffed bottles of 1-butanol in ascending order to test for the participant's sensitivity, and then were presented with commonly found odors to see how well they were able to correctly recognize from a list which odor it was. This was part of the screening process to determine if the subjects had normal sense of smell to be part of the study

Odorants

The following is a list of fragrance materials used along with their CAS#: Ambroxan 6790-58-5 , Cedrol 77-53-2, Cyclohexyl Salicylate 25485-88-5, Damascone 57378-68-4, Galaxolide 1222-05-5, Herbane, Javanol 198404-98-7, Karanal 117933-89-8, Muscenone 82356-51-2, Patchouli Alcohol 5986-55-0 , Prismylate 122760-85-4, Sandalore 65113-99-7, Veltol 4940-11-8, and Velvione 37609-25-9.

Apparatus

The Chemosensory Perception Lab has developed a 8-station Vapor Delivery Device (VDD8) for measuring human odor thresholds. The VDD8 allows for a broad range of delivered concentrations, accommodates solvent-free delivery below a part per trillion, and adapts to various psychophysical methodologies (Schmidt and Cain, 2010). Its design encourages thousands of judgments to be collected per day from subjects being tested simultaneously and provides capacity for 3-alternative forced-choice testing.

Figure 2 shows the schematic diagram of the VDD8. Vapors are typically generated by using a direct injection with a syringe pump, but due to the high potency of the odorants, vapors were generated by over-the-surface-saturation. This technique allowed for constant flow and avoided fluctuations in vapor stream that occur at low injection rates of the syringe pump. The feed stream, combined from the saturator and make-up flow, entered the attenuator first which also served for further dilution if necessary, and then entered the distribution manifold to split into the 8 streams leading to its station. The streams were mixed with constant stream of background flow.

During the span of an experiment, samples were taken from the saturator hourly with a syringe and injected into a gas chromatograph with an FID to check for stable vapor concentration. These values were compared to a static headspace concentration provided by IFF to confirm accuracy. The vapor concentrations at each cone were calculated by dividing the vapor phase concentration inside the saturator by the total dilution factor of all the flows. This was determined experimentally by comparing the GC area counts measured from the saturator with that of test station (Sampling Port B). Some test odorants did not yield peaks in the GC, hence methanol was used as a substitute to determine the dilution factors with the appropriate settings.

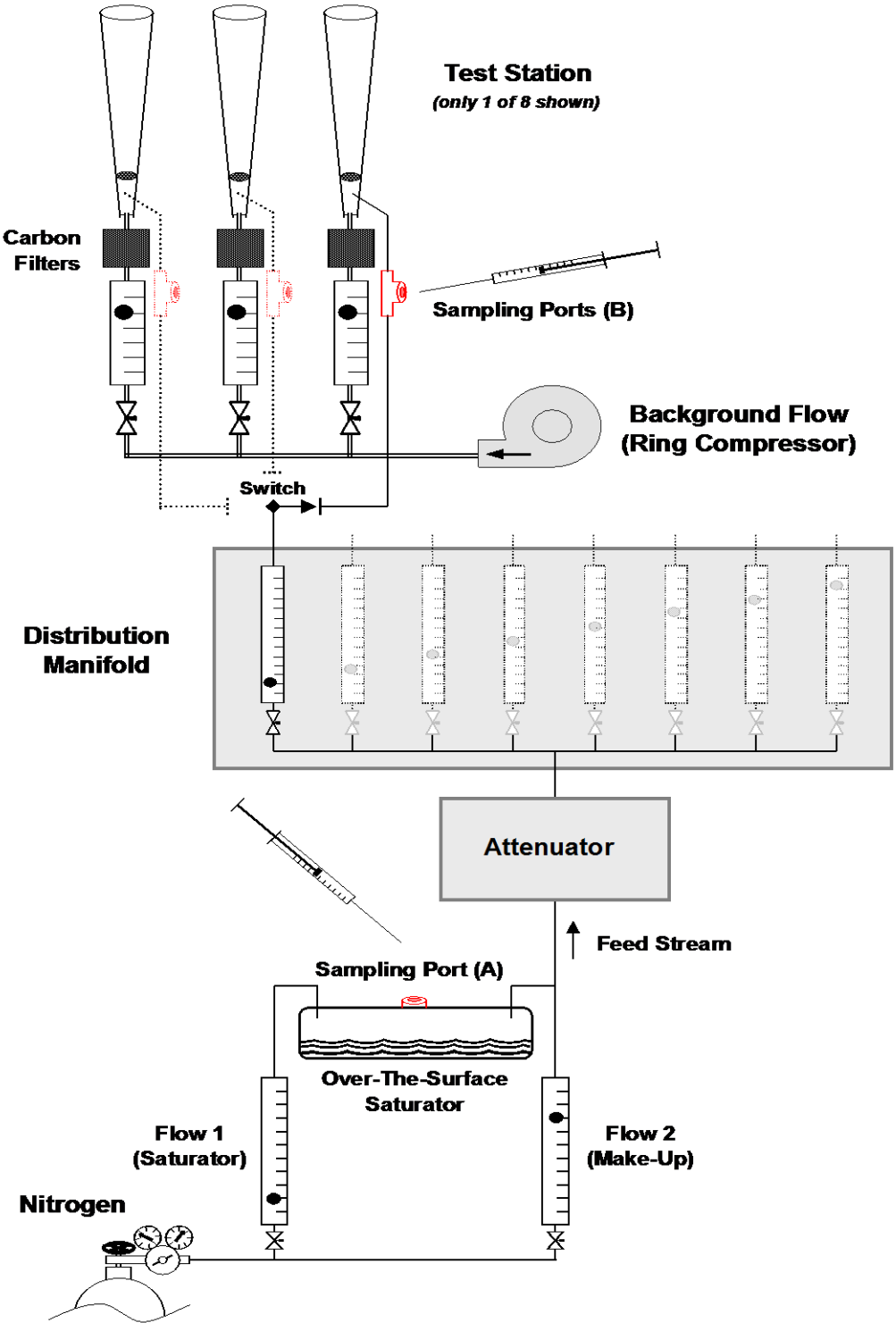


Figure 2. Schematic Diagram of the VDD8.

Experimental Design

Prior to the day of odorant testing, pilot testing was done to find an appropriate range for detection, and samples were taken to assess stability. Once this range was found, fine tuning was done by adjusting the flow of the device to have 50% detection range within the middle of the eight stations.

Figure 3 shows subjects sniffing the cones during a test day. Subjects typically completed 15-20 passes per session lasting four hours. Each pass consisted of sniffing starting from station 8 to station 1, in ascending order of concentration. Each pass took approximately 4 minutes to complete, with a 5 second interval between successive cones and 15 seconds between adjacent stations.

Figure 3. Subjects sniffing the cones of the VDD8 during test day. There is a computer controlled speaker system to control the timing.

During each pass, subjects were presented with three cones at each station, with one being the active cone. On the response sheet, the subject was asked to mark which cone had the stimulus, and give a confidence rating between 1 and 5, with 1 being the lowest confidence (Figure 4). Subjects were then also asked to circle their confidence rating at which they could determine the quality of the odor, or the recognition point. After each pass, the experimenter randomized the position of the active cones at each station. A 5 minute downtime in between passes to allow the odorant to adjust to the change in active cones.

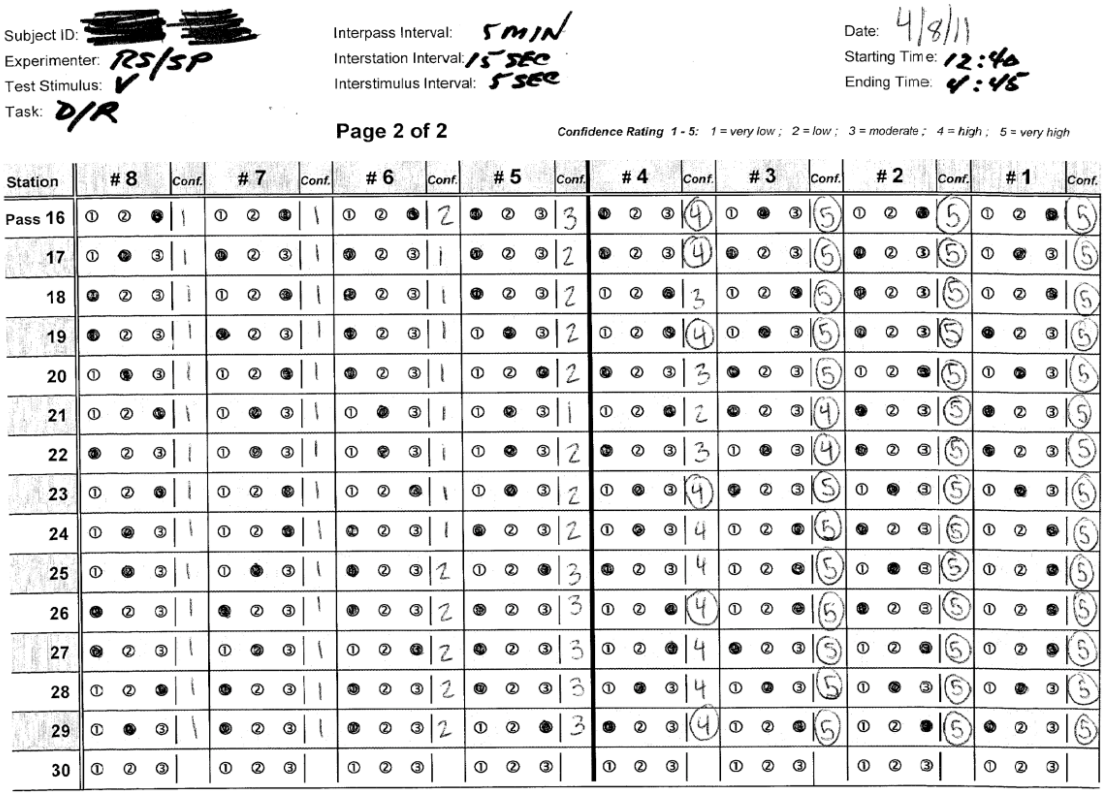


Figure 4. A subject's response sheet.

