Chem. Senses 28: 25–32, 2003

Detection Thresholds for Phenyl Ethyl Alcohol Using Serial Dilutions in Different Solvents

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Abstract

Detection thresholds are typically obtained by presenting a subject with serial dilutions of an odorant. Many factors, including the solvent used to dilute the odorant, can influence the measurement of detection thresholds. Differences have been reported in detection thresholds for phenyl ethyl alcohol (PEA) when different solvents are used. In this study we used gas chromatography (GC) to investigate further the effect of solvent on odor detection thresholds. We used a single ascending method and serial dilutions of PEA in four different solvents—liquid paraffin (LP), mineral oil (MO), propylene glycol (PG) and dipropylene glycol (DPG)—to determine the PEA thresholds for 31 adult subjects. For each solvent, we prepared eight serial log base 10 step dilutions (1–8), with corresponding liquid PEA concentrations of 6.3×10^{1}–6.3×10^{-6} (% v/v). We found that the threshold concentrations for PEA in LP (step 6.5) and PEA in MO (step 5.5) were significantly lower ($P < 0.05$) than for PEA in PG (step 4.0) and DPG (step 4.0). We then used GC to measure both the liquid and gas PEA concentrations for the dilution steps prepared with LP and PG. Although there were large threshold differences in the liquid concentrations of PEA in LP and PG, the headspace gas concentrations of PEA were the same. These results demonstrate the importance of determining the gas concentration of odorant stimuli when performing odor threshold measurements, in particular when comparing odor detection thresholds obtained using different solvents.

Key words: gas chromatography, headspace gas, human detection threshold, olfaction, partition coefficient

Introduction

Odor detection thresholds are often used to assess olfactory function in psychophysical studies (Doty *et al.*, 1986; Heywood and Costanzo, 1986; Stevens *et al.*, 1988) and for the clinical evaluation of patients with chemosensory disorders (Doty *et al.*, 1987; Deems *et al.*, 1991). Odor threshold concentrations provide a measure of the lower limits of olfactory detection and sensitivity. Test methods used to determine odor thresholds vary depending on the accuracy required, time constraints, available resources and type of study. Threshold testing may employ a variety of forced-choice or non-forced-choice methods, single ascending or staircase presentations, squeeze bottles or olfactometers, serial step dilutions, different solvents and different odorants (Doty and Kobal, 1995). Phenyl ethyl alcohol (PEA) is frequently selected as a test odorant because it has a pleasant rose-like smell and does not produce intranasal trigeminal sensations (Doty *et al.*, 1978; Cometto-Muniz and Cain, 1990). Two common solvents used to dilute PEA are propylene glycol (PG) and mineral oil (MO). Pierce *et al.* (Pierce *et al.*, 1996) reported a significant difference in PEA detection thresholds when they used these two solvents. To explain the different detection thresholds obtained, they hypothesized that the gas concentrations of PEA above the serial dilutions varied depending on the solvent.

In this study, we compared detection threshold measurements obtained for PEA diluted in four different solvents. We then used gas chromatography (GC) to obtain direct measurement of both the gas and liquid concentrations of PEA. An analysis was then performed to determine if there were significant differences in the detection thresholds obtained with the four solvents and if differences could be explained by gas to solvent concentration ratios (e.g. gas–solvent partition coefficients).

Materials and methods

Subjects

Thirty-one healthy adult subjects participated in the study (12 female, 19 male; mean age = 30.7 ± 12.2 SD). Subjects were excluded if they had a history of nasal disease, head

trauma or nasal congestion. Nasal airflow was evaluated prior to testing and subjects with obstructed or congested airflow were excluded.

Stimuli

PEA was employed as the test odorant. PEA has a sweet, floral (rose-like) odor and is considered a good odorant for detection threshold testing. We used four different solvents to dilute the PEA: dipropylene glycol (DPG), liquid paraffin (LP), MO and PG. PG and DPG are pure chemicals, while LP and MO are mixtures of liquid hydrocarbons. LP and MO are often considered to be the same compounds, but can vary in composition depending on the supplier and the method of manufacturing. DPG is commonly used as a diluent for perfumes and is a good solvent for organic odorants. The chemical properties of PEA and the four solvents used are listed in Table 1. Serial log base 10 step dilutions (1–8) with corresponding liquid concentrations of PEA of 63% (v/v)–6.3×10^{-6}% (v/v) were prepared with the four solvents (Table 2). At the higher concentration dilution steps (steps 1 and 2), PEA did not dissolve completely in two of the solvents (LP and MO) and separate layers were visible.

