CHAPTER 2

The olfactory system is invariant to odorant volatility

2.1 Abstract

It is sometimes held that volatility is a primary determinant of our ability to smell any given compound. Sometimes, corrections are even made for volatility when comparing olfactory responses to odorants at any given concentration in solution (Brockerhoff and Grant, 1999). These corrections do not significantly reduce the dynamic range of the amplitude of olfactory responses, though. I show here that if the odorant source is a solution whose composition is similar to that of the mucosa covering olfactory receptors, and in equilibrium, the system is invariant to the volatility of the odorant, i.e., two compounds present in a solution in equal activities will reach the olfactory mucosa in equal activities even if one is significantly more volatile than the other. This is due to the fact that the solubilization process is the inverse of the evaporation process if the activity coefficients of the odorants are similar for the source and the mucosa, and their strong dependencies on volatility therefore cancel each other out. Furthermore, even when the compositions are not similar, odorant activity at the mucosa is not an explicit function of the volatility, and depends only on the activity coefficients of the odorant at the source and the olfactory mucosa —the gas phase acts merely as a carrier. I extend the analysis to solid odor sources, for which the same concept holds. Interestingly, the same holds not only for noses whose receptors are immersed in solution, but also for artificial noses for which odorants are detected by sorption into a polymer phase.

2.2 Introduction

The olfactory system faces a seemingly formidable challenge in reconstructing the concentrations of olfactory objects, as well as the composition of simple mixtures, given that different substances have vastly different volatilities[1], and thus the relationship between the concentration found in the gas entering the nose or surrounding an antenna on the one hand, and the concentration at the source on the other, will vary for each substance. In addition, the olfactory system faces another daunting challenge, that of being sensitive to a huge dynamic range: all the way from single or a few molecules (Kaissling and Thorson, 1980) to saturation (inside a flower for a bee, for example).

If two odorants of different volatilities are present in aqueous solution (called *source* hereafter) in equal concentrations, what will their concentrations in the mucosa covering odor receptors be after evaporating from the source and reaching a nose? I show here that the aqueous mucosa solves the problem of invariance to volatility and reduces the dynamic range the rest of the olfactory system must cope with, by factoring out the volatility of the odorant. The reasoning is that the gaseous phase acts merely as a carrier medium between a solid or liquid source and a liquid target, the olfactory mucosa. If the activities coefficients of the odorants in the mucosa and in the source solution are similar enough, the process of evaporation into the carrier gas is the inverse of the process of solubilization from the carrier gas into the mucosa, and the effects of the two tend to cancel each other out. This means that two odorants will dissolve in an aqueous medium such as the olfactory mucosa in concentrations equal to their concentrations at the source[2], even if the two have different volatilities, provided that they both are dilute enough and have similar enough activity coefficients in the source and in the mucosa. Thus, for physiologists interested in the concentration reaching olfactory receptors, the most relevant concentration, contrary to previous belief, is that at the source

1. Throughout, the terms volatility and vapor pressure are used interchangeably, and specify vapor pressure in the pure state.

2. Excluding active effects such as that of any odorant binding proteins with affinity for the odorants in question, which would change an odorant's activity coefficient in the mucosa.

solution, and not that in the air carrier.

2.3 The olfactory epithelium is covered by an aqueous mucosa

Before odorant molecules reach olfactory receptors, they must first cross an aqueous interface. In vertebrates, the olfactory epithelium is covered by the nasal mucosa, which is 5–30 micrometers deep depending on the species and living environment (Menco, 1980). In insects, olfactory receptor neurons (ORNs) are covered by the sensillar lymph, which lies below the cuticular walls of the sensilla (Pelosi and Maida, 1995). The functions of this mucosa have remained a puzzle. In vertebrates, it has been proposed that they serve a protective function for the olfactory receptor neurons (Pelosi, 2001). In insects, though, where the cuticular walls of the sensilla provide more of a protective layer than the mucosa itself, its presence is puzzling.

2.4 In equilibrium, the concentration of an odorant in the mucosa is given by the fraction of its partial pressure over the vapor pressure of the pure odorant

The concentration of an odorant in an aqueous phase in equilibrium with the vapor phase[1] is governed by the partition coefficient between the vapor and the solution, which dictates that the concentration in the aqueous phase is proportional to the ratio of the partial pressure of the vapor over the vapor pressure of the odorant:

$$a_{mucosa} = g_{mucosa}\, x_{mucosa} = p / p^* , \qquad \text{(Equation 1)}$$

where *a* is the odorant activity, x is the equilibrium mole fraction of the odorant, g is the odorant activity coefficient in the mucosa, p is the partial pressure of the odorant in the airspace above the

