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A Cut-off in Ocular Chemesthesis from Vapors of Homologous Alkylbenzenes and 2-Ketones as Revealed by Concentration-Detection Functions

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Abstract

Studies of homologous series of environmental vapors have shown that their chemesthetic (i.e., sensory irritation) potency increases with carbon chain length (that is, their detection thresholds decrease) until they reach a homolog that fails to be detected, even at vapor saturation. All ensuing homologs cannot be detected either. In this investigation, we measured concentration-detection (i.e., psychometric) functions for ocular chemesthesis from homologous alkylbenzenes (pentyl, hexyl, and heptyl benzene) and 2-ketones (undecanone, dodecanone, and tridecanone). Using a three-alternative forced-choice procedure against air blanks, we tested a total of 18 to 24 subjects, about half of them females, average age 31 years, ranging from 18 to 56 years. Stimuli were generated and presented by a computer-controlled, vapor delivery device whose output was quantified by gas chromatography. Exposure time was 6 sec and delivery flow 2.5 L/min. Within the context of present and previous findings, the outcome indicated that the functions for heptylbenzene and 2-tridecanone reached a plateau where further increases in concentration did not enhance detection. We conclude that: a) a cut-off point in ocular chemesthetic detection is reached along homologous alkylbenzenes and 2-ketones at the level of heptylbenzene and 2-tridecanone, respectively, and b) the observed effect rests on the homologs exceeding a critical molecular size (or dimension) rather than on them failing to achieve a high enough vapor concentration.

Keywords: Eye irritation – Ocular chemesthesis – n-Alkylbenzenes – 2-Ketones – Concentration-detection functions – Molecular cut-off – Structure-activity relationships

Introduction

Chemosensory irritation has long been recognized as a very common, and often early, symptom of potentially dangerous exposure to airborne chemicals (Alarie, 1973a; Alarie, 1973b; Nielsen, 1991; Nielsen and Hansen, 1993; Nielsen *et al.*, 2007). The sensory detection of a wide variety of compounds by means other than olfaction and taste (i.e., gustation) was originally included under the concept of a common chemical sense (Keele, 1962). More recently, it has been labeled chemical nociception (Lee *et al.*, 2005) or chemically-induced somesthesis, from where the convenient term "chemesthesis" arises (Green *et al.*, 1990; Green and Lawless, 1991; Bryant and Silver, 2000). Humans exposed to environmental vapors experience chemesthetic sensations in exposed mucosae, usually the eyes, nose, and throat (Doty *et al.*, 2004). These sensations are often pungent, i.e., sharp, and include: stinging, piquancy, tingling, irritation, burning, cooling or freshness, prickling, and the like. In the specific case of the ocular and nasal mucosae, chemesthesis is mediated by the trigeminal nerve system (Finger *et al.*, 1999; Doty and Cometto-Muñiz, 2003).

In mice, chemesthesis produced by volatile organic compounds (VOCs) has been measured by recording the airborne concentration depressing the respiratory rate by 50%, labeled RD_{50}. These values can be used to extrapolate the data to humans (Schaper, 1993; Kuwabara *et al.*, 2007; Nielsen *et al.*, 2007). In humans, thresholds for nasal chemesthesis from VOCs have been measured by testing subjects lacking olfaction (i.e., anosmics) (Cometto-Muñiz and Cain, 1990; Cometto-Muñiz and Cain, 1991; Cometto-Muñiz and Cain, 1993; Cometto-Muñiz and Cain, 1994; Cometto-Muñiz *et al.*, 1998a; Cometto-Muñiz *et al.*, 1998b). Alternatively, human nasal trigeminal thresholds have also been measured by employing a nasal lateralization (i.e.,

localization) technique that requires subjects to identify the nostril receiving a VOC when the contralateral nostril receives just air (Wysocki *et al.*, 1997; Cometto-Muñiz and Cain, 1998; Dalton *et al.*, 2000). The two approaches aim to eliminate the interference from the sense of smell in assessing human nasal chemesthetic sensitivity to VOCs, see reviews in (Cometto-Muñiz, 2001; Doty *et al.*, 2004). Human thresholds for ocular chemesthesis (eye irritation) from vapors also provide information on trigeminal chemesthetic sensitivity, unbiased by smell, e.g., (Cometto-Muñiz and Cain, 1991; Cometto-Muñiz *et al.*, 1998b). To a first approximation, nasal and ocular trigeminal sensitivity to VOCs fall close to each other (Cometto-Muñiz and Cain, 1995).

Systematic studies measuring human chemesthetic thresholds for members of a variety of homologous chemical series found that sensitivity increases (i.e., thresholds decrease) with carbon chain length (Cometto-Muñiz, 2001). This trend is interrupted (i.e., cut-off) upon reaching a homolog that fails to elicit chemesthesis, even at vapor saturation. All ensuing larger homologs also fail. We have labeled this point in the series the cut-off point, and the shortest homolog that fails to be detected via chemesthesis, the cut-off homolog. In the present study we sought to identify the cut-off homolog for eye irritation among alkylbenzenes and 2-ketones by testing a range of concentrations up to vapor saturation and plotting concentration-detection (i.e., psychometric) functions. Previous results guided the present selection of three contiguous members from each series that were likely to bracket the cut-off point (Cometto-Muñiz and Cain, 1993; Cometto-Muñiz and Cain, 1994; Cometto-Muñiz *et al.*, 2006).