Procedures

Each subject was given four different odor detection threshold tests using PEA diluted in LP, MO, PG and DPG. Each test was administered twice for each of the solvents for a total of eight detection threshold measurements. The order of the solvent tested was randomized. Detection threshold measurements were obtained using a single ascending non-forced-choice method widely used in Japan (Takagi, 1989). The dilution step at which the odorant stimulus was first detected was used to define detection threshold. Prior to testing, subjects were instructed to say 'yes' only when they were sure they had detected the odor. If unsure, the subjects were instructed not to guess. The average of the two PEA threshold measurements was defined as the subject's threshold for that solvent.

Absorbent perfumer's paper strips were used for odorant presentation. A 10 ml volume of each stimulus dilution was placed in a 26 ml glass scintillation vial. The tip of the paper strip was dipped into the vial containing the odorant liquid

Table 1 Properties of phenyl ethyl alcohol and liquid solvents

Chemical	Company	CAS[a]	Molecular formula	Mol. wt	Density (g/cm^3)	Boiling point (°C)	Vapor pressure[b] (mmHg, 23°C)
Phenyl ethyl alcohol	Acros	60-12-8	$C_8H_{10}O$	122.17	1.0202	220	0.0735
Propylene glycol	Acros	57-55-6	$C_3H_8O_2$	76.09	1.0361	188	0.1071
Dipropylene glycol	IFF	25265-71-8	$C_6H_{14}O_3$	134.18	1.0206	232	0.0269
Mineral oil	Squibb	8042-47-5	mixture	–	0.85	>323	<0.0001[c]
Liquid paraffin	Acros	8042-47-5	mixture	–	0.85	>302	<0.0001[c]

[a]CAS: the American Chemical Society's Chemical Abstracts Service (CAS) registry number.

[b]American Institute of Chemical Engineers DIPPR database (v. 1.5).

[c]Estimated from distillation data.

Table 2 Concentration of phenyl ethyl alcohol

	Concentration (% v/v)	Log *C* (v/v)	g/cm^3	mol/cm^3
Pure liquid	100	0.00	1.0202	8.35×10^{-3}
Dilution step				
1	63.1	–0.20	6.44×10^{-1}	5.27×10^{-3}
2	6.31	–1.20	6.44×10^{-2}	5.27×10^{-4}
3	0.631	–2.20	6.44×10^{-3}	5.27×10^{-5}
4	0.0631	–3.20	6.44×10^{-4}	5.27×10^{-6}
5	0.00631	–4.20	6.44×10^{-5}	5.27×10^{-7}
6	0.000631	–5.20	6.44×10^{-6}	5.27×10^{-8}
7	0.0000631	–6.20	6.44×10^{-7}	5.27×10^{-9}
8	0.00000631	–7.20	6.44×10^{-8}	5.27×10^{-10}
Saturated gas[a]			4.86×10^{-7}	3.98×10^{-9}

[a]Caclulated from $PV = nRT$ at 23C, 1 atm.

dilution and then presented to the subject by placing the strip under their nose. Subjects were allowed to sniff the tip of the paper strip three or four times and, if they detected the odorant, that dilution step was recorded as the detection threshold. Each paper strip was used only once and then discarded into a narrow-necked glass bottle kept under a ventilation hood. To reduce room air contamination further, the odorant vials were also kept in the ventilation hood.

Data analysis

The averages of the two threshold measurements for each of the four solvents were determined for each subject (Figure 1) and the data subjected to an analysis of variance (ANOVA). Differences in the median threshold values among the four treatment groups were analyzed using Tukey's test. All statistical analyses were performed using SPSS v. 10. SigmaPlot v. 8.0 was used to generate the graphs.