Data Analysis

Results are summarized as detection probability, recognition probability and confidence rating as a function of stimulus concentration. Detection and recognition probability (P) were corrected for chance. P would lie between 0, chance detection, and 1.0, perfect detection. The equation for chance correction was:

$$P = (m \cdot p(c) - 1)/(m-1) \qquad (2)$$

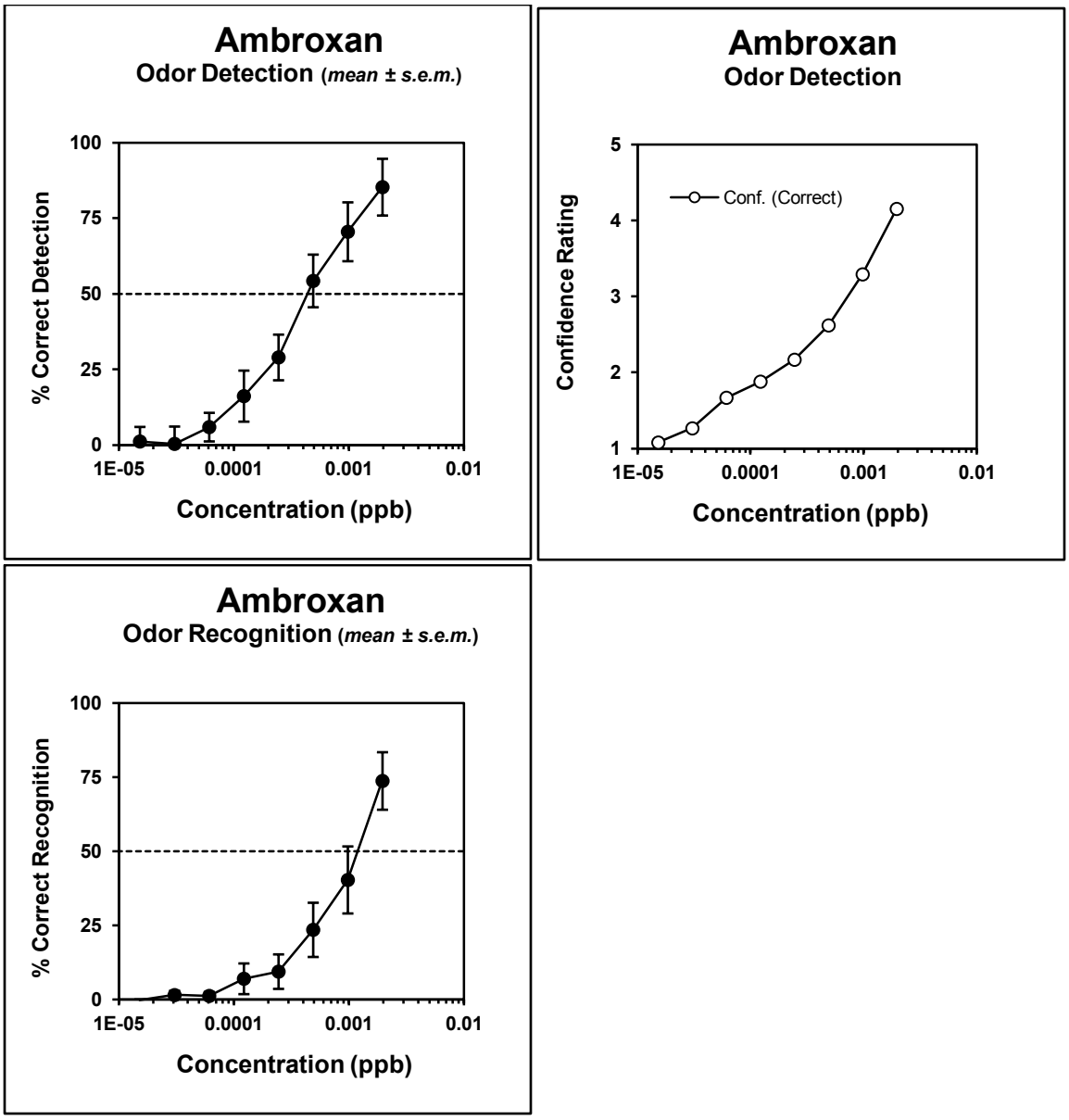
P is the detectability corrected for chance, m is the number of choices of per trial, and p(c) is the proportion correct (number of correct trials/total number of trials) (Macmillan and Creelman 1991). Proportions were computed for individual subjects based upon their 10-17 replicate judgments and then these were averaged across subjects. The error bars in the results show variation across subjects

RESULTS

Detection and Recognition Functions

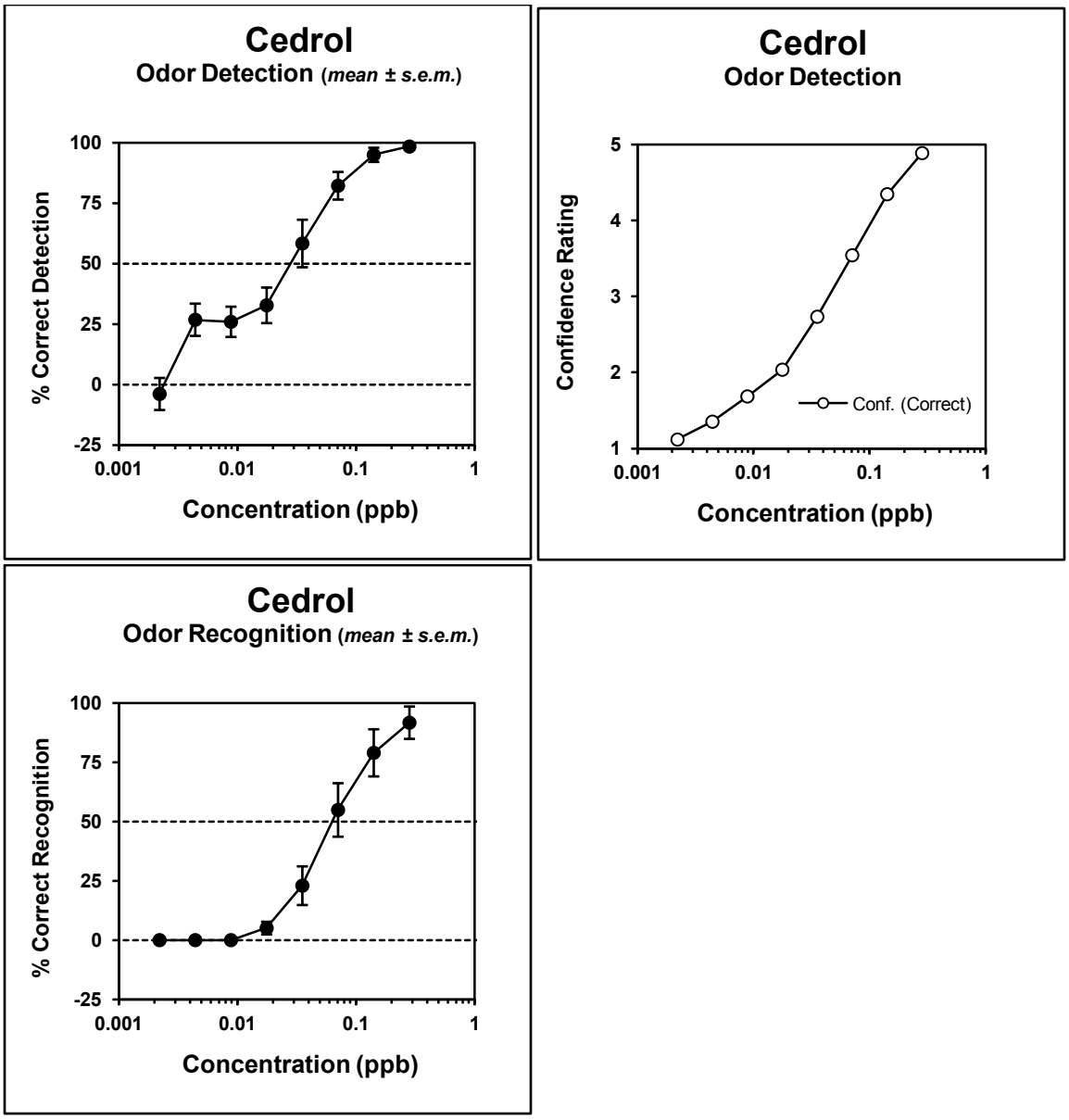
Figure 5 shows the results from the odor detection threshold testing of the 14 chemicals. They are plots of detection, recognition, and confidence rating as a function of vapor concentration. The function in the upper left section of the figure represents the concentration-detection function of the chemical. Each point represents the average correct detection of the subjects per concentration. The 50% line represents the detection threshold. The function on the lower left section represents the recognition function. Each point represents the average correct for recognition, and the recognition threshold is the 50% point. In most cases, the results were in the shape of a sigmoid curve. The function on the upper right section shows the average of the confidence ratings reported by the subjects per concentration. Confidence ratings increased as proportion of correct responses increased. There was good correspondence with growth of confidence and detection. The table below each figure presents the number of subjects for each chemical, and the detection and recognition threshold.

The results show that this psychometric study generally resulted in an ogive curve with 50% correct as the threshold. The chemicals displayed very high potency, with various thresholds in the parts per trillion. Herbane had the highest detection threshold of all at 4.1 ppb whereas Patchouli Alcohol was lowest at 0.44 ppt.



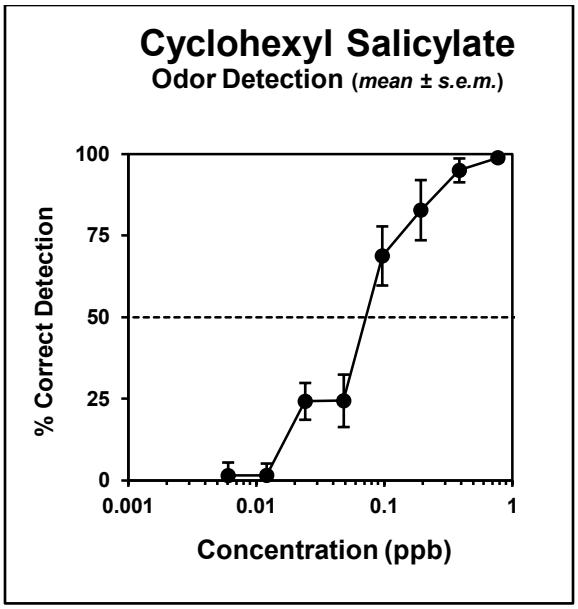
Number of Subjects	13
Detection Threshold	0.49 ppt
Recognition Threshold	1.1 ppt
Recognition : Detection	2.3:1

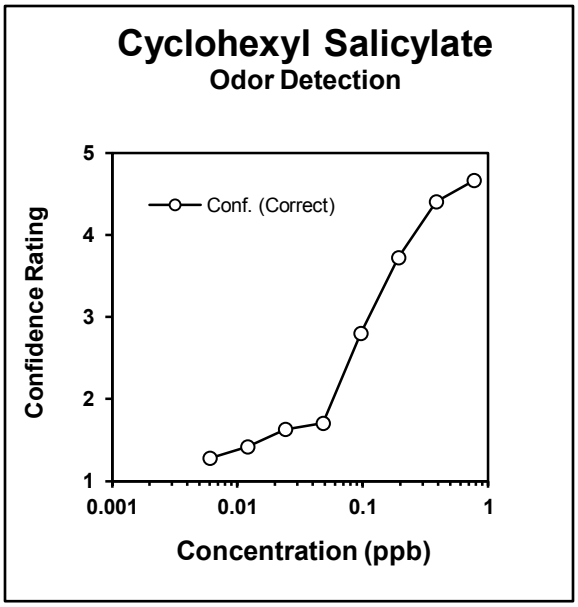
Figure 5. Psychophysical functions for 14 odorants. Functions include both the raw mean results with standard error of the mean for detection and recognition functions for each odorant. The function on the right shows the confidence ratings of correct detection reported by subjects.