Experiment 1: Alkylbenzenes

An institutional review board at the University of California, San Diego, approved the protocol for all experiments described here. All participants provided written informed consent.

Materials and Methods

Subjects. We recruited 18 subjects (9 female). Their average age (±SD) was 34 (±13) years, ranging from 18 to 56 years. All participants scored in the normosmic range of a standardized clinical olfactory test (Cain, 1989) (no standardized test is available for either nasal or ocular chemesthetic sensitivity). Three males were smokers. Three females used contact lenses but refrained from wearing them on testing days. Data from the few smokers and contacts users was within the range of that from nonsmokers and non-users of contacts.

A subset of 14 participants (6 female) from the group described above had available time to complete at least 20 trials per concentration (half with each eye) for all three alkylbenzenes (see Procedure). Data presented below for individual subjects refers to this more comprehensively tested group.

Stimuli. The chemicals tested (purity in parenthesis) were: pentylbenzene (99%), hexylbenzene (98%), and heptylbenzene (98%). Medical Air U.S.P. (humidified to 50% relative humidity, RH) served as blanks (see Procedure). Table 1 presents the actual vapor concentrations in nitrogen (N_2) tested for each chemical as determined by gas chromatography (flame ionization detector, FID), and the nominal concentrations as determined by the ratio “stimulus flow / total (blank air + stimulus) flow” (see Apparatus).

Insert Table 1 about here

Apparatus. Stimuli and blanks were delivered to the subject's eye from a dynamic dilution, vapor delivery device (VDD). The instrument is described in detail in a recent publication (Cometto-Muñiz *et al.*, 2007a) and is based on a vacuum system analogous to that devised by Kobal (Kobal, 1985). Total flowrate to the eye, presented via a glass eyepiece (Cometto-Muñiz *et al.*, 2005b), always equaled 2.5 L/min, and time of exposure was set to 6 sec.

Procedure. Each subject participated in three sessions, one per chemical. A session lasted between 2 and 3 hours and included two 5-10 min breaks. Order of presentation of chemicals across sessions and subjects was randomized. Within a session, a trial consisted of a three-alternative forced-choice procedure (3AFC) between a stimulus and two blanks. Order of stimulus and blanks was also randomized. Participants wore noseclips to avoid odor cues and had to decide which of the three presentations felt different (typically stronger) to the tested eye. In addition, they also rated the confidence in their decision on a scale from 1 (not confident at all, guessing) to 5 (extremely confident). The experimenter started the session by presenting a trial including the lowest concentration of the selected chemical to one eye (randomly selected) and then to the other. The procedure continued in ascending concentration (always alternating the tested eye) until the highest concentration (i.e., 100%v/v) was reached. A rest period of at least 3 minutes ensued before the whole process was repeated. No procedures were used to rinse the eyes either between trials or during the rest period. Testing continued until 20 trials per concentration (half with each eye) were completed.

Data analysis. The outcome was summarized as plots of detection probability, i.e., detectability, or confidence rating as a function of vapor concentration (log ppm by volume) for each chemical. Detectability (P) was corrected for chance and took a value between P=0.0 (chance detection) and P=1.0 (perfect detection), according to (Macmillan and Creelman, 1991):

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

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

Statistical significance was tested by analysis of variance (ANOVA) for repeated measurements (software: SuperANOVA v.1.11, Abacus Concepts, Inc., Berkeley, CA). Adjustment factors correcting for the inherent correlation of repeated measurements via either the conservative Greenhouse-Geiser or the liberal Hunyh-Feldt epsilon values left all reported significant factors and interactions still significant at $p \leq 0.01$.

Results

Figure 1 (upper section) shows the concentration-detection functions and confidence ratings for each alkylbenzene. Pentyl benzene and, to some extent, hexylbenzene showed increasing detection with concentration, reaching at full vapor strength P≈0.60 and P≈0.26, respectively. In contrast, heptylbenzene showed a flat function around chance detection (i.e., P≈0.0) barely reaching P≈0.17 at full vapor strength. Confidence ratings reflected the trend in detectability.

A look at the individual data from the 14 subjects that completed all three chemicals provided additional insight (Figure 1, lower section). No gender differences in detectability emerged. Despite individual variability, most subjects shared the group pattern observed for each alkylbenzene. Thus, for pentylbenzene, the majority of participants showed a sharp increase in detection with concentration, many of them reaching detectabilities close to perfect detection. For hexylbenzene, only three subjects continued to show clearly such trend. Finally, for heptylbenzene only two subjects showed the trend, with all other subjects either failing altogether to increase detection with concentration or reaching a plateau where further increases in concentration did not produce enhanced detection. A two-way ANOVA for repeated measurements performed on the individual data from the 14 subjects common to the three chemicals gave statistical support to the results. The ANOVA showed that the factors alkylbenzene (three levels) {$F(2,26)=17.4$, $p<0.0001$}, concentration (five levels) {$F(4,52)=28.1$, $p<0.0001$), and their interaction {$F(8,104)=5.20$, $p<0.0001$) were all significant. Contrast tests revealed that the function for pentylbenzene was significantly different from those of hexylbenzene ($p=0.0008$) and heptylbenzene ($p<0.0001$), and that the difference between the functions for hexyl and heptylbenzene came very close to significance ($p=0.055$).