Gas chromatography

We used GC to determine the PEA headspace gas concentrations for each of the PEA dilutions. A Hewlett Packard 5880A GC fitted with a Carbopack column (Supelco, Bellfonte, PA) and flame ionization detector (FID) was used to obtain PEA measurements for both liquid and gas concentrations. The GC was calibrated by injecting measured amounts of PEA dissolved in high-purity ethanol (Table 3). A PEA concentration–response calibration curve was obtained by plotting the area of the GC peaks (peak area) for each of the PEA liquid concentration standards (Figure 2). Measurements of PEA in the four different solvents were obtained by injecting 0.5 µl liquid samples into the GC injection port. The gas PEA concentration levels (Figure 3A) were obtained by GC measurements of the headspace gas above the 10 ml liquid samples. To obtain headspace samples, dilution step vials were covered with a layer of parafilm and a Hamilton gas-tight syringe was used to withdraw 5 ml gas samples of the headspace. Since the retention times of PEA and PG were relatively close, oven temperature programming was used (1 min at 160°C, then 10/min to 185°C, then 5/min to 200°C) to obtain a good separation of the peaks. Gas-tight syringes were used to inject both the liquid and gas samples. Between samples, the injection syringes were washed first with ethanol and then acetone to avoid cross-sample contamination. Injection volumes for each PEA dilution sample were 0.5 µl for liquid samples and 5 ml for gas samples (23°C). Data consisted of the average of three to five repeated GC measurements for a given dilution step (Figure 3).

Measurement of gas–solvent partition coefficients for PEA

The gas–solvent partition coefficient (K_{gs}) is defined as the ratio of the number of moles of odorant in the gas phase to

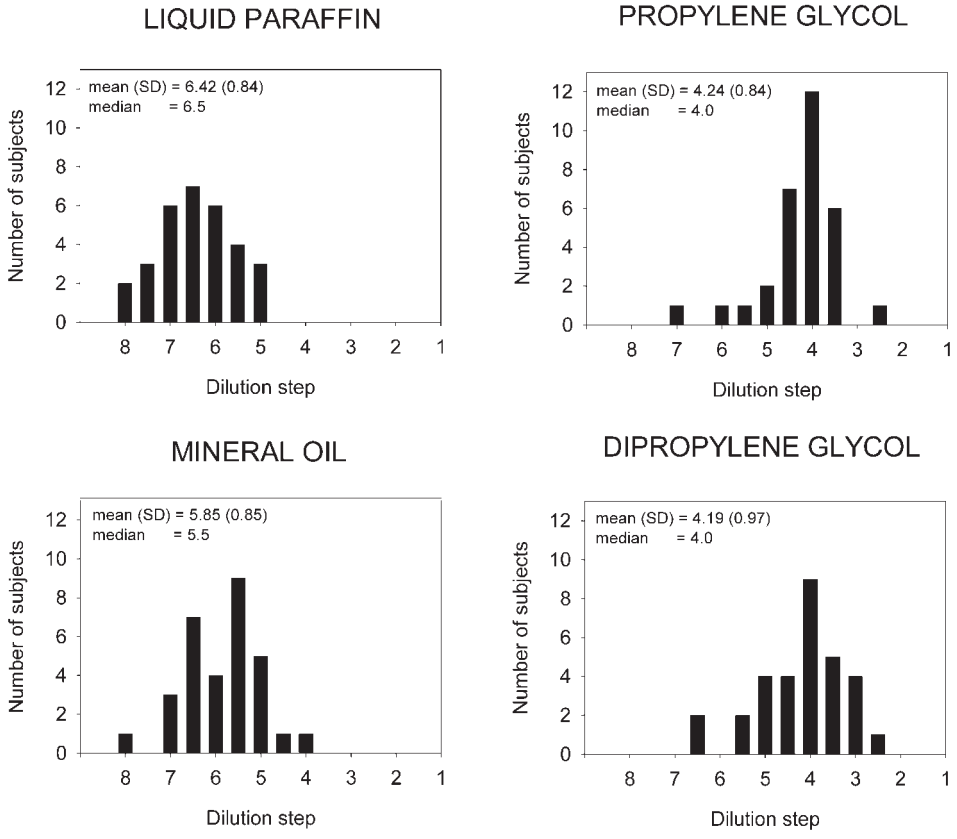

Figure 1 Distribution of detection thresholds for 31 subjects tested with log step dilutions of PEA in four solvents.