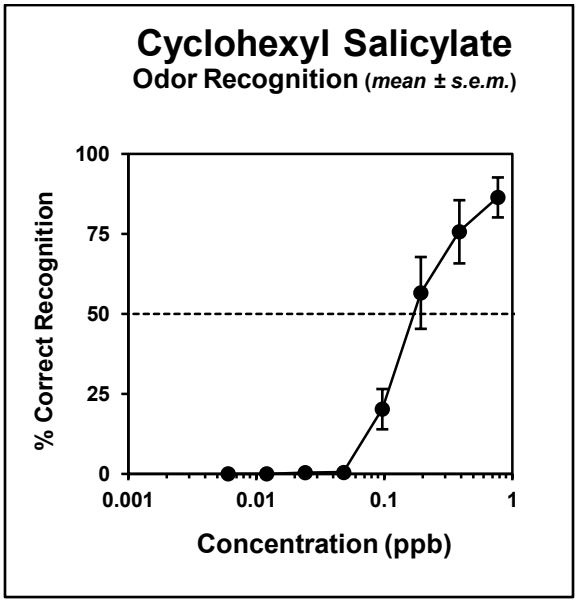


Number of Subjects	11
Detection Threshold	22 ppt
Recognition Threshold	72 ppt
Recognition : Detection	3.3:1

Figure 5. Psychophysical functions for 14 odorants, Continued.

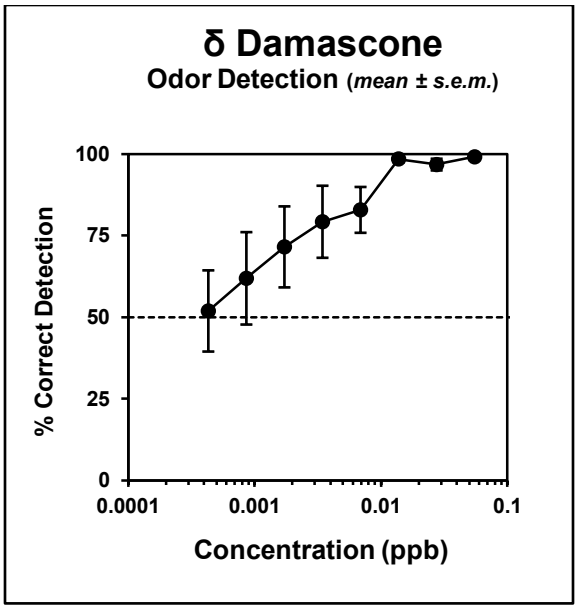


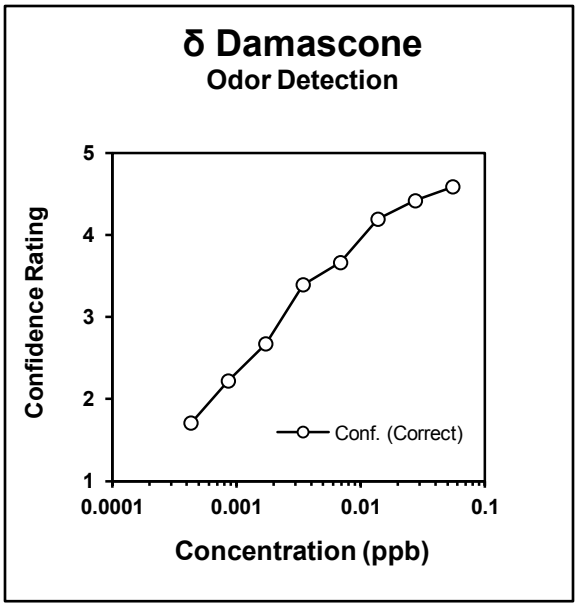


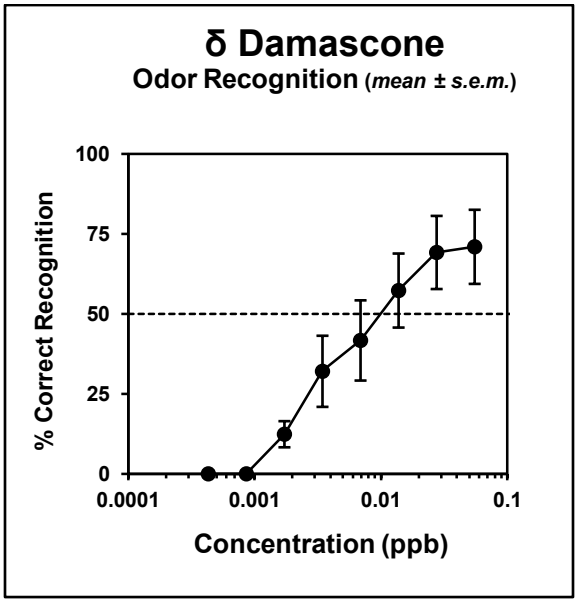


Number of Subjects	13
Detection Threshold	72 ppt
Recognition Threshold	244 ppt
Recognition : Detection	3.4:1

Figure 5. Psychophysical functions for 14 odorants, Continued.

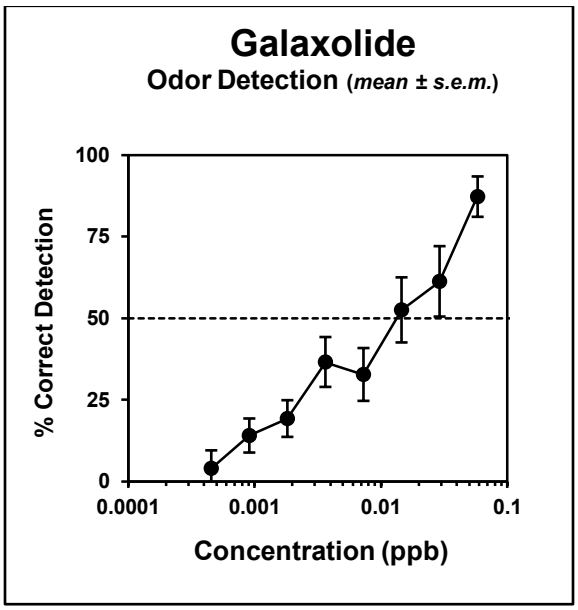


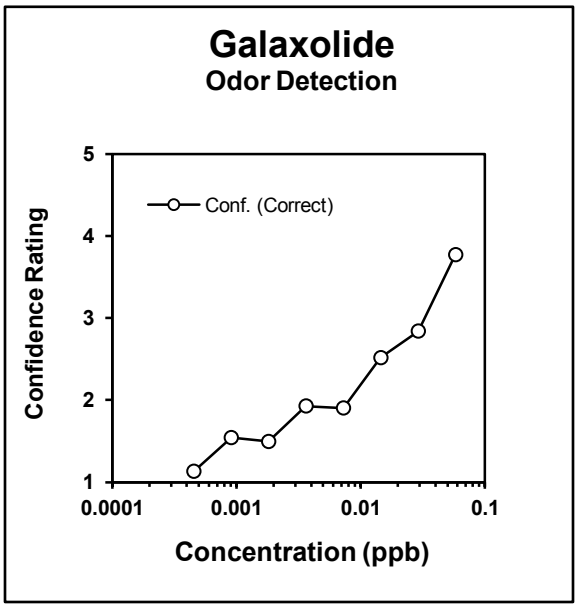


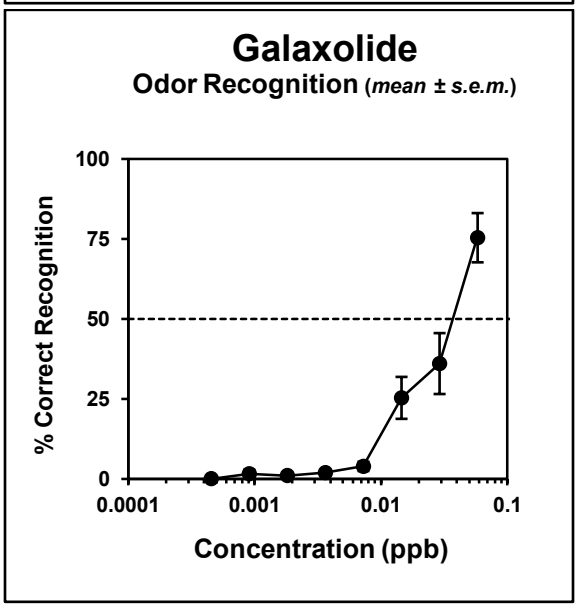


Number of Subjects	10
Detection Threshold	0.50 ppt
Recognition Threshold	10.4 ppt
Recognition : Detection	21:1

Figure 5. Psychophysical functions for 14 odorants, Continued.