Insert Figure 1 about here

Experiment 2: Ketones

Materials and Methods

Subjects. We recruited 24 subjects (13 female). Their average age (±SD) was 28 (±9) years, ranging from 18 to 52 years. All participants were normosmics (Cain, 1989), non-users of contact lenses, and, except for one occasional smoker (male, 24 years old), nonsmokers.

A subset of 18 participants (9 female) from the group described above had available time to complete at least 20 trials per concentration (half with each eye) for all three ketones (see Procedure). Data presented below for individual subjects refers to this more comprehensively tested group.

Stimuli. The chemicals tested (purity in parenthesis) were: 2-undecanone (98+%), 2-dodecanone (98+%), and 2-tridecanone (98%). Medical Air U.S.P. (humidified to 50% relative humidity, RH) served as blanks (see Procedure). Table 2 presents the actual vapor concentrations in N_2 tested for each chemical as determined by gas chromatography (flame ionization detector, FID), and the nominal concentrations as determined by the ratio “stimulus flow / total (blank air + stimulus) flow” (see Apparatus).

Insert Table 2 about here

Apparatus. Same as in Experiment 1.

Procedure. Same as in Experiment 1.

Data analysis. Same as in Experiment 1.

Results

Figure 2 (upper section) presents the concentration-detection functions for each of the three ketones. Only 2-undecanone and, to a minor extent, 2-dodecanone showed an increase in detection with concentration, reaching at full vapor strength P=0.45 and P=0.18, respectively. 2-Tridecanone failed to be detected across the entire concentration range. Confidence ratings reflected the trend in detectability.

Using the data from the 18 participants tested on all three ketones, we plotted individual concentration-detection functions (Figure 2, lower section). No gender differences in detectability emerged. For 2-undecanone, most subjects increased detection with an increase in concentration, and some of them reached high levels of detectability ($P > 0.80$). For 2-dodecanone, only 3 subjects continued to show this trend (albeit one of them reached a detectability plateau at $P = 0.7$ where a further increase in concentration failed to increase detection). For 2-tridecanone, none of the subjects consistently increased detection with concentration, and all performed around chance detection across the entire concentration range. We performed a two-way ANOVA for repeated measurements on the data from these 18 participants, using the factors ketones (three levels) and concentration (five levels). The results revealed significance differences for ketones $\{F(2,34)=20.4,\ p<0.0001\}$, for concentration $\{F(4,68)=7.0,\ p<0.0001\}$, and for their interaction $\{F(8,136)=5.7,\ p<0.0001\}$. Contrast tests revealed that the function for 2-undecanone differed significantly from those for 2-dodecanone ($p=0.0017$) and 2-tridecanone ($p<0.0001$), and that the function for 2-dodecanone differed significantly from that for 2-tridecanone ($p=0.0055$). Thus, the outcome of the ANOVA gave statistical support to the differences among psychometric functions shown in Figure 2.

Insert Figure 2 about here

Discussion

At least two reasons could explain the appearance of a cut-off effect in the chemesthetic detection of homologous VOCs (Cometto-Muñiz *et al.*, 1998a). One possibility is that a homolog is reached whose vapor saturation at room temperature (≈23°C) falls below the threshold concentration for detection. This represents a limitation based on vapor concentration. Another possibility is that a homolog is reached whose molecular dimensions exceed a critical value that allows it to reach or bind effectively to the appropriate receptor(s). This represents a limitation based on molecular size or structure. Previous work employed two strategies to investigate the issue. One strategy applied a well-established quantitative structure-activity relationship (QSAR) (Abraham *et al.*, 1998; Abraham *et al.*, 2001; Abraham *et al.*, 2003) to predict the chemesthetic threshold concentration of the cut-off homolog and establish whether it was indeed higher than the saturated vapor concentration of that homolog at 23°C (Cometto-Muñiz *et al.*, 1998a). The other strategy tested whether the detectability of the cut-off homolog could be significantly increased by presenting the vapor at an even higher concentration, for example, the saturated vapor at 37°C (body temperature) (Cometto-Muñiz *et al.*, 2005b; Cometto-Muñiz *et al.*, 2006; Cometto-Muñiz *et al.*, 2007b). Results from both strategies pointed out to a limitation based on molecular dimension (size) as the reason for the cut-off, at least for the six series studied: acetate esters, n-alcohols, n-alkylbenzenes, 2-ketones, carboxylic acids and aliphatic aldehydes.

To gather additional evidence on the basis for the cut-off effect in chemesthetic detection of VOCs, we recently pursued an additional strategy, that is to measure concentration-detection (i.e., psychometric) functions for homologs approaching the cut-off point (Cometto-Muñiz *et al.*, 2007a). We reasoned that, under a limitation based on molecular size, the function for a cut-off homolog should reach a plateau (well below P=1.0) where further increases in concentration would fail to increase detectability. This is the strategy employed here for the three homologous alkylbenzenes and ketones.