Table 3 Solutions used to generate GC calibration curve

GC calibration solutions	Concentration			0.5 μl sample	
	% v/v	g/cm^3	mol/cm^3	g	mol
Pure PEA	100	1.02×10^{0}	8.35×10^{-3}	5.10×10^{-4}	4.18×10^{-6}
PEA in ethanol	1	1.02×10^{-2}	8.35×10^{-5}	5.10×10^{-6}	4.18×10^{-8}
PEA in ethanol	0.01	1.02×10^{-4}	8.35×10^{-7}	5.10×10^{-8}	4.18×10^{-10}
PEA in ethanol	0.631[a]	6.44×10^{-3}	5.27×10^{-5}	3.22×10^{-6}	2.63×10^{-8}

[a]Dilution step 3.

the number of moles in the liquid phase for a given solution. To obtain K_{gs} values, we first measured the gas headspace and liquid concentrations of PEA for dilutions in three different solvents. We obtained GC measurements of PEA in LP, PG and ethanol at dilution step 3. PEA concentrations (mol/cm^3) for both gas and liquid samples were calculated using the GC concentration–response calibration curve for PEA (Figure 2). The PEA K_{gs} values were then obtained by calculating the ratio of the gas to solvent PEA concentrations at dilution step 3 (Table 4).

Results

Detection thresholds for human subjects

Figure 1 gives the PEA detection thresholds for 31 subjects obtained for each of the four solvents. The mean, standard deviation (SD) and median values are given for each histogram. A Friedman ANOVA revealed that the threshold data were not normally distributed and therefore median values were used for the statistical analysis of the four groups. A comparison of the four groups demonstrated that the median threshold values for PEA diluted in LP (step 6.5) and in MO (step 5.5) were significantly different from those in PG (step 4.0) and in DPG (step 4.0) (Tukey's test, $P < 0.05$). The threshold concentration for PEA in LP was >100 times lower (2.5 log steps) than for PEA in PG. There was no significant difference between LP and MO or between PG and DPG. We found no differences between the detection thresholds for males and females. Age-related changes in threshold were not found in this study; however, only two subjects were over the age of 50.

GC measurements

Figure 2 gives the GC calibration curve obtained for PEA using the standard liquid dilutions of PEA listed in Table 3. Sample dilutions of PEA equivalent to dilution step 3 (0.631% v/v) were also measured and plotted on the calibration curve (Figure 2, open symbols). These measurements fell within the 95% confidence interval of the predicted calibration curve. A peak area of 10 on the PEA calibration curve represents 1.75 10^{-11} mol of PEA. Figure 3A shows

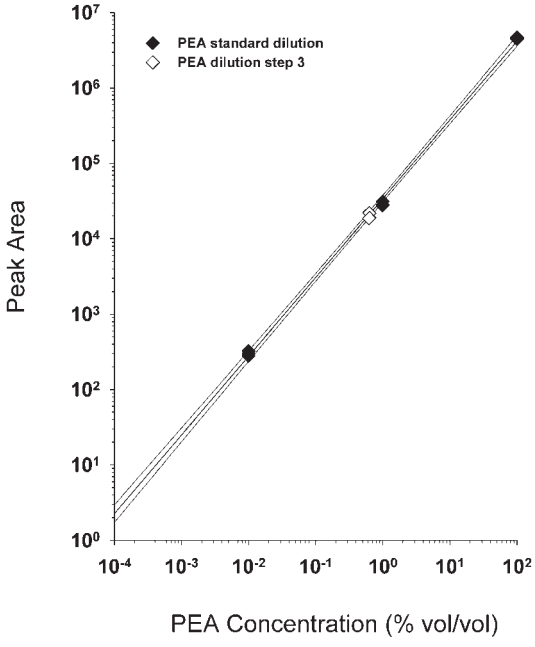

Figure 2 GC calibration curve for PEA. Filled symbols represent the GC measurements of three 0.5 µl samples of 100%, 1% and 0.01% standard solutions of PEA diluted in ethanol. The graph shows the regression curve and 95% confidence intervals. Open symbols represent three independent measurements of a 0.63% dilution of PEA in ethanol (dilution step 3).

the results of the 5 ml headspace gas sample concentration measurements. The linear regression lines and their 95% confidence limits for PEA in LP and PEA in PG at different dilution steps are shown. A comparison of gas samples obtained at the same liquid dilution (step 3) revealed that the gas concentration of PEA in LP is higher than that of PEA in PG. Gas concentrations for PEA in LP were consistently higher than PEA in PG at all dilution steps. However, when the mean detection thresholds for PEA diluted in the two different solvents (step 6.42 for LP and step 4.24 for PG) were plotted in Figure 3A, the values for the gas concentrations (1.96×10^{-12} and 1.75×10^{-12} mol/cm^3, respectively) were found to be the same using the 95% confidence intervals. These findings demonstrate that