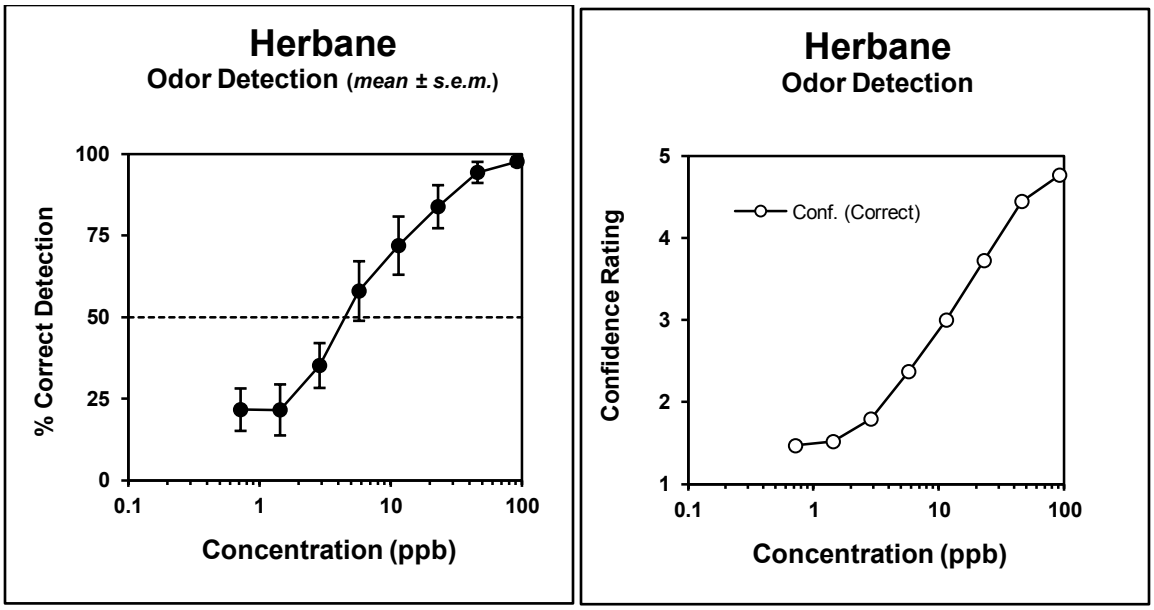






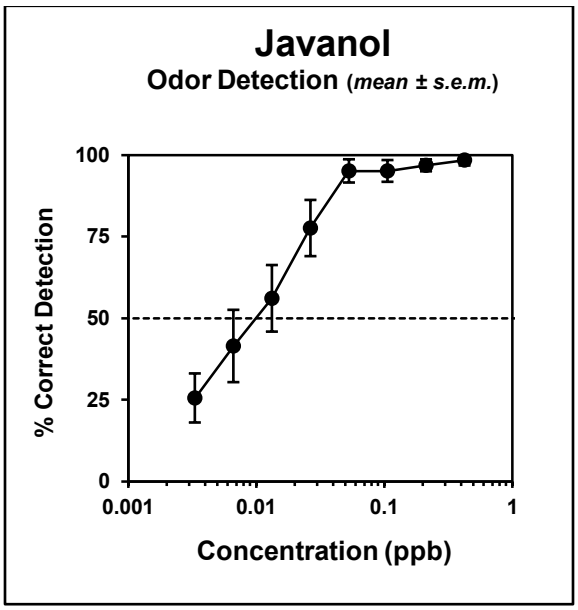
Number of Subjects	14
Detection Threshold	11 ppt
Recognition Threshold	35 ppt
Recognition : Detection	3.3:1

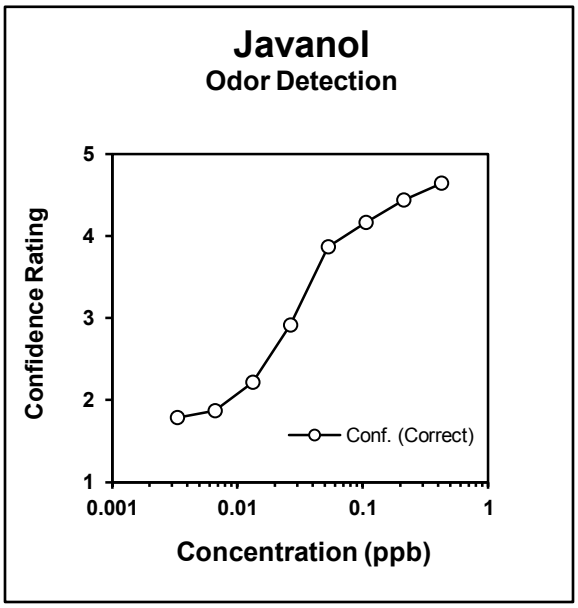
Figure 5. Psychophysical functions for 14 odorants, Continued.

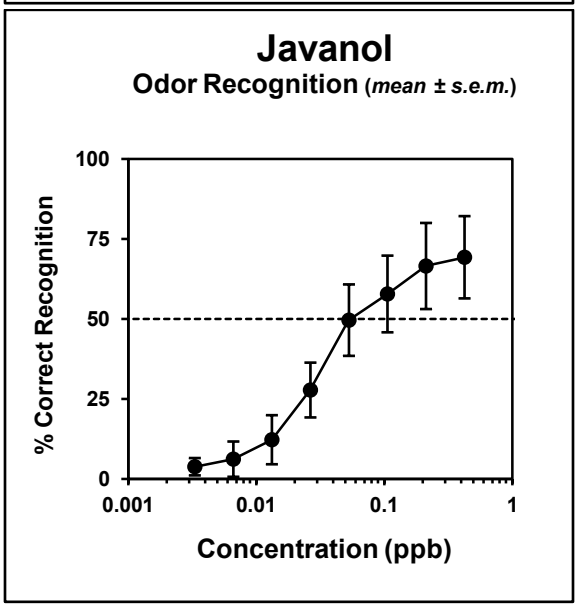


Number of Subjects	13
Detection Threshold	4.1 ppb
Recognition Threshold	n/a
Recognition : Detection	n/a

Figure 5. Psychophysical functions for 14 odorants, Continued. Recognition data was not collected for Herbane.

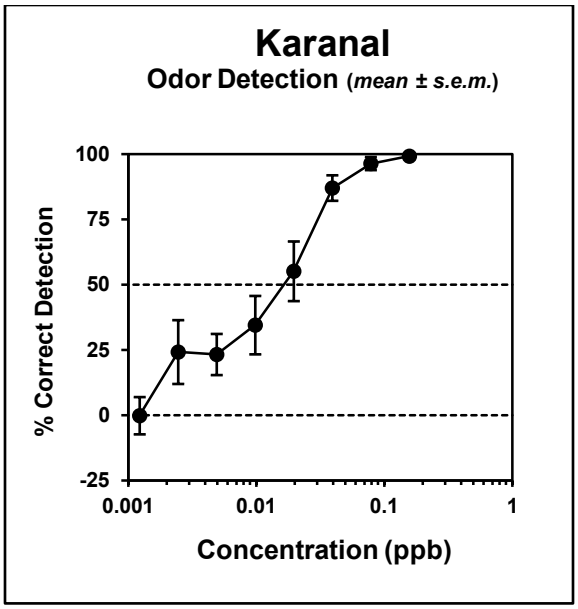


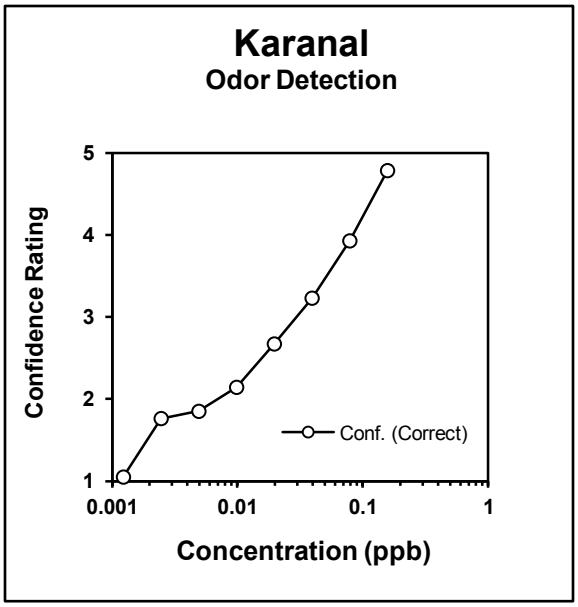


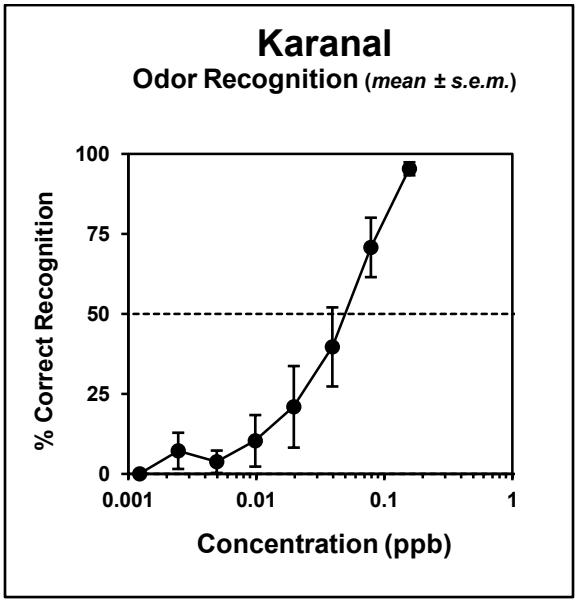


Number of Subjects	10
Detection Threshold	8.3 ppt
Recognition Threshold	97 ppt
Recognition : Detection	12:1

Figure 5. Psychophysical functions for 14 odorants, Continued.