1. Equilibrium between phases is assumed throughout the discussion.

epithelium, and p* is the vapor pressure of the pure odorant (Doleman et al., 1998). If the activity coefficients, which account for the specific solvation interactions between the sorbent phase and the odorant molecules, are similar for odorants within a homologous series, then the concentration of any member of the homologous odorant series in the mucosa will be primarily determined by the fraction of the pressure at the nose over the vapor pressure of the pure odorant, as opposed to being determined primarily by the absolute concentration of the odorant in the vapor phase (Moulton and Eayrs, 1960; Amoore and Butter, 1978; Doleman et al., 1998). Indeed, the olfactory detection thresholds for homologous series of alkanes and alcohols are to a first approximation a constant fraction of the vapor pressure of the odorant both in humans (Mullins, 1955; Cometto-Muniz and Cain, 1990; Doleman et al., 1998) and rats (Moulton and Eayrs, 1960). This relationship has also been reported for electrophysiological thresholds in the olfactory mucosa of the frog (Ottoson, 1958) and the trigeminal nerve of the rat (Silver et al., 1986).

What determines, then, the fraction of the vapor pressure of an odorant in the gas phase? The process of evaporation of an odorant from a solution to air is the inverse of the sorption of that odorant into the solution:

$$p_{source} = g_{source} \cdot x_{source} \cdot p^* \ , \qquad \text{(Equation 2)}$$

which is just a rearrangement of equation 1, with the subindices changed from mucosa to source.

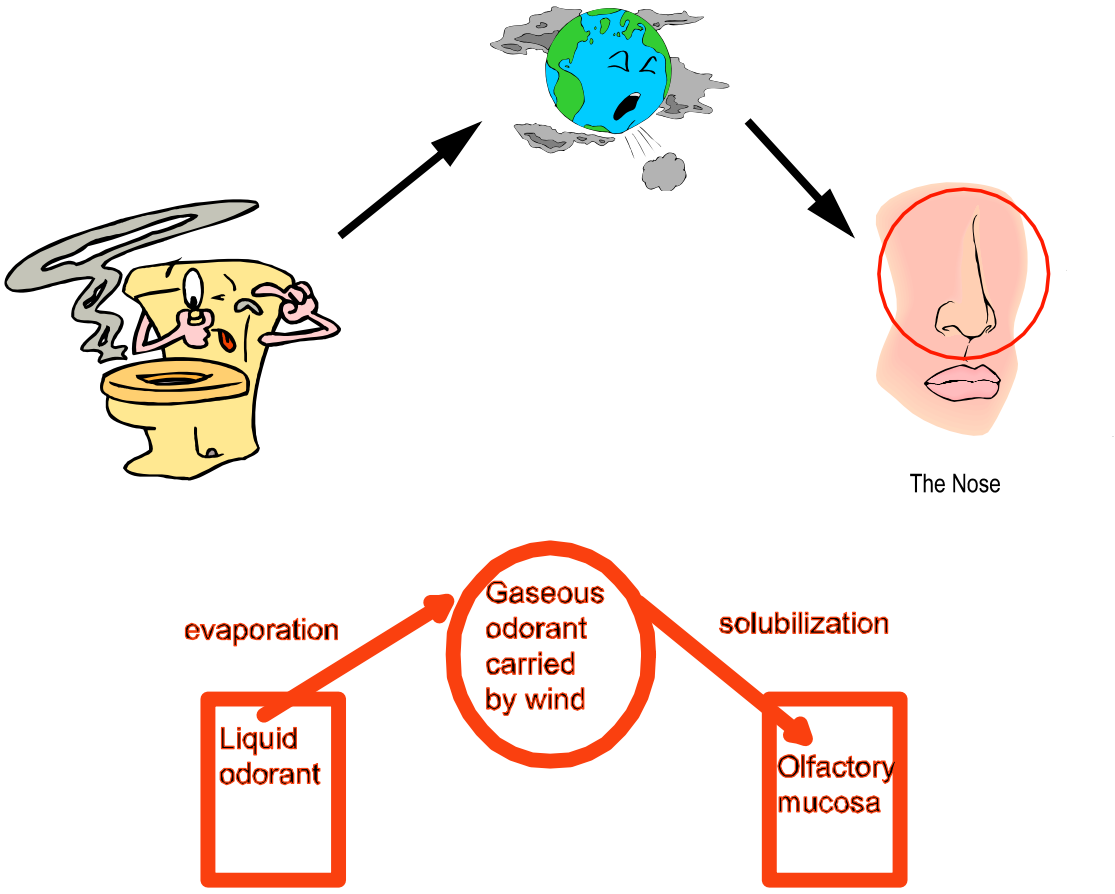
2.5 Transport of odorant molecules from source to nose

In most olfactory environments, the simple diffusion of odorant molecules is a negligible means of dispersing odorants (Murlis and Jones, 1982; Murlis et al., 1992). Odors of distant objects are brought to the nose by wind, following the path of the air packet into which they evaporate (Murlis and Jones, 1982). This means that, while the frequency of the packets decreases with distance from the source (Murlis et al., 2000), the absolute concentration in each packet varies less (Murlis et al.,

2000), and even after dilution into surrounding air, the relative concentrations of different components will remain constant. The partial pressure of an odorant above the mucosa will then be

$$p_{mucosa} = d \cdot p_{source} \quad , \qquad \text{(Equation 3)}$$

where d stands for a dilution factor $(0<d<=1)$[1].