The psychometric functions for the alkylbenzenes (Figure 1) revealed that the detectability of heptylbenzene, even at full strength (100% v/v), only reached P=0.17. Nevertheless, there were no indications of a detectability plateau (as defined above) across the concentration range tested. This range can be extended by adding data from a previous study of heptylbenzene that tested its detection by eye irritation at two concentrations: vapor saturation at 23°C and at 37°C (Cometto-Muñiz *et al.*, 2006) (Figure 3). Then, it becomes clear that the ocular chemesthetic detectability of heptylbenzene indeed reaches a plateau at P≈0.20, particularly considering that chemesthetic psychometric functions grow from slightly above chance to perfect detection within one order of magnitude as seen here for pentylbenzene (Figure 1) and in previous studies for other VOCs (Cometto-Muñiz *et al.*, 1999; Cometto-Muñiz *et al.*, 2002). In Figure 3, an increase in concentration from about 26 ppm to 141 ppm fails to increase detectability further. Thus, the present results for alkylbenzenes provide additional support to the notion of a cut-off based on molecular dimension(s) at the level of heptylbenzene.

Insert Figure 3 about here

Regarding the ketones, the psychometric functions obtained suggest a cut-off at the level of 2-dodecanone or 2-tridecanone (Figure 2). For this series, previous detectability data for 2-dodecanone is available only for vapor saturation at 23°C, whereas for 2-tridecanone it is available for vapor saturation at 23 and at 37 °C (Cometto-Muñiz *et al.*, 2006). Figure 4 merges these previous values with the present psychometric functions. The outcome in terms of reaching a detectability plateau is not evident for 2-dodecanone but it is quite clear for 2-tridecanone, again in the context of the characteristic steepness of chemesthetic functions as shown here for 2-undecanone (Figure 2) and as seen for other compounds (Cometto-Muñiz *et al.*, 1999; Cometto-Muñiz *et al.*, 2002). The present results for 2-ketones also indicate that the cut-off observed at the level of 2-tridecanone is based on molecular dimension(s). In turn, Table 3 lists the particular cut-off homolog found for each of the series tested so far, and includes the mucosal site probed and the corresponding references.

Insert Figure 4 and Table 3 about here

The chemesthetic detection of general VOCs in the eyes and nose is achieved via polymodal nociceptors within free nerve endings of C- and A-delta fibers from the trigeminal nerve that innervate the ocular and nasal mucosae (Doty et al., 2004). Nevertheless, the molecular mechanisms involved have not been clearly established. Probable participants are members of a family of transient receptor potential (TRP) channels (Pedersen *et al.*, 2005; Ramsey *et al.*, 2006), involved in a wide variety of sensory systems, including temperature, touch, taste, and chemical nociception, among others (Clapham, 2003; Voets and Nilius, 2003; Voets *et al.*, 2005; Nilius, 2007). For example, subtypes of TRP channels are implicated in the detection of typical pungent stimuli such as capsaicin, piperine, isothiocyanates, acrolein, thiosulfinates,

cinamaldehyde and menthol, to name a few (Julius, 2005; Bautista *et al.*, 2006; Bandell *et al.*, 2007). Thus, TRPs are strong candidates to play a role in the detection of the wide structural variety of environmental VOCs capable of eliciting chemesthetic sensations, such as the alkylbenzenes and ketones studied here. Additional relevant receptors include acid-sensing ion channels (ASIC), purinergic receptors (P2X), and nicotinic acetylcholine receptors (nAChR), all present in trigeminal nerve fibers (Silver *et al.*, 2006). Furthermore, other not yet identified receptors and mechanisms are also likely to participate in the detection of airborne irritants (Symanowicz *et al.*, 2004; Inoue and Bryant, 2005).

Ultimately, the overall effect of nociceptive agents, endogenous and exogenous, rests on the combined input of multiple channels and mechanisms as has been pointed out (Tominaga *et al.*, 1998; Garle and Fry, 2003; Voets and Nilius, 2003; Ramsey *et al.*, 2006). In this context, studies probing the integrated, psychophysical outcome of human chemesthetic detection within a structure-activity approach become particularly relevant. A quite successful quantitative structure-activity relationship (QSAR) has been developed for human ocular and nasal chemesthesis, as measured psychophysically (Abraham *et al.*, 1998; Abraham *et al.*, 2003). In line with the evidence discussed above, this model shows that the chemesthetic potency of VOCs, at least up to the cut-off point, is largely based on “selective” processes. These processes involve transfer-driven effects in which gradual structural changes in the VOC evoke predictable, and rather small, changes in biological activity. They reflect the transfer of the irritant from the vapor phase through various biological phases (biophases) until reaching the receptor(s) environment(s). In contrast, the observation of the cut-off effect indicates the appearance of a “specific” process, that is, one in which a small structural change in the VOC evoke a less predictable, and often large, change in activity. A cut-off effect fits this

category, and it is not presently contemplated in the otherwise successful QSARs cited above.

Results at the molecular and psychophysical level, as discussed, suggest that chemesthesis rests on processes integrating many mechanisms, and can be evoked by a large variety of chemical stimuli (Cometto-Muñiz, 2001). Many, if not all, of these pathways rely on receptors involving protein structures (Owsianik *et al.*, 2006; Ramsey *et al.*, 2006). It is, then, not surprising that potential irritants might reach a critical molecular size, i.e., the cut-off point, beyond which they fail to activate the relevant receptors or to fit into the binding pocket of a receptive macromolecule. The present experiments are part of a project that aims to identify the cut-off homologs across a broad range of homologous series and to ascertain whether the phenomenon is based on restrictions related to molecular dimension or to vapor concentration. These and previous results favor the first interpretation (Cometto-Muñiz *et al.*, 1998a; Cometto-Muñiz *et al.*, 2005b; Cometto-Muñiz *et al.*, 2006; Cometto-Muñiz *et al.*, 2007a; Cometto-Muñiz *et al.*, 2007b). With the help of molecular modeling programs, and once the cut-off homologs are identified across as many series as possible, their critical dimensions could be quantified and the outcome incorporated as an additional parameter into the established QSAR, expanding its applicability to VOCs located beyond the cut-off boundaries.