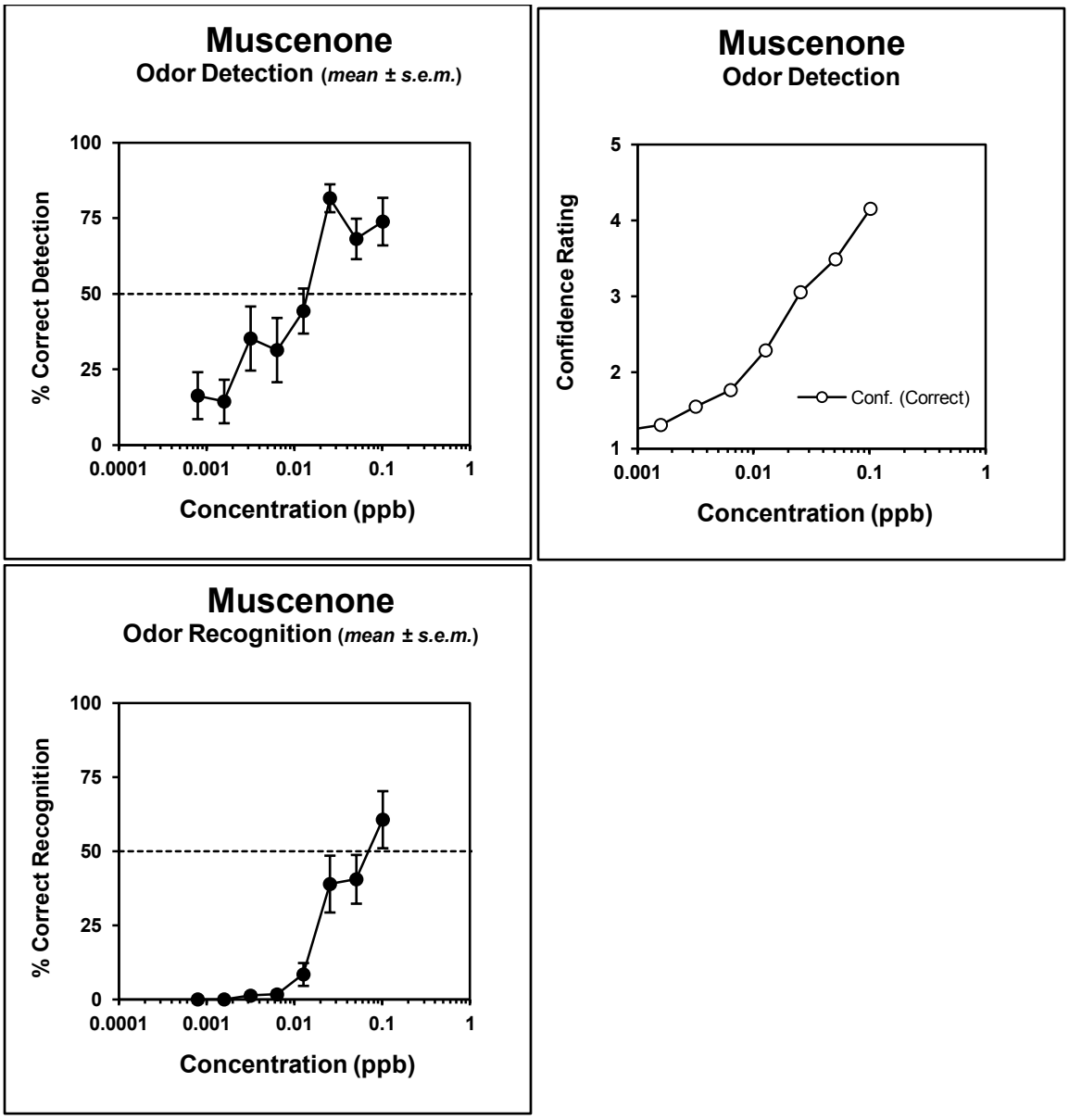






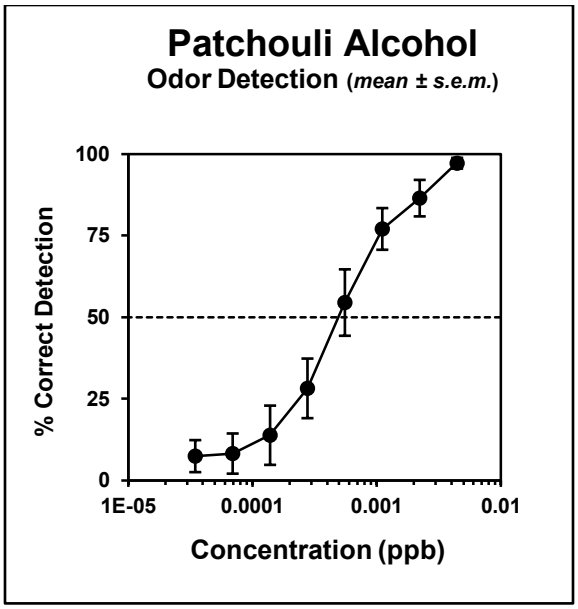
Number of Subjects	10
Detection Threshold	12 ppt
Recognition Threshold	36 ppt
Recognition : Detection	3.1:1

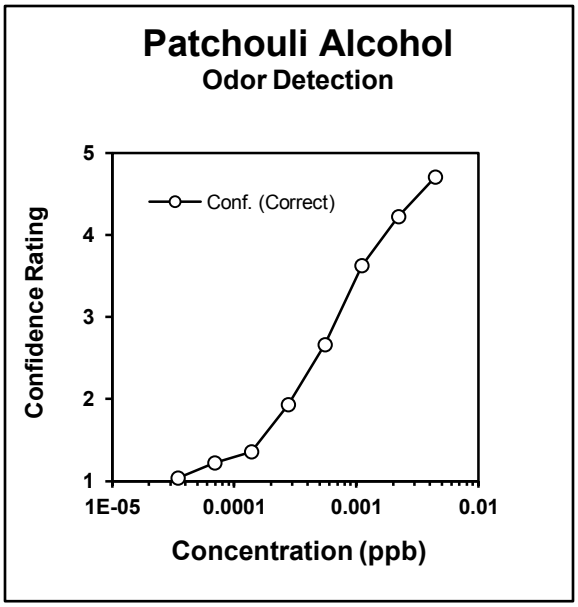
Figure 5. Psychophysical functions for 14 odorants, Continued.

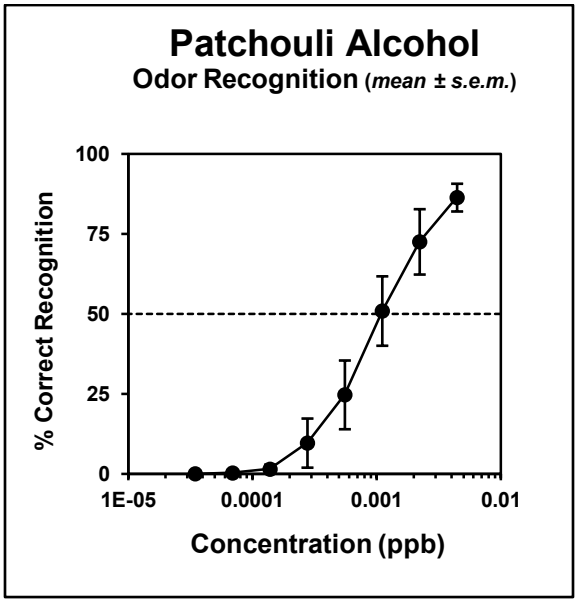


Number of Subjects	11
Detection Threshold	13 ppt
Recognition Threshold	61 ppt
Recognition : Detection	4.9:1

Figure 5. Psychophysical functions for 14 odorants, Continued.

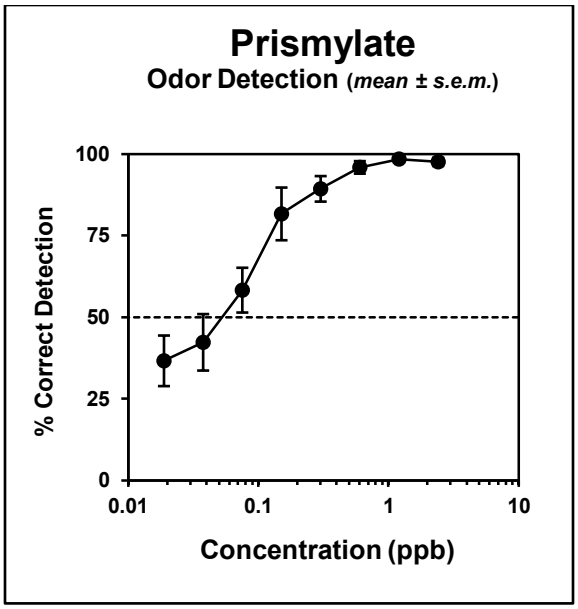


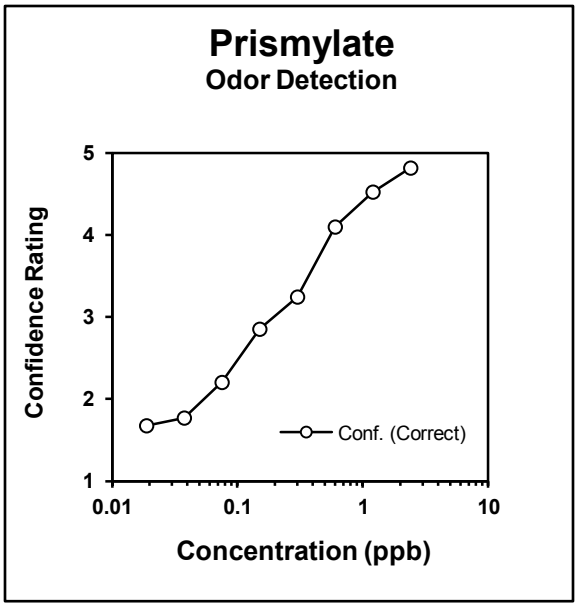


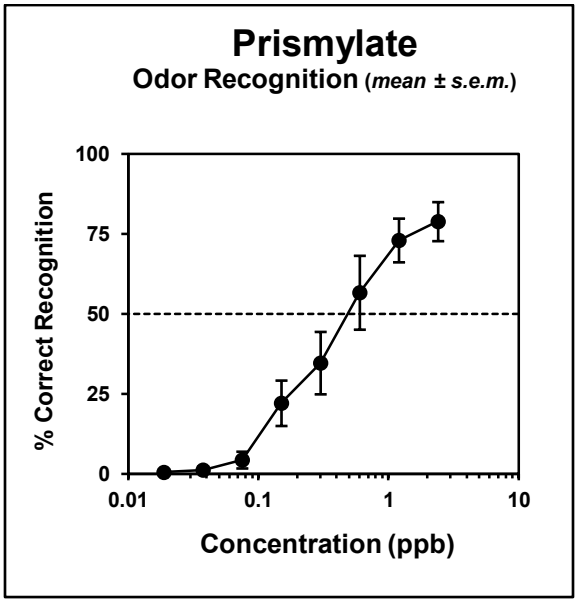


Number of Subjects	13
Detection Threshold	0.44 ppt
Recognition Threshold	1.2 ppt
Recognition : Detection	2.8:1

Figure 5. Psychophysical functions for 14 odorants, Continued.