Solubilization is the inverse process of evaporation, making the net process of odor transport independent of volatility.

1. This dilution factor accounts for the failure of the vapor to reach saturation before being swept by an air current, dilution by mixture with air during travel from odor source to the nose, and failure of the odor to reach saturation in the mucosa if the odor is present only transiently. Because this dilution is to a first approximation homogeneous for all odorants (Murlis et al., 1992), it does not affect their relative concentrations.

2.6 Invariance to volatility

The above considerations have an interesting consequence. It is sometimes said that more volatile odorants will reach odor receptors in higher concentrations and thus are more effective olfactory stimulants (e.g., Brockerhoff and Grant, 1999, but see Mullins, 1955). In reality, however, the volatility (p^*) appears in the *numerator* of equation 2 determining the concentration of an odorant in the vapor phase, but it appears in the *denominator* in equation 1 determining the concentration of an odorant in the mucosa. Thus, in the relationship between the concentration in the liquid source and that in the mucosa, the volatility appears in both the numerator and the denominator, and therefore cancels out. Combining equations 1 and 3, then

$$a_{mucosa} = g_{mucosa} \cdot x_{mucosa} = p_{mucosa} / p^* = d \cdot p_{source} / p^* \qquad \text{(Equation 4)}$$

Combining equations 2 and 4:

$$x_{mucosa} = d \cdot (g_{source} / g_{mucosa}) \cdot x_{source} \qquad \text{(Equation 5)}$$

The presence of an aqueous mucosa over the epithelium has, then, the important consequence that the concentration encountered by the olfactory system is independent of the volatility of the different components[1,2].

A second consequence of the presence of the mucosa and its invariance to volatility is a dramatic compression of the dynamic range of concentrations faced by the olfactory system, compared to what it would be if detection happened directly in the gaseous phase. For a system already facing a dynamic range of many dB's, such a compression could play a fundamental role in the ability of animals to respond to concentrations ranging from a few molecules of a distant source to the saturated

1. Of course, a substance with *zero* volatility will never reach the mucosa, so this discussion holds for non-zero volatilities.

2. It is possible in principle that an implicit dependence on volatility is contained in the dependence on the activity coefficient ratios for non-ideal solutions.

vapor pressure faced by, say, an insect inside a flower.

For dilute odorants (and the olfactory system is sensitive to very low concentrations (Kaissling and Thorson, 1980; Devos et al., 1990)), the relationship in equation 5 is even simpler.

2.7 Raoult's law

When the components in a solution are chemically similar, the mixture is called an ideal solution and the vapor pressure of each component can be approximated by Raoult's law:

$$p_A = x_A \cdot p^*_A \qquad \text{(Equation 6)}$$

or rearranging:

$$p_A / p^*_A = x_A \quad , \qquad \text{(Equation 7)}$$

where p_A stands for the vapor pressure of component A, p^*_A is the vapor pressure of A as a pure liquid, and x_A stands for the mole fraction of A in the liquid (see Atkins, 1989).

When the components are dissimilar, strong deviations from Raoult's law can occur. Even then, though, the law becomes increasingly accurate as x_A approaches unity. In other words, the law is a good approximation for the solvent so long as the solution is dilute.

2.8 Henry's law

In real non-ideal solutions, the solute does not follow Raoult's law. The English chemist William Henry realized that the vapor pressure of the solute in a dilute solution is proportional to the mole fraction, even if the proportionality constant is not the vapor pressure of the pure liquid (Atkins, 1989). This is a simple consequence of the fact that any curve can be approximated with arbitrary

accuracy by a straight line in the limit of its length going to zero (see, for example, Feynman, 1965); Henry's empirical discovery was that the range of validity of this approximation was sufficiently broad for what came to be called *ideal dilute solutions* so that it was useful.