Conflict of Interest Statement

There are no conflicts of interest.

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References

Abraham, M.H., Gola, J.M.R., Cometto-Muñiz, 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., Hassanisadi, M., Jalali-Heravi, M., Ghafourian, T., Cain, W.S., Cometto-Muñiz, J.E., 2003. Draize rabbit eye test compatibility with eye irritation thresholds in humans: a quantitative structure-activity relationship analysis. *Toxicol. Sci.* **76**, 384-391.

Abraham, M.H., Kumarsingh, R., Cometto-Muñiz, J.E., Cain, W.S., 1998. An algorithm for nasal pungency thresholds in man. *Arch. Toxicol.* **72**, 227-232.

Alarie, Y., 1973a. Sensory irritation by airborne chemicals. *CRC Crit. Rev. Toxicol.* **2**, 299-363.

Alarie, Y., 1973b. Sensory irritation of the upper airways by airborne chemicals. *Toxicol. Appl. Pharmacol.* **24**, 279-297.

Bandell, M., Macpherson, L.J., Patapoutian, A., 2007. From chills to chilis: mechanisms for thermosensation and chemesthesis via thermoTRPs. *Curr. Opin. Neurobiol.* **17**, 490-497.

Bautista, D.M., Jordt, S.E., Nikai, T., Tsuruda, P.R., Read, A.J., Poblete, J., Yamoah, E.N., Basbaum, A.I., Julius, D., 2006. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell* **124**, 1269-1282.

Bryant, B., Silver, W.L., 2000. Chemesthesis: The Common Chemical Sense. In: Finger, T.E., Silver, W.L., Restrepo, D. (Eds.), *The Neurobiology of Taste and Smell. 2nd Edition*. Wiley-Liss, New York, pp. 73-100.

Cain, W.S., 1989. Testing olfaction in a clinical setting. *Ear Nose Throat J.* **68**, 316-328.

Cain, W.S., Lee, N.S., Wise, P.M., Schmidt, R., Ahn, B.H., Cometto-Muñiz, J.E., Abraham, M.H., 2006. Chemesthesis from volatile organic compounds: Psychophysical and neural responses. *Physiol. Behav.* **88**, 317-324.

Clapham, D.E., 2003. TRP channels as cellular sensors. *Nature* **426**, 517-524.

Cometto-Muñiz, J.E., 2001. Physicochemical basis for odor and irritation potency of VOCs. In: Spengler, J.D., Samet, J.M., McCarthy, J.F. (Eds.), *Indoor Air Quality Handbook*. McGraw-Hill, New York, pp. 20.21-20.21.

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., 1995. Relative sensitivity of the ocular trigeminal, nasal trigeminal and olfactory systems to airborne chemicals. *Chem. Senses* **20**, 191-198.

Cometto-Muñiz, J.E., Cain, W.S., 1998. Trigeminal and olfactory sensitivity: comparison of modalities and methods of measurement. *Int. Arch. Occup. Environ. Health* **71**, 105-110.

Cometto-Muñiz, J.E., Cain, W.S., Abraham, M.H., 1998a. 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., 2005a. Determinants for nasal trigeminal detection of volatile organic compounds. *Chem. Senses* **30**, 627-642.

Cometto-Muñiz, J.E., Cain, W.S., Abraham, M.H., 2005b. Molecular restrictions for human eye irritation by chemical vapors. *Toxicol. Appl. Pharmacol.* **207**, 232-243.

Cometto-Muñiz, J.E., Cain, W.S., Abraham, M.H., Gola, J.M.R., 1999. Chemosensory detectability of 1-butanol and 2-heptanone singly and in binary mixtures. *Physiol. Behav.* **67**, 269-276.

Cometto-Muñiz, J.E., Cain, W.S., Abraham, M.H., Gola, J.M.R., 2002. Psychometric functions for the olfactory and trigeminal detectability of butyl acetate and toluene. *J. Appl. Toxicol.* **22**, 25-30.

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

Cometto-Muñiz, J.E., Cain, W.S., Abraham, M.H., Sánchez-Moreno, R., 2006. Chemical boundaries for detection of eye irritation in humans from homologous vapors. *Toxicol. Sci.* **91**, 600-609.

Cometto-Muñiz, J.E., Cain, W.S., Abraham, M.H., Sánchez-Moreno, R., 2007a. Concentration-detection functions for eye irritation evoked by homologous n-alcohols and acetates approaching a cut-off point. *Exp. Brain Res.* **182**, 71-79.

Cometto-Muñiz, J.E., Cain, W.S., Abraham, M.H., Sánchez-Moreno, R., 2007b. Cut-off in detection of eye irritation from vapors of homologous carboxylic acids and aliphatic aldehydes. *Neuroscience* **145**, 1130-1137.

Dalton, P.H., Dilks, D.D., Banton, M.I., 2000. Evaluation of odor and sensory irritation thresholds for methyl isobutyl ketone in humans. *Am. Ind. Hyg. Assoc. J.* **61**, 340-350.