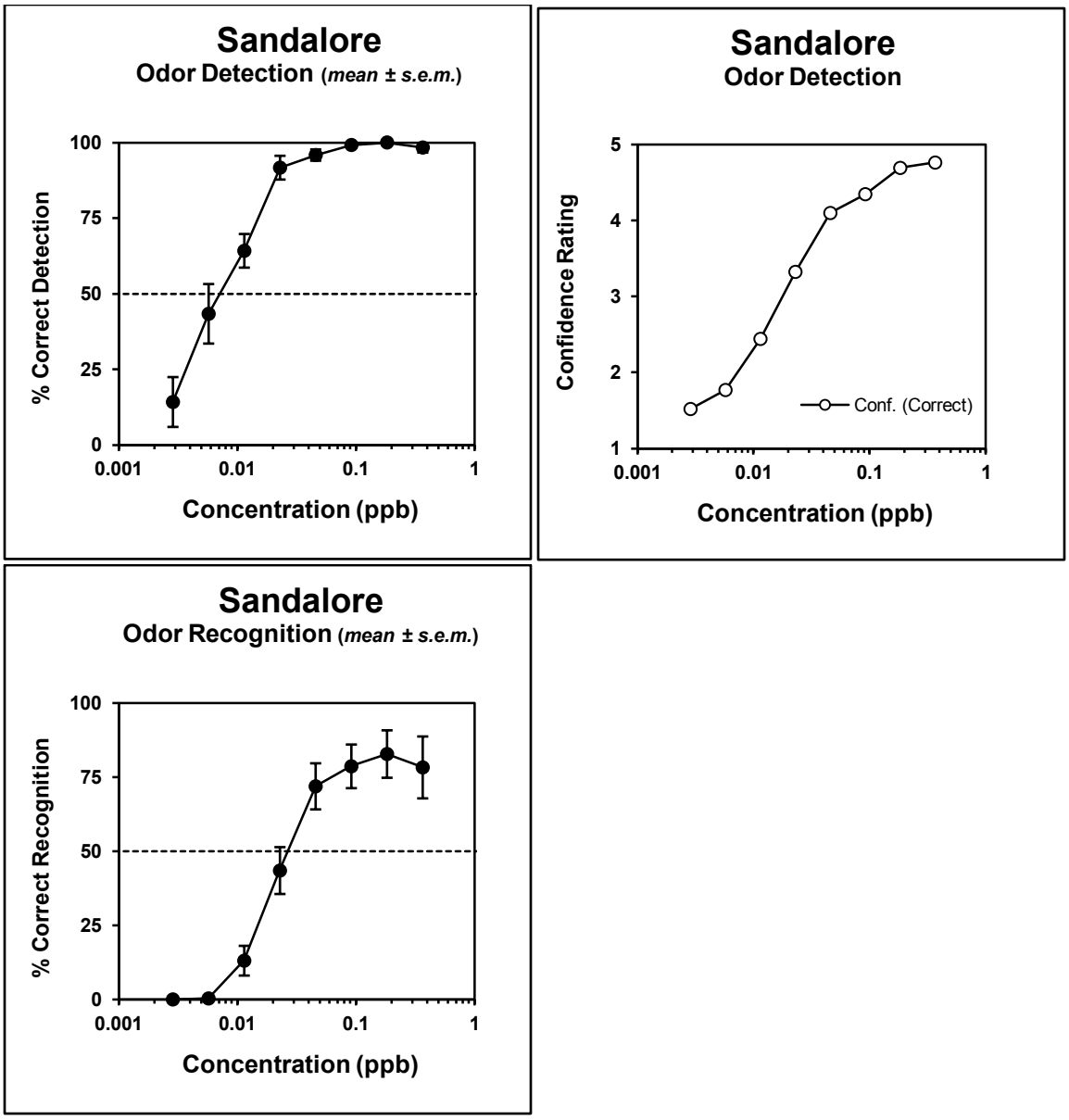






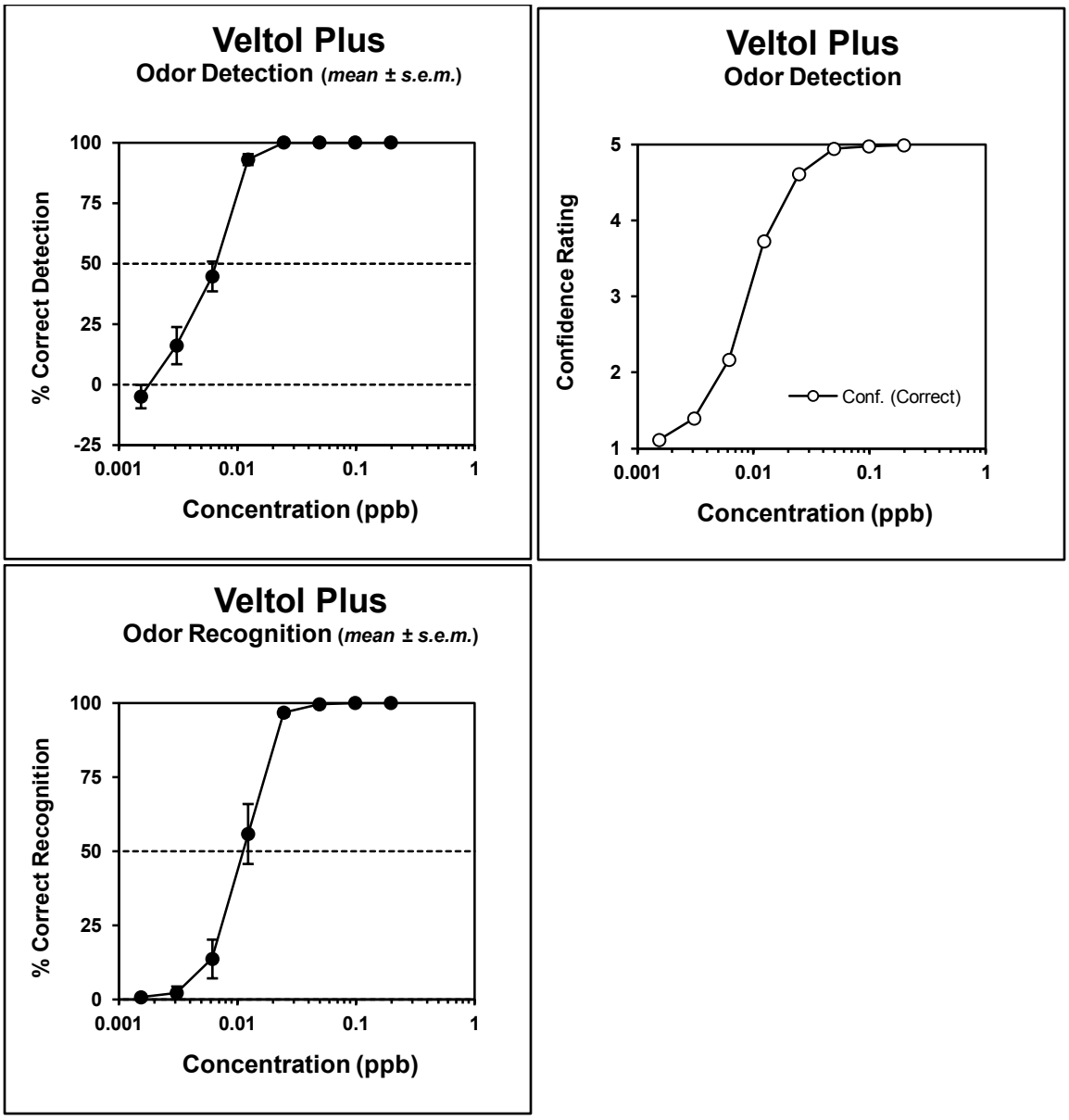
Number of Subjects	10
Detection Threshold	39 ppt
Recognition Threshold	589 ppt
Recognition : Detection	15:1

Figure 5. Psychophysical functions for 14 odorants, Continued.



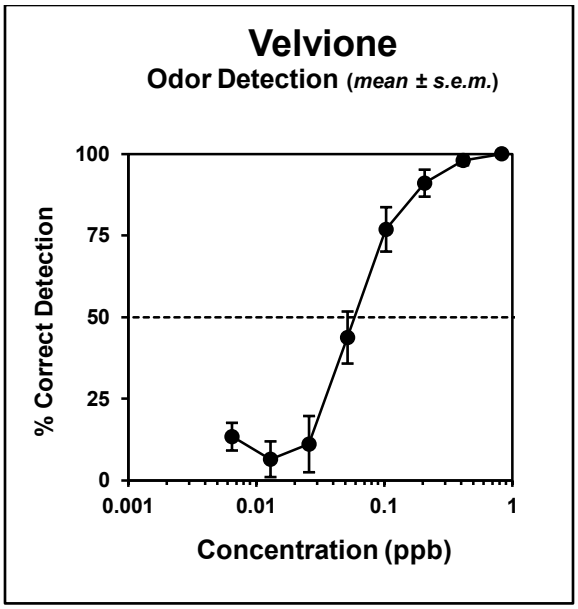
Number of Subjects	10
Detection Threshold	7.4 ppt
Recognition Threshold	43 ppt
Recognition : Detection	5.7:1

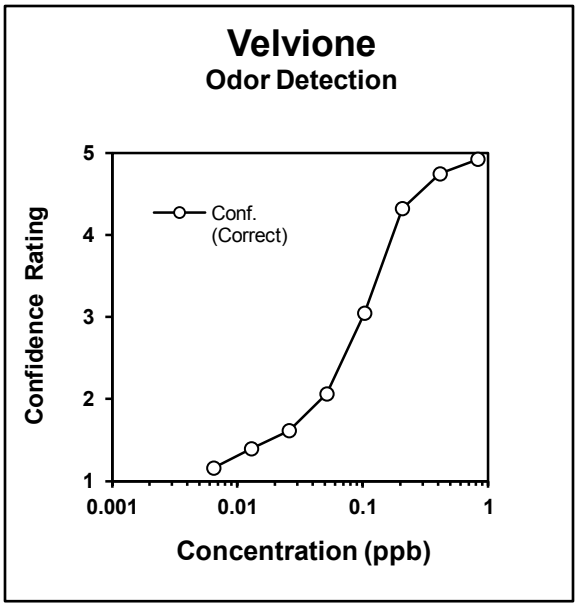
Figure 5. Psychophysical functions for 14 odorants, Continued.

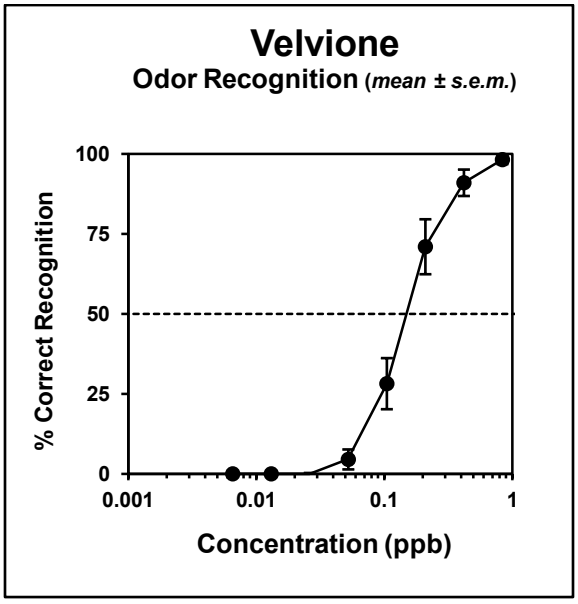


Number of Subjects	12
Detection Threshold	5.9 ppt
Recognition Threshold	9.7 ppt
Recognition : Detection	1.6:1

Figure 5. Psychophysical functions for 14 odorants, Continued.