2.9 The concentration in the mucosa for ideal solutions

For ideal solutions, the odor-dependent activity coefficients are unity, and thus, activities are equal to concentrations in the solution. Equation 5 simplifies and becomes independent of the odor and linear in the concentration at the source. The relative concentrations of components of a mixture in the epithelium then equal the relative concentrations in the original liquid odorant:

$$x_{mucosa} = d \cdot x_{source} \qquad \text{(Equation 8)}^{1}$$

or, for ideal dilute solutions, per Henry's law,

$$x_{mucosa} = d \cdot k \cdot x_{source} = k' \cdot x_{source} \qquad \text{(Equation 9)}$$

with k and k' constants for any given set of mixture components.

2.10 Solid-state odor sources

The independence of the concentration of odorants in the mucosa with respect to volatility holds for solid odor sources as well as liquid ones. The process of solubilization in the mucosa is of course

1. Slight differences between the relative concentrations of two odorants at the source and those at the epithelium may remain due to failure to reach equilibrium and differential kinetics of absorption of components – especially at high flow rates through the nose—, differential diffusion of components, temperature differences between source and mucosa and differential contamination due to different presence of components in the background. Diffusion is a negligible component of odorant kinetics, though (Murlis et al., 1992).

the same whether the source was a solid or a liquid. What is then the vapor pressure created by a solid in equilibrium with its surroundings? At the temperature of fusion (T_{fusion}), solid and liquid phases are in equilibrium with each other, which means their chemical potentials are equal to each other. Since the pressure of the vapor in equilibrium with a phase is dependent on the chemical potential of the phase, the pressure of the vapor created by sublimation of a solid (p^{subl}) is equal to that created by vaporization of a liquid (p^{vap}) at T_{fusion}:

$$p^{subl}_{Tfusion} = p^{vap}_{Tfusion} \qquad \text{(Equation 10)}$$

But, p^{subl} and p^{vap} are both (different) functions of the temperature (Atkins, 1989):

$$p^{subl} = p^{subl}_{T_{fusion}} e - C_{subl} \quad , \qquad \text{(Equation 11)}$$

where p^{subl}_0 is p^{subl} at $T=T_0$ and

$$C_{subl} = \frac{\Delta H_{subl}}{R}\left(\frac{1}{T} - \frac{1}{T_{fusion}}\right) \quad , \qquad \text{(Equation 12)}^1$$

where ΔH_{subl} is the enthalpy of sublimation and R is the gas constant. Likewise,

$$p^{vap} = p^{vap}_{Tfusion} \cdot e^{-c}_{vap} \quad , \qquad \text{(Equation 13)}$$

where p^{vap}_0 is p^{vap} at $T=T_0$ and

1. Assuming ΔH_{vap} and ΔH_{subl} to be independent of temperature in the range T_{fusion} - $T_{ambient}$.

$$C_{vap} = \frac{\Delta H_{vap}}{R}\left(\frac{1}{T} - \frac{1}{T_{fusion}}\right) \quad , \qquad \text{(Equation 14)}$$

where ΔH_{vap} is the enthalpy of vaporization. Combining equations 10-14 to obtain p^{subl} as a function of p^{vap}, we arrive at

$$p^{subl} = p^{vap} \cdot e^{\frac{-(\Delta H_{subl} - \Delta H_{vap})}{R}(\frac{1}{T} - \frac{1}{T_{fusion}})} \qquad \text{(Equation 15)}$$

Thus, the pressure of a vapor in equilibrium with a solid odorant is proportional to its vapor pressure in liquid form, with the proportionality constant dependent on the temperature, the fusion temperature and the difference between the enthalpy of sublimation and that of vaporization. So, just like in equation 2 for liquid odorants, the vapor pressure is in the numerator of this equation, and thus the concentration of odor in the mucosa resulting from a solid source is independent of its volatility:

$$x_{mucosa} = \frac{d}{\gamma_{mu}\cos a} e^{\frac{-(\Delta H_{subl} - \Delta H_{vap})}{R}(\frac{1}{T} - \frac{1}{T_{fusion}})} \qquad \text{(Equation 16)}$$

2.11 Discussion

In summary, the problem of reconstructing an odorant's composition at the source in the face of varying volatility of components turns out to be solved by the very physics of the process by which an odorant reaches the olfactory epithelium of land animals. The olfactory mucosa, exploiting the fact that most behaviorally relevant odor sources are solid or liquid rather than gaseous[1], achieves

invariance to the volatility of the odorant by virtue of inverting the process of evaporation of an odor from its source to the atmosphere. This allows the mucosa to act as a gain control mechanism to maintain the concentration of different odorants in the mucosa at more similar levels than would be the case were odor receptors detecting odorant concentration in the vapor phase. In doing so, the mucosa dramatically compresses the dynamic range faced by olfactory receptors, facilitating their task of recognition across many orders of magnitude of concentration.