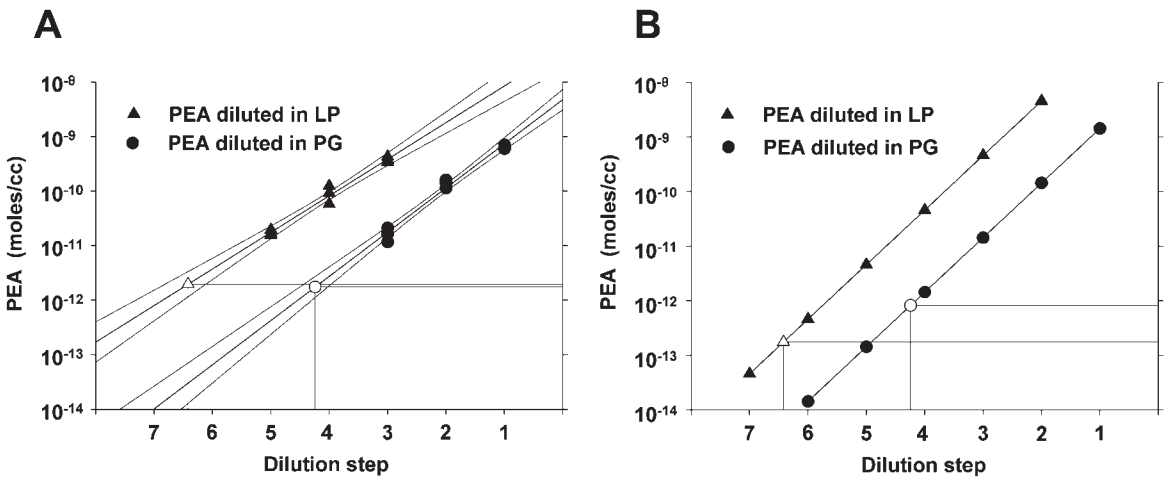

Figure 3 Comparison of headspace gas concentrations for PEA diluted in two solvents, LP and PG. **(A)** Regression lines and 95% confidence intervals for PEA gas concentrations based on GC measurements of headspace gas for dilution steps 1–5 (filled symbols). Although the mean detection threshold obtained for PEA in LP (step 6.42) was lower than for PEA in PG (step 4.24), the concentrations of PEA in the headspace gas (open symbols) at these two threshold values were the same (1.96×10^{-12} and 1.75×10^{-12}mol/cm^3). **(B)** PEA concentrations calculated from the gas–solvent partition coefficients (K_{gs}) obtained for PEA in LP and PG (Table 4). Note that the gas concentration of PEA in LP (1.75×10^{-13} mol/cm^3) at detection threshold (open symbol) was lower that for PEA in PG (8.24×10^{-13} mol/cm^3).

Table 4 Gas–solvent partition coefficients for PEA

Solvent	Dilution step	PEA in gas (mol/cm^3)		PEA in solvent (mol/cm^3)		Partition coefficient (K_{gs})
		Mean	SD	Mean	SD	
LP	3	3.77×10^{-10}	5.38×10^{-11}	4.32×10^{-5}	1.00×10^{-5}	8.73×10^{-6}
PG	3	1.64×10^{-11}	4.37×10^{-12}	6.02×10^{-5}	1.53×10^{-5}	2.73×10^{-7}
Ethanol	3	5.40×10^{-11}	1.94×10^{-11}	5.24×10^{-5}	5.15×10^{-6}	1.03×10^{-6}

while the detection thresholds described by liquid dilution step concentrations may be very different for different solvents, the actual gas concentrations at detection threshold can be the same.

Gas–solvent partition coefficients for PEA

Table 4 gives three gas–solvent partition coefficients (K_{gs}) calculated from the GC measurements of both the gas and liquid concentrations of PEA dilutions. The partition coefficient for PEA in LP (8.73×10^{-6}) is 32 times higher than that for PEA in PG (2.73×10^{-7}). This reflects the relative increase in PEA gas concentrations obtained when PEA is diluted in LP. These gas–solvent partition coefficients can be used to estimate gas concentrations for different liquid dilutions. Figure 3B shows PEA gas concentration values predicted from the gas–solvent partition coefficients. The gas concentration at detection threshold predicted for PEA in PG is similar to that measured by GC. However, for PEA in LP, the predicted gas concentration was much lower than that measured by GC.