Number of Subjects	13
Detection Threshold	52 ppt
Recognition Threshold	160 ppt
Recognition : Detection	3.1:1

Figure 5. Psychophysical functions for 14 odorants, Continued.

Prediction of ODT via Physicochemical Properties

Abraham et al have previously used equations for correlating odor threshold values for 64 varied compounds, including esters, aldehydes, ketones, alcohols, carboxylic acids, aromatic hydrocarbons, terpenes, and a number of other VOCs (Abraham et al 2002). The few compounds that were outliers to the equation might be left out. Descriptors and coefficients were optimized for predicted values to yield the following equation:

$$\log(1/ODT) = -5.27 + 0.51\mathbf{E} + 1.96\mathbf{S} + 1.48\mathbf{A} + 1.53\mathbf{B} + 0.72\mathbf{L} \qquad (3)$$

$$n = 50,\ r^2 = 0.78,\ SD = 0.57$$

In 2011, Abraham et al erected an equation with 353 compounds, including the data set obtained by Nagata (2003) and values obtained by Cometto-Muñiz and Cain (Cometto-Muñiz and Cain 1990, 1991, 1993, 1994; Cometto-Muñiz, Cain, and Abraham 1998; Cometto-Muñiz, Cain, Abraham et al. 1998):

$$\log(1/ODT) = -1.56 + 0.39\mathbf{E} + 0.57\mathbf{S} + 1.10\mathbf{A} + 1.36\mathbf{B} + 0.58\mathbf{L} + 3.82\mathbf{M} + 1.94\mathbf{AL} + 1.46\mathbf{AC} + 1.31\mathbf{UE} - 2.33\mathbf{C1} + 1.67\mathbf{C1AL} + 2.57\mathbf{C1AC} + 1.83\mathbf{C2AL} + 0.93\mathbf{C2AC} - 0.79\mathbf{HS} \qquad (4)$$

$$n = 353,\ r^2 = 0.76,\ SD = 0.82$$

The new variables introduced in equation 4 are indicator variables (Table 1). Each symbol represents a series of compounds. Series such as the aldehydes and mercaptans were found to be more potent than calculated, and a simple indicator variable brings the points in line with the other compounds (Abraham et al 2011). The physicochemical descriptors for the test chemicals were obtained by Abraham et al

through empirical or computational methods (Table 2). Table 3 lists the odorants and the ODT and log(1/ODT) values. With these values and equations, we were able to predict the ODTs and compare them with our observed ODTs.

Table 1. The Indicator variables used in equation 4.

Symbol	Variable
M	Mercaptans
AL	Aldehydes
AC	Carboxylic acids
UE	Unsaturated esters
C1	The Cometto-Muniz and Cain data set
C1AL	Aldehydes in the Cometto-Muniz and Cain data set
C1AC	Carboxylic acids in the Cometto-Muniz and Cain data set
C2	The Cometto-Muniz and Abraham data set
C2AL	Aldehydes in the Cometto-Muniz and Abraham data set
C2AC	Carboxylic acids in the Cometto-Muniz and Abraham data set
HS	The Hellman and Small data set

Table 2. Solvation parameters used in the present work.

Material Name	E	S	A	B	L
Galaxolide	1.090	1.15	0.00	0.63	9.476
Patchouli Alc	0.800	0.58	0.32	0.61	7.660
Veltol Plus	0.930	1.13	0.16	0.80	5.079
Muscenone	0.650	0.90	0.00	0.60	8.590
Javanol	0.820	0.96	0.37	0.48	8.059
Sandalore	0.550	0.87	0.37	0.61	7.630
Ambroxan	0.630	0.64	0.00	0.50	7.874
Damascone Delta	0.700	1.01	0.00	0.67	7.120
Velvione	0.650	0.90	0.00	0.59	8.600
Karanal	0.640	1.04	0.00	0.74	9.177
Cedrol Cryst	0.800	0.68	0.32	0.58	7.755
Cyclohexyl Sal	1.200	1.29	0.02	0.47	7.920
Prismylate	0.970	1.06	0.00	0.55	7.602
Herbane	0.900	1.46	0.00	0.79	7.590

Table 3. Values of observed log(1/ODT) with ODT in p.p.m. and ODT in p.p.b.

Material Name	ODT (ppb)	log(1/ODT)
Galaxolide	0.011	4.959
Patchouli Alc	0.00044	6.357
Veltol Plus	0.005	5.301
Muscenone	0.013	4.886
Javanol BHT	0.0083	5.081
Sandalore	0.0074	5.131
Ambroxan	0.00049	6.310
Damascone Delta	0.00050	6.301
Velvione	0.052	4.284
Karanal	0.012	4.921
Cedrol Cryst	0.022	4.658
Cyclohexyl Sal	0.072	4.143
Prismylate	0.039	4.409
Herbane	4.1	2.387

DISCUSSION

QSAR for Odor Detection Thresholds

Previous studies have shown that ODTs of humans can be predicted to a degree by a QSAR based on a solvation equation model (Abraham et al 2002). To further investigate odor potency in detail, we have to understand how olfactory perception is processed, where the stimuli and the receptor surface interact. It is not a simple transfer from the gas phase to receptor phase (Abraham et al 2001). Covering the receptors is a layer of mucus 10 to 30 μm thick that the molecules must first diffuse through (Hornung and Mozell, 1981). After the molecules are transported into the mucosa, it can then interact with the receptors of the neuron. This area is often referred to as the receptor biophase (Abraham et al 2002). The model works best for biological responses that depend on "selective" effects, those that control transfer of VOC from air to the nasal mucus before reaching the receptors. Solvation energy falls into the category that controls selective effects. Small structural changes in a VOC may elicit changes in biological activity that are often predictable and gradual and hence specific to the modification. The model does not really account for such "specific" effects, which depend on the VOC possessing a defined structure or functional group. Applying QSAR to ODTs from 60 VOCs showed that selective transfer accounts for 77% of the total effect (Abraham et al 2002).

The results of the predicted ODTs are shown in Table 4 for equations 3 and 4. The data obtained here shows that there was considerable variability, hence the values were compared by showing the ratios and approximate factors. Table 5 shows the ratio and approximate factor between the calculated and experimental values.

Table 4. Predicted ODT and log(1/ODT) values.

	Equation 3		Equation 4		Experimental	
Material	**log(1/ODT)**	**ODT (ppb)**	**log(1/ODT)**	**ODT (ppb)**	**log(1/ODT)**	**ODT (ppb)**
Galaxolide	5.355	0.004	5.880	0.001	4.959	0.011
Patchouli Alc	3.220	0.603	4.712	0.019	6.357	0.00044
Veltol Plus	2.552	2.805	3.662	0.218	5.301	0.005
Muscenone	3.954	0.111	5.008	0.010	4.886	0.013
Javanol	4.138	0.073	5.047	0.009	5.081	0.0083
Sandalore	3.713	0.194	4.816	0.015	5.131	0.0074
Ambroxan	2.764	1.723	4.301	0.050	6.310	0.00049
Damascone Delta	3.239	0.576	4.333	0.046	6.301	0.00050
Velvione	3.946	0.113	5.000	0.010	4.284	0.052
Karanal	4.862	0.014	5.614	0.002	4.921	0.012
Cedrol Cryst	3.439	0.364	4.783	0.016	4.658	0.022
Cyclohexyl Sal	4.345	0.045	4.907	0.012	4.143	0.072
Prismylate	3.640	0.229	4.586	0.026	4.409	0.039
Herbane	4.747	0.018	5.105	0.008	2.387	4.100

Table 5. Ratios and factors of calculated vs. experimental values of ODT.

	Equation 3 Calc vs Exp	Equation 3 Calc vs Exp	Equation 4 Calc vs Exp	Equation 4 Calc vs Exp
Material	**ODT Ratio 3**	**Approxim. Factor**	**ODT Ratio 4**	**Approxim. Factor**
Galaxolide	0.4	2.5	0.1	8.3
Patchouli Alc	1369.8	1369.8	44.1	44.1
Veltol Plus	561.1	561.1	43.6	43.6
Muscenone	8.6	8.6	0.8	1.3
Javanol	8.8	8.8	1.1	1.1
Sandalore	26.2	26.2	2.1	2.1
Ambroxan	3517.2	3517.2	102.1	102.1
Damascone Delta	1152.3	1152.3	93.0	93.0
Velvione	2.2	2.2	0.2	5.2
Karanal	1.1	1.1	0.2	4.9
Cedrol Cryst	16.6	16.6	0.7	1.3
Cyclohexyl Sal	0.6	0.6	0.2	5.8
Prismylate	5.9	5.9	0.7	1.5
Herbane	0.004	229	0.002	522

Firstly, the data shows that Equation 4 was a better estimator for the ODTs than Equation 3. Equation 3 resulted in values in which the ratios were over 3000, whereas Equation 4 did not exceed 102.1. Additionally, some of the ODT ratios derived from Equation 4 were close to the value of 1. Equation 4 incorporated additional ODT data sets that allowed a more general equation to be applied for wider range of odorants.

We ultimately used Equation 4 to plot the calculated vs. observed log(1/ODT) of the fragrance compounds to understand how well the points fit in comparison to the previous data sets. Figure 6 shows the plot of log(1/ODT) observed vs. calculated for the multiple data sets in blue, a total of 353 chemicals. The majority of the original data points lay within the range of -1 to 3 log(1/ODT) that represented odorants with lower potency. The new data points still fell within the cluster of the previous data points, but within range of higher potency that only a few of the previous points covered. The original R^2 correlation value was 0.76, but with the inclusion of the fragrance compounds, the value improved to 0.78.

It was encouraging to see how the model was able to predict some thresholds closely. The new data points lay within the cluster of the previous data sets and even improved the correlation value. Furthermore, the fragrance chemicals were much more complex, potent, and may have had properties that the model did not account for. No indicator variable was used, yet the points still fit near the regression line with the original data points. The equation does reasonably well, but it can possibly be improved upon by the inclusion of additional data sets that will take more time. There is still much further study needed to learn about the model and other complex fragrances.