Doty, R.L., Cometto-Muñiz, J.E., 2003. Trigeminal chemosensation. In: Doty, R.L. (Ed.), *Handbook of Olfaction and Gustation (2nd Edition)*. Marcel Dekker, New York, pp. 981-1000.

Doty, R.L., Cometto-Muñiz, J.E., Jalowayski, A.A., Dalton, P., Kendal-Reed, M., Hodgson, M., 2004. Assessment of upper respiratory tract and ocular irritative effects of volatile chemicals in humans. *Crit. Rev. Toxicol.* **34**, 85-142.

Finger, T.E., Silver, W.L., Bryant, B., 1999. Trigeminal nerve. In: Adelman, G., Smith, B.H. (Eds.), *Encyclopedia of Neuroscience*. Elsevier, Amsterdam, pp. 2069-2071.

Garle, M.J., Fry, J.R., 2003. Sensory nerves, neurogenic inflammation and pain: missing components of alternative irritation strategies? A review and a potential strategy. *Altern. Lab. Anim.* **31**, 295-316.

Green, B.G., Lawless, H.T., 1991. The psychophysics of somatosensory chemoreception in the nose and mouth. In: Getchell, T.V., Doty, R.L., Bartoshuk, L.M., Snow Jr., J.B. (Eds.), *Smell and Taste in Health and Disease*. Raven Press, New York, pp. 235-253.

Green, B.G., Mason, J.R., Kare, M.R., 1990. Preface. In: Green, B.G., Mason, J.R., Kare, M.R. (Eds.), *Chemical Senses. Vol. 2: Irritation*. Marcel Dekker, Inc., New York, pp. v-vii.

Inoue, T., Bryant, B.P., 2005. Multiple types of sensory neurons respond to irritating volatile organic compounds (VOCs): calcium fluorimetry of trigeminal ganglion neurons. *Pain* **117**, 193-203.

Julius, D., 2005. From peppers to peppermints: natural products as probes of the pain pathway. *Harvey Lect.* **101**, 89-115.

Keele, C.A., 1962. The common chemical sense and its receptors. *Arch. Int. Pharmacodyn. Ther.* **139**, 547-557.

Kobal, G., 1985. Pain-related electrical potentials of the human nasal mucosa elicited by chemical stimulation. *Pain* **22**, 151-163.

Kuwabara, Y., Alexeeff, G.V., Broadwin, R., Salmon, A.G., 2007. Evaluation and application of the RD50 for determining acceptable exposure levels of airborne

sensory irritants for the general public. *Environ. Health Perspect.* **115**, 1609-1616.

Lee, Y., Lee, C.H., Oh, U., 2005. Painful channels in sensory neurons. *Mol. Cells* **20**, 315-324.

Macmillan, N.A., Creelman, C.D., 1991. *Detection theory: A user's guide*. Cambridge University Press, Cambridge.

Nielsen, G.D., 1991. Mechanisms of activation of the sensory irritant receptor by airborne chemicals. *Crit. Rev. Toxicol.* **21**, 183-208.

Nielsen, G.D., Hansen, L.F., 1993. Sensory irritation of the upper respiratory tract. *Pharmacol. Toxicol.* **72 Suppl 3**, 32-35.

Nielsen, G.D., Wolkoff, P., Alarie, Y., 2007. Sensory irritation: risk assessment approaches. *Regul. Toxicol. Pharmacol.* **48**, 6-18.

Nilius, B., 2007. Transient receptor potential (TRP) cation channels: rewarding unique proteins. *Bull. Mem. Acad. R. Med. Belg.* **162**, 244-253.

Owsianik, G., D'Hoedt, D., Voets, T., Nilius, B., 2006. Structure-function relationship of the TRP channel superfamily. *Rev. Physiol. Biochem. Pharmacol.* **156**, 61-90.

Pedersen, S.F., Owsianik, G., Nilius, B., 2005. TRP channels: an overview. *Cell Calcium* **38**, 233-252.

Ramsey, I.S., Delling, M., Clapham, D.E., 2006. An introduction to TRP channels. *Annu. Rev. Physiol.* **68**, 619-647.

Schaper, M., 1993. Development of a database for sensory irritants and its use in establishing occupational exposure limits. *Am. Ind. Hyg. Assoc. J.* **54**, 488-544.

Silver, W.L., Clapp, T.R., Stone, L.M., Kinnamon, S.C., 2006. TRPV1 receptors and nasal trigeminal chemesthesis. *Chem. Senses* **31**, 807-812.

Symanowicz, P.T., Gianutsos, G., Morris, J.B., 2004. Lack of role for the vanilloid receptor in response to several inspired irritant air pollutants in the C57Bl/6J mouse. *Neurosci. Lett.* **362**, 150-153.

Tominaga, M., Caterina, M.J., Malmberg, A.B., Rosen, T.A., Gilbert, H., Skinner, K., Raumann, B.E., Basbaum, A.I., Julius, D., 1998. The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* **21**, 531-543.

Voets, T., Nilius, B., 2003. TRPs make sense. *J. Membr. Biol.* **192**, 1-8.

Voets, T., Talavera, K., Owsianik, G., Nilius, B., 2005. Sensing with TRP channels. *Nat. Chem. Biol.* **1**, 85-92.