Discussion

Measurement of olfactory thresholds

Odor detection thresholds are frequently used to assess olfactory function. They provide an estimate of the lower limit of detection and reflect the minimum number of odor molecules needed to elicit a response from the nervous system. Since the amount of odorant (gas concentration) actually reaching the sensory receptors is rarely known (Rawson, 2000), measurements of odor detection thresholds only approximate the actual detection limits. Many factors contribute to the variability in the detection threshold values published in the literature. Differences in assessment methods, delivery systems (Doty *et al.*, 1986), number of dilutions steps used, odorant type (Cometto-Muniz and Cain, 1995) and solvent all influence the measurement of threshold values. The stimulus concentration at threshold is often reported as the liquid concentration of an odorant diluted in a solvent. Serial dilution steps provide a convenient way to measure olfactory thresholds and are often employed in clinical evaluations (Doty *et al.*, 1984; Cain

et al., 1988). Unfortunately, there is no agreement on the best solvent to use for odorant dilutions. Since the type of solvent used to dilute an odorant can have an effect on the gas concentration above the mixture, it is important to know the relationship between the odorant and the solvent. Our measurements of detection thresholds for PEA using serial dilution steps showed a significant difference in thresholds for PEA diluted in LP and PG. Gas chromatographic measurements revealed that there were differences in the gas headspace concentrations of PEA dilution steps prepared in different solvents, even though the concentrations in the liquid dilutions were identical. Interestingly, GC measurements of the gas concentrations for the different dilution steps corresponding to the detection thresholds for PEA in LP and PG were the same. If gas headspace concentrations were routinely given when publishing odor detection thresholds, differences due to solvents would not be an issue. However, when thresholds are reported as the concentration in a liquid solution, then the solvent used can be an important factor contributing to differences in threshold values. Direct measurement of the headspace gas concentration above a stimulus solution provides a better estimate of the number of odorant molecules delivered in the stimulus than does the concentration of the odorant diluted in the solvent. Unfortunately, for most detection threshold measurements it is not practical to perform a GC analysis of the headspace gas concentrations and stimulus threshold values are reported as the concentration of the odorant in the solution. It would be helpful if there were established methods for preparing serial odorant dilutions using a single standard solvent.

In this study, the gas concentrations of PEA measured at detection threshold using GC methods were 1.96×10^{-12} mol/cm^3 (0.044 p.p.m.) for PEA in LP and 1.75×10^{-12} mol/cm^3 (0.039 p.p.m.) for PEA in PG. These data fall within the range of gas concentrations (0.0065–7.5 p.p.m.) for PEA detection thresholds previously reported (Stahl, 1973; Cometto-Muniz and Cain, 1990; Devos *et al.*, 1990). Variability in published threshold values for a given odorant may reflect differences in the methodology used to measure and define detection thresholds. It is difficult to interpret detection threshold data obtained using different methods without knowing the gas concentration of the stimulus.

Partition coefficients

Measurements of gas concentrations are preferred when determining olfactory thresholds. However, such measurements are time-consuming and often require expensive equipment. The gas concentrations of an odorant stimulus can in some cases be calculated from basic gas laws. Raoult's law ($P_a = P_{vap}x_a$) can be applied to ideal solutions and calculates the partial pressure of a chemical (P_a) from its vapor pressure (P_{vap}) and the mole fraction x_a of the chemical in the mixture. One advantage of Raoult's law is that there are published tables giving the vapor pressures for a wide range of odorant compounds (Lide, 2001). However, Raoult's law can not be applied to many odorant mixtures because they often do not behave as ideal solutions (Amoore, 1978). Henry's law ($P_j = K_j x_j$) provides an accurate description of most gases dissolved in solution. It predicts the partial pressure of a solute (P_j) in the vapor phase, when the mole fraction (x_j) of the solute in solution and Henry's constant (K_j) are known. Henry's law works best for solutions with low concentrations of solute and low vapor pressures. However, it becomes a poor predictor when the concentration of the solution increases. Partition coefficients (K_{gs}) that describe the distribution of a solute between the gas and liquid phases can also be used to predict gas concentrations. Partition coefficients for odorant chemicals can be determined experimentally by calculating the ratio of the concentrations in the gas and solvent phases (C_g/C_s). Partition coefficients for solvents such as octanol and water are available in published tables (Lide, 2001). However, for many organic solvents the gas–solvent partition coefficients are not available and must be determined experimentally.