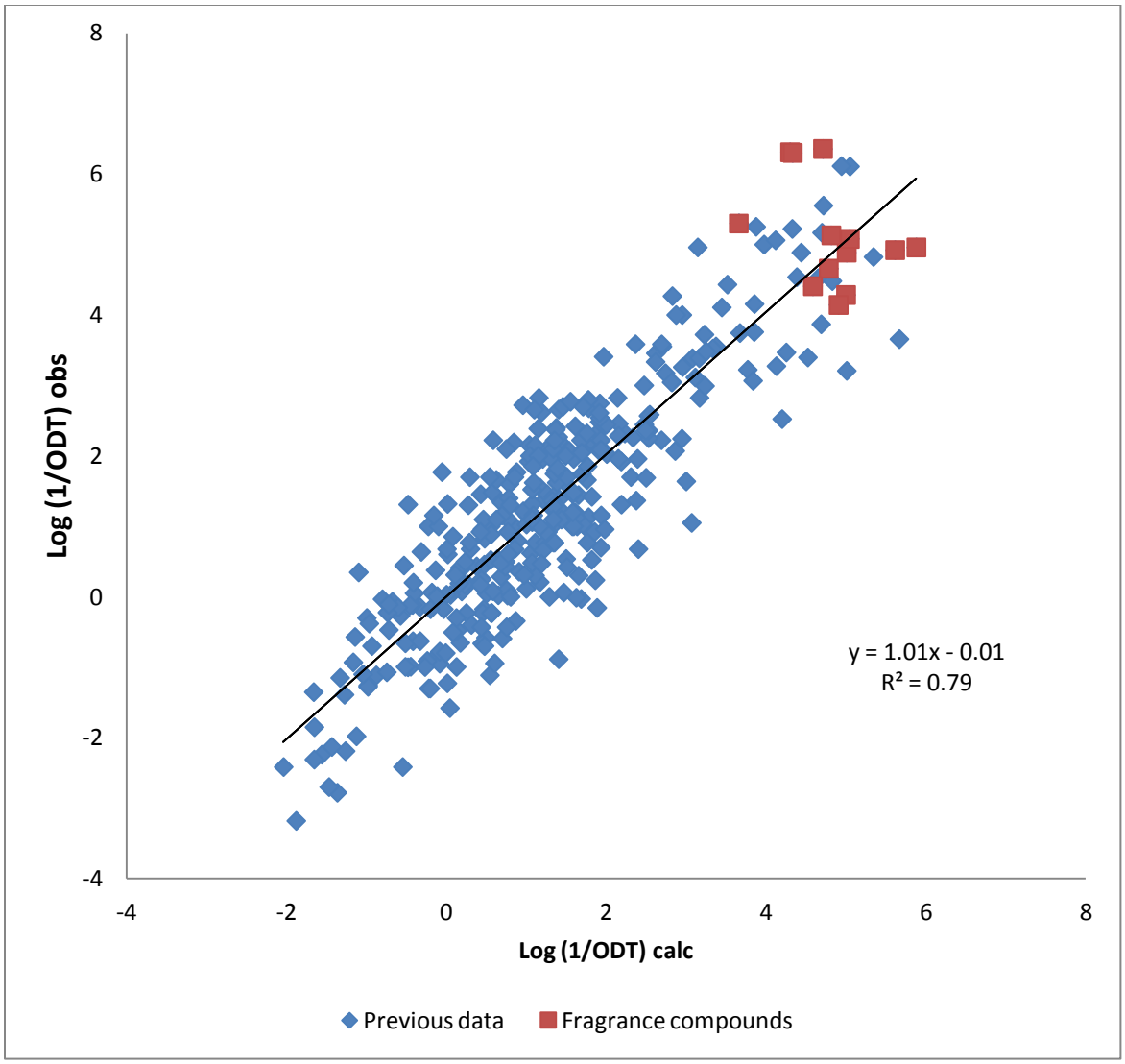


Figure 6. Plot of calculated log(1/ODT) versus observed log(1/ODT) from Equation 4. The blue represents previous data points, and the red represents points obtained from this study.

Relationship between the Detection and Recognition Threshold

There are current methodologies that are accepted by scientists for determining odor detection points, such as the three alternative forced-choice procedure with an ascending concentration approach used for this study, and many studies have been done to find the detection thresholds of many odorants. However, the same does not apply for recognition thresholds. There are relatively few studies done and they are unsupported by accepted methodologies. Our current methodology is also unsupported, but we still attempted to investigate the recognition point that may help us to gain insight for future improvements or studies.

The results show that for most of the odorants (Table 6), recognition data lies between two to four times in magnitude above the detection threshold. As expected, the recognition point for the odorants was not much higher than the detection point. During testing, most subjects could distinguish the qualities of the odor one to two stations from where they can detect the odorant.

Table 6. Recognition to detection ratio of the listed materials.

Odorant	Recognition to Detection Ratio
Ambroxan	2.3 : 1
Cedrol	3.3 : 1
Cyclohexyl Salicylate	3.4 : 1
δ Damascone	21 : 1
Galaxolide	3.3 : 1
Javanol	12 : 1
Karanal	3.1 : 1
Muscenone	4.9 : 1
Patchouli Alcohol	2.8 : 1
Prismylate	15 : 1
Sandalore	5.7 : 1
Veltol Plus	1.6 : 1
Velvione	3.1 : 1

A few studies have attempted to find the difference in magnitude between detection and recognition of odorants, and they have shown values much greater, up to 100 times the magnitude. According to Dalton (2002), the concentration has to be increased by as much as a factor of 3 above detection before the subject can recognize the quality. Additionally, the values obtained by Cain et al fall surprisingly close within our factor ranged between two and four. With accumulation of considerable data on odor detection, Cain et al found a general rule for converting the detection data into recognition threshold. They used a performance criterion of 84% detection to conclude that the recognition thresholds exceeds the detection by a factor of three (Cain et al 2009).

Some of the threshold ratios showed a greater gap than the expected values between factors two and four. This may be possibly due to the fact that our current methodology is inadequate. We can greatly improve our results by creating a performance based test for recognition, for example, ask the subjects to choose a scent that matches with the chemical being tested. Another possibility may be due to new subjects who were not fully acclimated to the testing instructions and environment, or subjects completely insensitive to the material, skewing the results. According to Buck, change in concentration of odors can change the receptor combinatorial code. At higher concentrations, additional olfactory receptors are activated, resulting in a change in the perception of odor (Buck 2005). This could occur at the stations with higher concentration, causing the subjects to be uncertain at which station they were able to recognize the odor.

Looking Forward

The study of odorant potency has practical justifications. With the emergence of a billion dollar fragrance industry, predicting odor detection thresholds has become relevant in order to determine the amount of chemical needed to achieve its smell. ODT testing is time consuming and expensive, and difficult to come up with a lot of data. Additionally, it is also important theoretically in the field of biology. By studying the behavior of the intact organism, we can learn about how sensitive the organism is to series of odorants. It helps us understand properties of molecules that make sense of smell work.

Our methodology for detection threshold testing is effective for producing accurate and reliable results. In future studies, we hope to contribute additional ODTs to future work on the linear solvation equations, and develop other effective methods of recognition testing to prove that the threshold is magnitude of 2-4 times the detection threshold.

REFERENCES

Abraham, M.H. (1996). The potency of gases and vapors: QSARS- Anesthesia, sensory irritation, and odor. *Indoor Air and Human Health,* 67-90.

Abraham, M.H., Gola, J.M., Cometto-Muniz, J.E., Cain, W.S. (2001). The correlation and prediction of VOC thresholds for nasal pungency, eye irritation and odour in humans. *Indoor Built Environ, 10* ,252-257.

Abraham, M.H., Gola, J.M., Cometto-Muniz, J.E., Cain, W.S. (2002). A model for odour thresholds. *Chem Senses, 27(2)*,95-104.

Abraham, M.H., Sanchez-Moreno, R., Cometto-Muniz, J.E., Cain WS. (2011). An algorithm for 353 odor detection thresholds in humans. *Chemical Senses Advance Access*.

Buck, L. and Axel, R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell, 65*, 175-187.

Buck, L. (2005). Unraveling the sense of smell (Nobel Lecture) Angew. *Chem. Int. Ed., 44*, 6128 – 6140. Retrieved from http://www.nobelprize.org/nobel_prizes/medicine/laureates/2004/press.html.

Cain, W.S. (1989). Testing olfaction in a clinical setting. *Ear, Nose, and Throat Journal, 68*.

Cain, W.S. (1992). Techniques in the quantitative study of human olfaction. *The Science of Olfaction,* 279-308.

Cain, W.S., Schmidt, R., Cometto- Muñiz, J.E. (2009). The recognition point in odor detection.

Cometto-Muñiz, J.E., Abraham, M.H. Human olfactory detection of homologous n-alcohols measured via concentration-response functions. *Pharmacology Biochemistry and Beahvior, 89(3),* 279-291.

Cometto-Muñiz, J.E., Cain, W.S. (1990). Thresholds for odor and nasal pungency. *Physiol. Behav, 48*, 719-725.

Cometto-Muñiz, J.E., Cain, W.S. (1991). Nasal pungency, odor, and eye irritation thresholds for homologous acetates. *Pharmacol Biochem Behav, 39*, 983-989.

Cometto-Muñiz, J.E., Cain, W.S. (1993). Efficacy of volatile organic compounds in evoking nasal pungency and odor. *Arch Environ Health. 48*, 309-314.

Cometto-Muñiz, J.E., Cain, W.S. (1994). Sensory reactions of nasal pungency and odor to volatile organic compounds. *The alkylbenzenes. Am Ind Hyg Assoc J., 55*, 811-817.

Cometto-Muñiz, J.E., Cain, W.S., Abraham, M.H. (1998). Nasal pungency and odor of homologous aldehydes and carboxylic acids. *Exp Brain Res., 118,* 180-188.

Cometto-Muñiz, J.E., Cain, W.S., Abraham, M.H. (2003). Quantification of chemical vapors in chemosensory research. *Chem Senses, 28(6),* 467-77.

Cometto-Muñiz, J.E., Cain, W.S., Abraham, M.H., Gil-Lostes, J. (2008). Concentration-detection functions for the odor of homologous n-acetate esters. *Physiology and Behavior, 95(5)*, 658-667.

Cometto-Muñiz, J.E., Cain, W.S., Abraham, M.H., Kumarsingh, R. (1998). Trigeminal and olfactory chemosensory impact on selected terpenes. *Pharmacol Biochem Behav., 60*, 765-770.

Dalton, P. (2002). Olfaction. *Stevens' Handbook of Experimental Psychology*.

Gescheider, G.A. (1997). *Psychophysics: the fundamentals*. New Jersey: Lawrence Erlbaum Associates.

Macmillan, N.A., Creelman, C.D. (1991). *Detection theory: A User's Guide*. Cambridge: Cambridge University Press.

Nagata, Y. (2003). Measurement of odor threshold by triangle odor bag method. Odor measurement review. *Tokyo(Japan): Office of Odor, Noise and Vibration, Environmental Management Bureau, Ministry of Environment*, 118-127.

Schmidt, R., Cain, W.S. (2010). Making scents: dynamic olfactometry for threshold measurement. *Chem Senses*, *35(2)*, 109-20.