A similar trend holds not only for olfactory thresholds but also for sorption into a carbon black-polymer sensor: the mean response intensity of electronic nose detectors is essentially constant for members of an homologous series if their activities are held constant (see Doleman et al., 1998). This suggests the mucosa does not need to be aqueous to create invariance to volatility: solubilization or adsorption into other media will have a similar effect. The critical step to achieve invariance to volatility is to make the odor receptors responsive not to the absolute concentration in the vapor, as mass spectroscopy or flame ionization detection is, but rather to the thermodynamic activity of the odorant. In addition, the more similar the composition of the mucosa to that of the odor source, the more similar the activity coefficients for different odorants will be for both phases, and the less the relative concentrations in the mucosa will differ from those at the source.

2.12 Thermodynamic equlibria versus particle counters

More generally, gas quantification processes can be separated into two classes: those counting par-

1. Even predicting the toxicity of a gas is better served by identifying the concentration of the toxin in aqueous solution, or better, its activity, than by measuring the gas' pressure in the vapor phase (see Ferguson, 1939). This does not apply to all gas measurement applications, though. To measure the amounts of different gases in the atmosphere that could absorb sunlight, for example, the relevant concentration is the absolute concentration in the vapor phase. Neither does it apply to all gas detectors: mass spectrometers, for example, measure absolute concentration in the gas phase.

ticles, such as nuclear, mass, photonic or electric processes of detection, including geiger counters, mass spectroscopy and flame ionization detection, and those relying on a thermodynamic equilibrium, such as biological noses and carbon-black polymer sensors. The former detect absolute concentration in the gas phase, whereas the latter detect activities. In evaluating a molecule's biological effect, such as in measuring toxicities, it is the activity that is more relevant. Furthermore, the invariance to volatility that characterizes the latter detector class, but not the former, makes the responses of thermodynamic detectors more correlated with a mixture's concentration in the original condensed phase than that of particle counters.

2.13 Predictions and empirical support

The analysis presented here suggests the prediction that, if olfactory detection thresholds for different odorants are predominantly driven by the concentration of odorant that reaches the mucosa (Doleman et al., 1998), then thresholds based on concentration of the odorant in solution should be significantly more similar for different odorants than thresholds based on concentration in air. A survey of the literature shows that this is indeed the case. While the human thresholds in g/l in air observed for n-butanol, pyridine and isovaleric acid vary over four orders of magnitude (four studies compiled by Laffort, 1963, cited in Amoore and Buttery, 1978), the thresholds in g/l in water are comparatively constant, varying by a factor of two or less (Amoore and Buttery, 1978) (Table 1).

A second prediction is that, despite decades of attempts to correlate olfactory thresholds with volatilies (Ottoson, 1958; Moulton and Eayrs, 1960; Brockerhoff and Grant, 1999), olfactory thresholds, measured in concentration at the source, should be independent of volatility. In other words, two ideal compounds of different volatilities but equal in all other regards will accumulate in the mucosa in concentrations proportional to those at the source and independent of their volatilities. This prediction is harder to test, because it is difficult to vary volatility without varying other physicochemical