In this study, the partition coefficients (K_{gs}) for PEA in LP and PG were determined from direct measurements of liquid and gas concentrations (Table 4). When PG is used as a solvent, the concentration response curve measured by GC (Figure 3A) and that calculated from the partition coefficient (Figure 3B) reveal only a slight difference in the estimates of PEA concentration at detection threshold. Therefore, the K_{gs} value for PEA in PG provides a good alternative for estimating gas concentrations for PEA diluted in PG. On the other hand, the threshold value estimated by K_{gs} for LP shows approximately one log unit difference from the gas concentration determined by GC measurement. This suggests that the K_{gs} value obtained for PEA in LP is not a good predictor of the gas concentration. It also suggests that the behavior of PEA dissolved in LP deviates from an ideal solution. The properties of a given solvent and its molecular interaction with the odorant or solute will determine the degree to which ideal gas concentrations can be achieved. Partition coefficients, like gas laws, have limited application and rarely provide a better alternative to direct GC measurement when attempting to determine the gas concentrations of odorant stimuli.

Selection of solvents

Perhaps one of the most overlooked factors in establishing a method to measure odorant detection thresholds is the selection of the solvent used to dilute the odorant. What are the criteria for selecting a solvent for an organic compound such as PEA? An ideal solvent for any odorant is one that is odorless, has a high solubility for organic compounds, is resistant to evaporation, stable for long time periods and safe for human subject testing. In this study, LP and MO were used as solvents. These two materials are not pure chemicals, but are mixtures of organic compounds obtained from the fractional distillation of oil. LP and MO are

essentially the same product, though they are often given different names by their manufacturers. Names for these compounds include paraffin oil, light mineral oil and Nujol, and these terms are often used interchangeably. It is impossible to provide a single mol. wt for these compounds since they are mixtures and therefore include a range of mol. wt constituents. However, the average mol. wt for LP and MO is heavier than for PG and the vapor pressure is much lower. Therefore, the gas concentrations of PEA solutions diluted in LP or MO are always likely to be higher than that for PG, even when the liquid concentrations of PEA in the solutions are the same. The stability, safety for use in the human body and low vapor pressure has made United States Pharmaceutical (USP) grade light MO the solvent of choice for many applications. The problem with using USP MO is that it is a mixture, not a pure chemical. The component chemicals are typically not disclosed and the precise ratio of compounds of different mol. wts is not known. In order to compare the results of detection thresholds obtained for an odorant diluted in MO obtained from two different sources, it is necessary to know how the MO was manufactured. Even for an MO product made by the same company, different lot numbers or production runs may have different properties. In this study we found that PEA did not dissolve in LP and MO at the higher concentrations (step 1, 63% v/v and 2, 6.3% v/v). Thus, the solubility in the solvent may approach ideal behavior at the lower range of concentrations, but become problematic at higher concentrations where intermolecular forces may deviate from an ideal solution. PG is a common solvent used for many organic compounds. It is a pure chemical and thus has several advantages compared to MO or LP. The solubility of organic compounds in PG is typically greater than in MO or LP.

Safety concerns regarding the use of this or any chemical, especially at high concentration levels, must also always be considered. This may be more of a concern for chemicals such as PG than for MO or LP.

There are many factors to be considered when selecting solvents for olfactory testing. As demonstrated in this study, solubility and partition coefficients can play an important role in the measurement of odorant detection threshold values.

Summary

The present study demonstrates how different solvents affect the gas concentrations of an odorant stimulus. The results show how the liquid concentrations of an odorant can differ by as much as several log units at odor detection thresholds, depending on the solvent, while the gas concentrations of the stimulus may actually be the same. Thus, it is important to know the gas concentration of a stimulus when measuring the detection threshold of an odorant. In cases where the direct measurement of gas concentrations is not feasible, gas–solvent partition coefficients, K_{gs}, may be useful.

Acknowledgements

We are grateful to Mr Carl Wolf for providing access to the gas chromatograph used in this study and for his expert technical advice regarding headspace gas measurements. We would like to thank Dr Steven Price for helpful discussions regarding partition coefficients and for his review of the manuscript. We also acknowledge International Flavors and Fragrances for their generous gift of a high purity grade of odorless dipropylene glycol.

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Accepted October 7, 2002