Wysocki, C.J., Dalton, P., Brody, M.J., Lawley, H.J., 1997. Acetone odor and irritation thresholds obtained from acetone-exposed factory workers and from control (occupationally unexposed) subjects. *Am. Ind. Hyg. Assoc. J.* **58**, 704-712.

Table 1. Concentrations tested for each alkylbenzene as determined by gas chromatography (flame ionization detector, FID) of samples taken at the outlet of the vapor delivery device (expressed in ppm and in log ppm by volume) and as determined by the ratio of stimulus / total (air+stimulus) flow (in % v/v) set with the device (see Apparatus). SD indicates standard deviation.

CHEMICAL	ppm ±SD	log ppm ±SD	% v/v
Pentylbenzene	24±1.5	1.375±0.027	20
	69±8.2	1.834±0.050	40
	132±17	2.118±0.061	60
	207±20	2.315±0.042	80
	245±31	2.386±0.057	100
Hexylbenzene	6.2±0.63	0.788±0.047	20
	19±2.4	1.273±0.057	40
	32±9.4	1.487±0.132	60
	50±14	1.682±0.121	80
	68±17	1.824±0.011	100
Heptylbenzene	2.7±1.7	0.332±0.324	20
	7.9±5.1	0.830±0.269	40
	13±4.2	1.096±0.141	60
	21±4.0	1.313±0.083	80
	27±4.7	1.432±0.070	100

Table 2. Concentrations tested for each ketone as determined by gas chromatography (flame ionization detector, FID) of samples taken at the outlet of the vapor delivery device (expressed in ppm and in log ppm by volume) and as determined by the ratio of stimulus / total (air+stimulus) flow (in % v/v) set with the device (see Apparatus). SD indicates standard deviation.

CHEMICAL	ppm ± SD	log ppm ± SD	% v/v
2-Undecanone	6.1 ± 1.5	0.769 ± 0.114	20
	17 ± 2.5	1.232 ± 0.065	40
	29 ± 9.2	1.434 ± 0.158	60
	37 ± 8.9	1.554 ± 0.112	80
	42 ± 15	1.598 ± 0.128	100
2-Dodecanone	1.6 ± 0.49	0.174 ± 0.143	20
	4.6 ± 0.99	0.650 ± 0.099	40
	5.9 ± 2.1	0.742 ± 0.192	60
	6.9 ± 1.5	0.829 ± 0.092	80
	8.1 ± 1.9	0.898 ± 0.089	100
2-Tridecanone	0.35*	-0.451*	20
	1.2 ± 0.50	0.034 ± 0.223	40
	2.4 ± 0.41	0.374 ± 0.075	60
	3.5 ± 0.73	0.535 ± 0.085	80
	4.1 ± 0.58	0.606 ± 0.063	100

* Value extrapolated from measurements at the higher concentrations.

Table 3. List of the particular cut-off homolog found for the homologous series studied so far, including the mucosal site tested and the corresponding citations.

Homologous series	Cut-off point for chemesthesis	Tested mucosa	References
n-Alcohols	1-Undecanol	Ocular	(Cometto-Muñiz *et al.*, 2005b; Cometto-Muñiz *et al.*, 2007a)
Esters (n-Acetates)	Decyl acetate	Ocular	(Cometto-Muñiz *et al.*, 2005b; Cometto-Muñiz *et al.*, 2007a)
	Decyl acetate	Nasal	(Cometto-Muñiz *et al.*, 2005a)
Esters (n-Butyrates)	Hexyl butyrate	Nasal	(Cain *et al.*, 2006)
2-Ketones	2-Tridecanone	Ocular	(Cometto-Muñiz *et al.*, 2006) (This study)
n-Alkylbenzenes	Heptyl benzene	Ocular	(Cometto-Muñiz *et al.*, 2006) (This study)
Carboxylic acids	Heptanoic acid	Ocular	(Cometto-Muñiz *et al.*, 2007b)
	Octanoic acid	Nasal	(Cometto-Muñiz *et al.*, 2005a)
Aldehydes	Dodecanal	Ocular	(Cometto-Muñiz *et al.*, 2007b)

Figure Legends

Figure 1. Upper section. Left. Group psychometric function (left y-axis, filled circles) and confidence ratings (right y-axis, empty squares) for the eye irritation evoked by pentylbenzene. Each point represents the average of 320 trials made by 16 subjects. Bars indicate standard error. Center. Same for hexylbenzene. Right. Same for heptylbenzene but where each point represents the average of 300 trials made by 15 subjects. Lower section. Individual functions from 14 subjects for eye irritation detection from pentylbenzene (left), hexylbenzene (center), and heptylbenzene (right). Each unique symbol represents the same subject across the three graphs. Each point represents the outcome of 20 trials per concentration for that subject. Filled circles joined by the thick line represent the average across the 14 subjects.

Figure 2. Upper section. Left. Group psychometric function (left y-axis, filled circles) and confidence ratings (right y-axis, empty squares) for the eye irritation evoked by 2-undecanone. Each point represents the average of 400 trials made by 24 subjects. Center. Same for 2-dodecanone. Right. Same for 2-tridecanone but where each point represents the average of 440 trials made by 24 subjects. Lower section. Individual functions from 18 subjects for eye irritation detection from 2-undecanone (left), 2-dodecanone (center), and 2-tridecanone (right). Each unique symbol represents the same subject across the three graphs. Each point represents the outcome of 20 trials per concentration for that subject. Filled circles joined by the thick line represent the average across the 18 subjects.