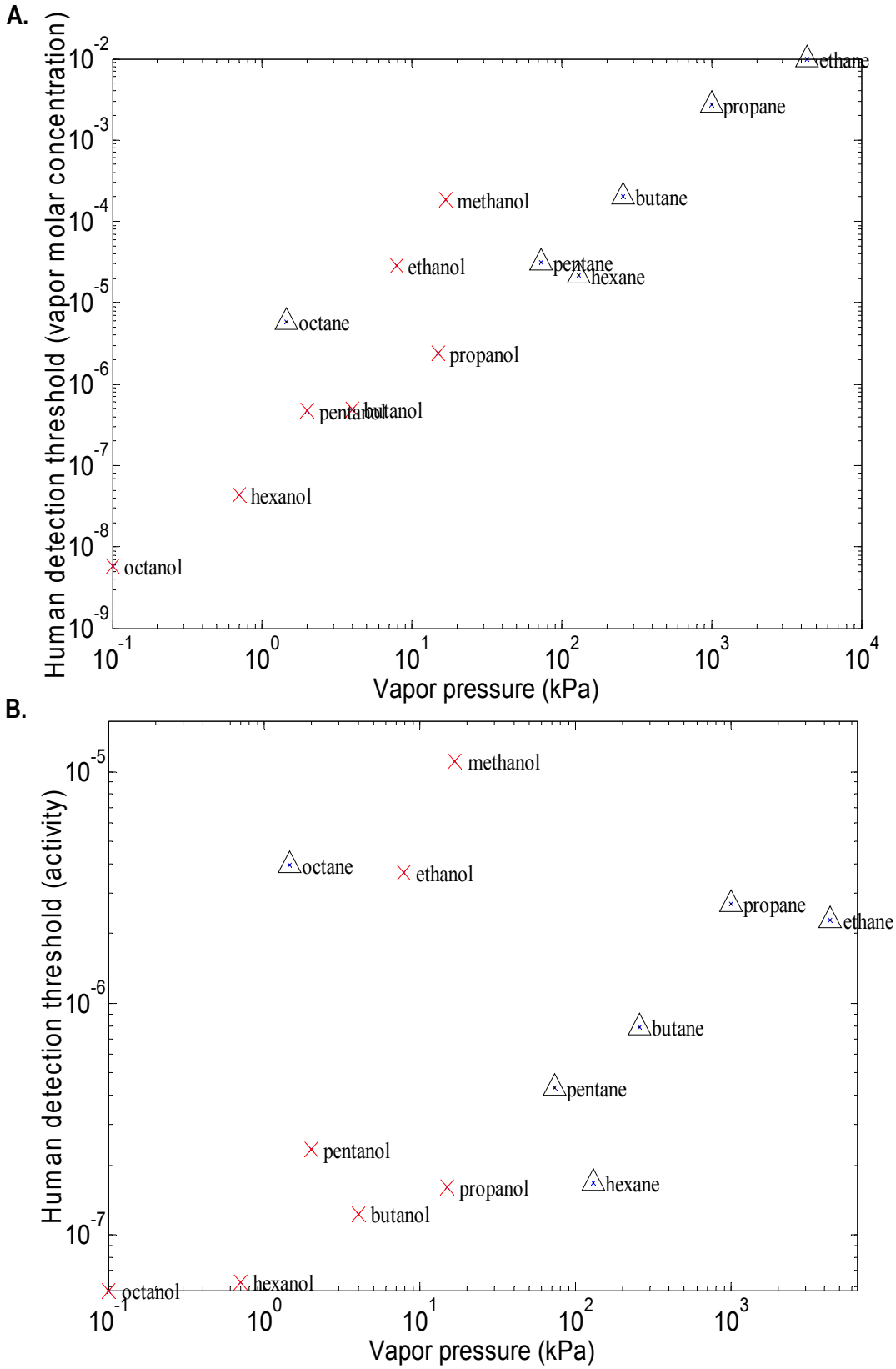


Figure 2.2. Human detection thresholds for 1-alcohols (red crosses) and n-alkanes (black triangles) are more tightly correlated with vapor pressures when measured in concentrations in the vapor (A)

than in thermodynamic activities (B). Activities were calculated as the ratio of the partial pressure in the vapor at threshold over the vapor pressure of the pure substances. Olfactory thresholds from Devos et al., 1990. Vapor pressures from the CRC Handbook (1999) and http://chemfinder.cambridgesoft.com/.

properties of the odorant that are bound to affect olfactory thresholds. One way to do that might be to change the composition of the carrier stream adding an insoluble gas to air in concentrations large enough to change the affinity of odorants for the vapor phase. This would have the effect of changing an odorant's vapor pressure, without a concomitant change in the physicochemical properties of odorants or their affinity for the sorbed phase. The prediction would be that odor detection thresholds (measured in activity, which can be approximated for ideal solutions by concentration of the source solution) would be unaffected (provided that the activity coefficients of the odorant are similar in the source and the mucosa).

TABLE 1. Comparison between aqueous and gaseous olfactory thresholds (adapted from Amoore and Buttery, 1978).

Compound	Water threshold (g/l)*	Air threshold (g/l)$^{+}$
n-Butanol	6.5×10^{-3}	4.3×10^{-5}
		1.0×10^{-6}
		3.2×10^{-6}
		3.7×10^{-5}
Pyridine	4.2×10^{-3}	4.1×10^{-5}
		3.2×10^{-5}
		3.7×10^{-6}
		3.9×10^{-8}
		7.4×10^{-7}
		1.6×10^{-7}
Isovaleric acid	1.2×10^{-4}	7.6×10^{-9}
	*: Amoore and Buttery, 1978.	+: From data compiled by Laffort (1963), where the originals are cited.

It is easier to show that olfactory thresholds are indeed less correlated with volatility when measured as concentration in solution than when measured as concentration in the vapor phase. Indeed, the correlation between a compound's volatility and its olfactory threshold measured as concentration in solution varies widely for different homologous series (for acids, Spearman rank correlation r=0.1, p>0.8, Fig. 2.3a; for aldehydes, r=0.7, p=0.19, Fig. 2.3b). Unfortunately, for decades, olfactory researchers have been biased toward measuring thresholds as concentrations in the vapor phase, partly because that obviates the need for specifying a solvent, which might be different for different odorants. Thus, thresholds measured in solution are scarce in the literature compared to the wealth of thresholds measured in the vapor (see Devos et al., 1990, for a compilation). Fortunately, we can use the ratio of thresholds in the vapor phase over the vapor pressure of the compound to calculate activities, since the activities in the vapor phase and in solution are the same in equilibrium. In man, thresholds measured as activities for 1-alcohols and n-alkanes have a much weaker dependency on volatility than that reported for thresholds measured as concentration in the vapor (Spearman rank correlation r=0.998, $p<10^{-4}$ for concentrations in vapor; r=0.035, p>0.1 for activities; see Fig. 2.2; compare Mullins, 1950 with Doleman et al., 1998, respectively)[1]. Detection thresholds, measured in activities, for the same homologous series (n-aliphatic alcohols) also show stronger dependencies on the species in which they are measured than on the volatility of the substance (Fig. 2.4). Finally, "correcting" for differences in volatility among olfactory stimuli (Brockerhoff and Grant, 1999) does not significantly reduce the variance in EAG responses induced by different odorants in a cone-

1. Performing the correlations separately for each series did not yield a better correlation for activities and vapor pressures of n-alkanes (r=0.03, p>0.9, down from 0.94, p<0.005 for concentrations in the vapor), but yielded a barely significant correlation for 1-alcohols (r=0.8, p<0.05, down from r=0.96, $p<4x10^{-4}$ for the vapor concentrations). Interestingly, this correlation was positive, not negative, meaning that, if anything, more volatile substances are less effective odorants.

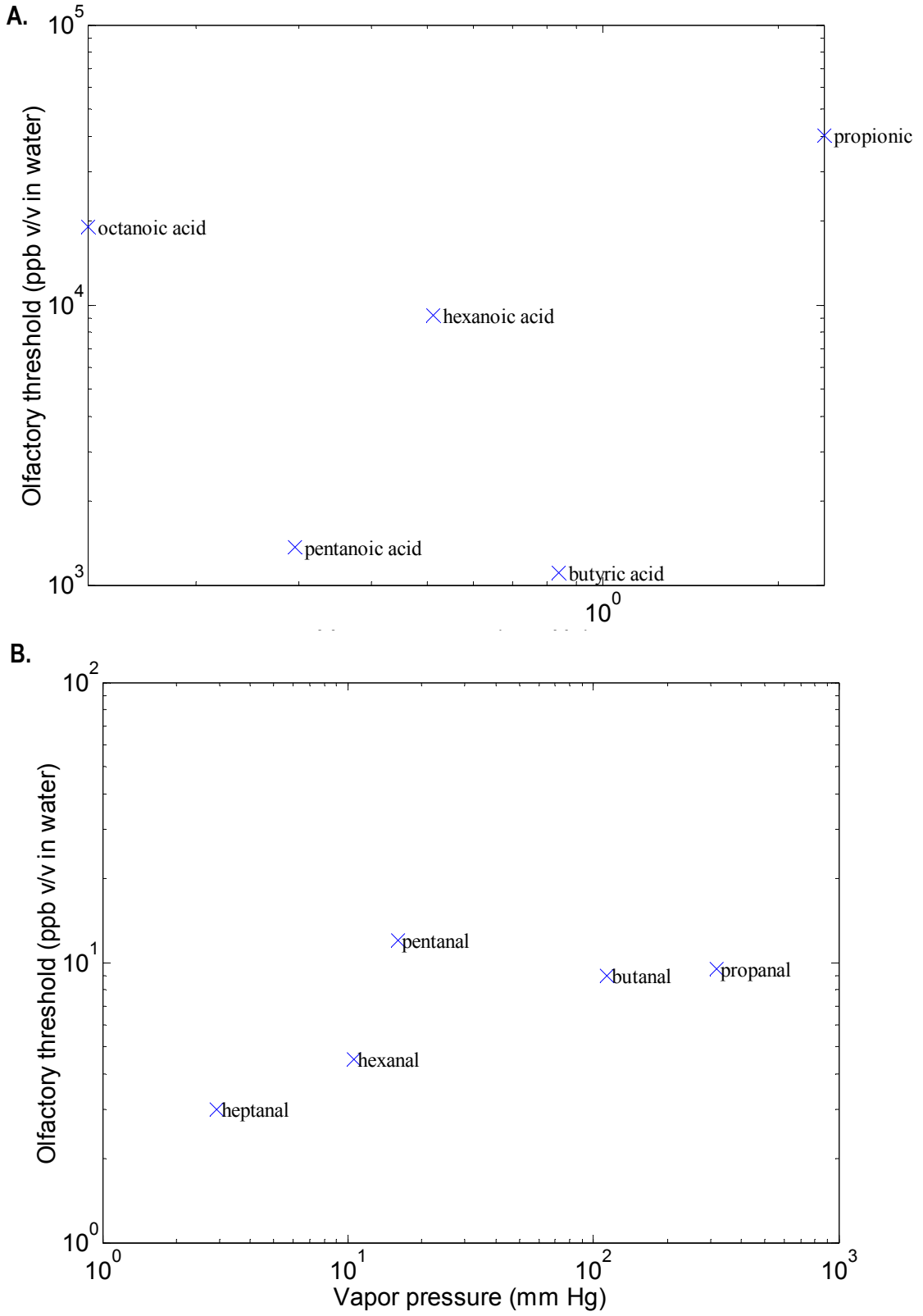


Figure 2.3. Human olfactory thresholds measured as concentrations in solution, as a function of volatility for a homologous series of (A) acids and (B) aldehydes.

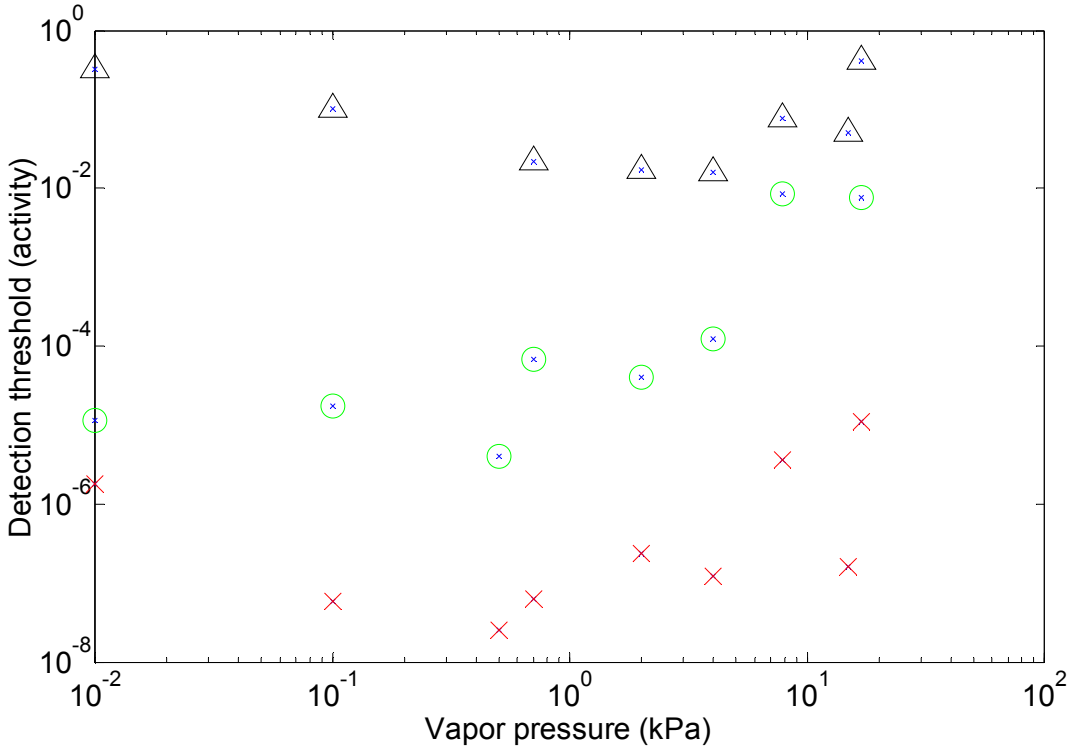


Figure 2.4. Relation between detection threshold, expressed as thermodynamic activities, for l-alcohols in humans (red crosses; Devos et al., 1990), rat (green circles; Moulton and Eayrs, 1960) and blowfly (black triangles; Dethier and Yost, 1951).

worm. Together, these observations suggest that olfactory detection thresholds are not primarily driven by volatility, if they depend on it at all.

Of course, detection thresholds depend on the affinity of the highest-affinity receptor for the compound in question, as well as, in some cases, on specific odor-binding proteins (Kim, Repp and Smith, 1998). The responses of the top-responding receptors, however, appear to be comparable for many molecules in homologous series, even when the identity of the top-responding receptors changes for different elements of each series (Fujimura et al., 1991). Furthermore, it is likely that olfaction operates under nonequilibrium conditions for at least some odorants. Olfactory stimulation is transient and intermittent, due both to the turbulent nature of odor plumes (Murlis et al., 1992) and to periodic sampling given by sniffing (Freeman, 1978; Gray and Skinner, 1988) in vertebrates or by

antennal movements in arthropods (Mellon, 1997). A complete characterization of the determinants of olfactory thresholds must await an *in vivo* characterization of each of the hundreds of receptor types, in its natural habitat of the mucosa and under conditions of intermittent stimulation.

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