Figure 3. A plot for heptylbenzene combining the psychometric function for ocular chemesthesis obtained here (circles) with previous detectability data for vapor saturation

at 23 and at 37 °C (squares) (Cometto-Muñiz *et al.*, 2006) clearly shows the emergence of a detection plateau at P≈ 0.2. Bars indicate standard error.

Figure 4. Left. A plot for 2-dodecanone combining the psychometric function for ocular chemesthesis obtained here (circles) with previous detectability data for vapor saturation at 23°C (square) (Cometto-Muñiz *et al.*, 2006). Right. Same for 2-tridecanone but where previous data includes detectability for vapor saturation at 23 and at 37 °C (squares) (Cometto-Muñiz *et al.*, 2006). The lack of a detectability value for 2-dodecanone saturated vapor at 37°C leaves the emergence of a plateau inconclusive, but for 2-tridecanone the plateau emerges clearly at P≈0.2. Bars indicate standard error.

FIGURE 1

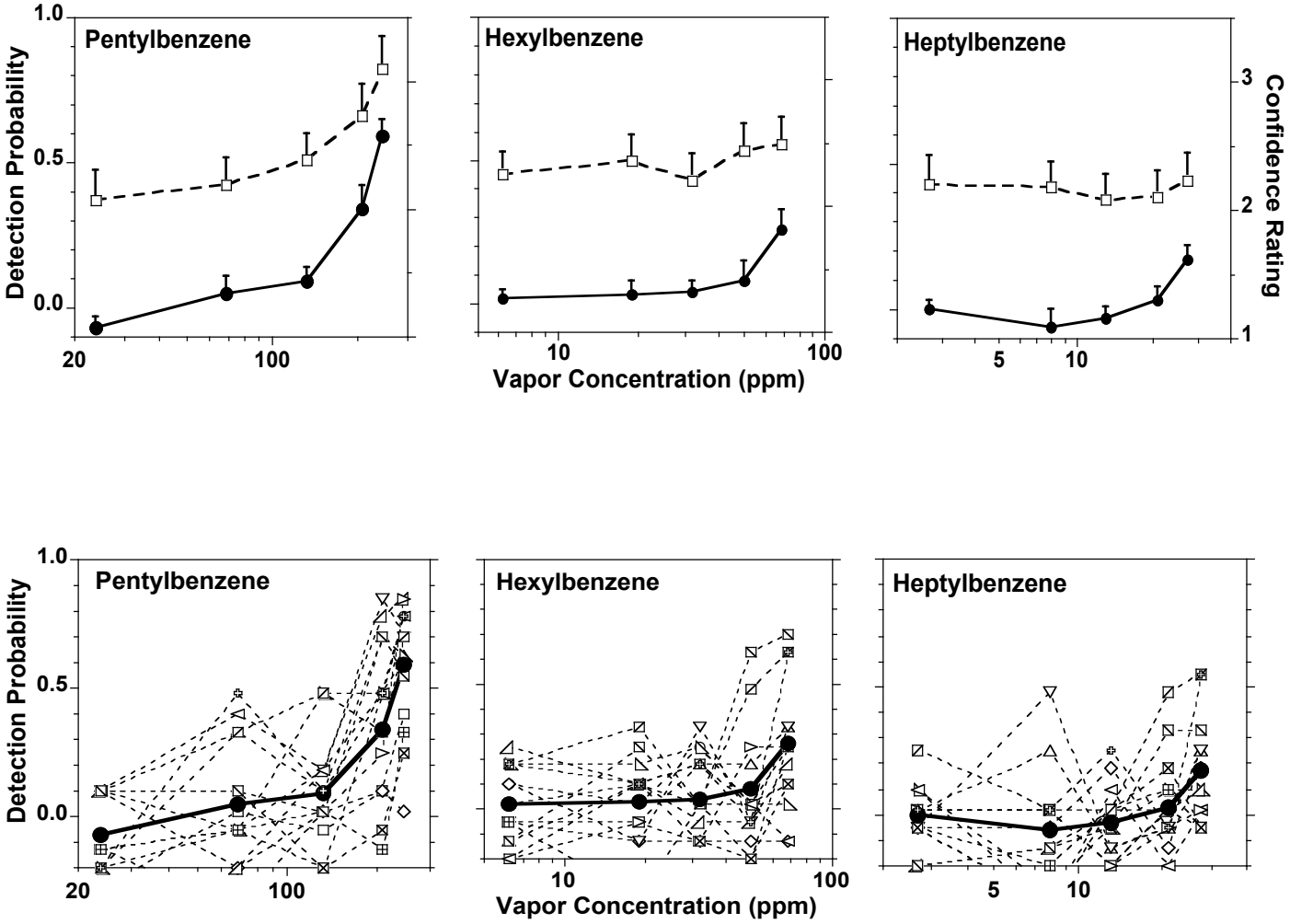

Pentylbenzene
Hexylbenzene
Heptylbenzene
Detection Probability
Confidence Rating
Vapor Concentration (ppm)
1.0
0.5
0.0
20
100
10
100
5
10
3
2
1
Pentylbenzene
Hexylbenzene
Heptylbenzene
Detection Probability
Vapor Concentration (ppm)
1.0
0.5
0.0
20
100
10
100
5
10

FIGURE 2

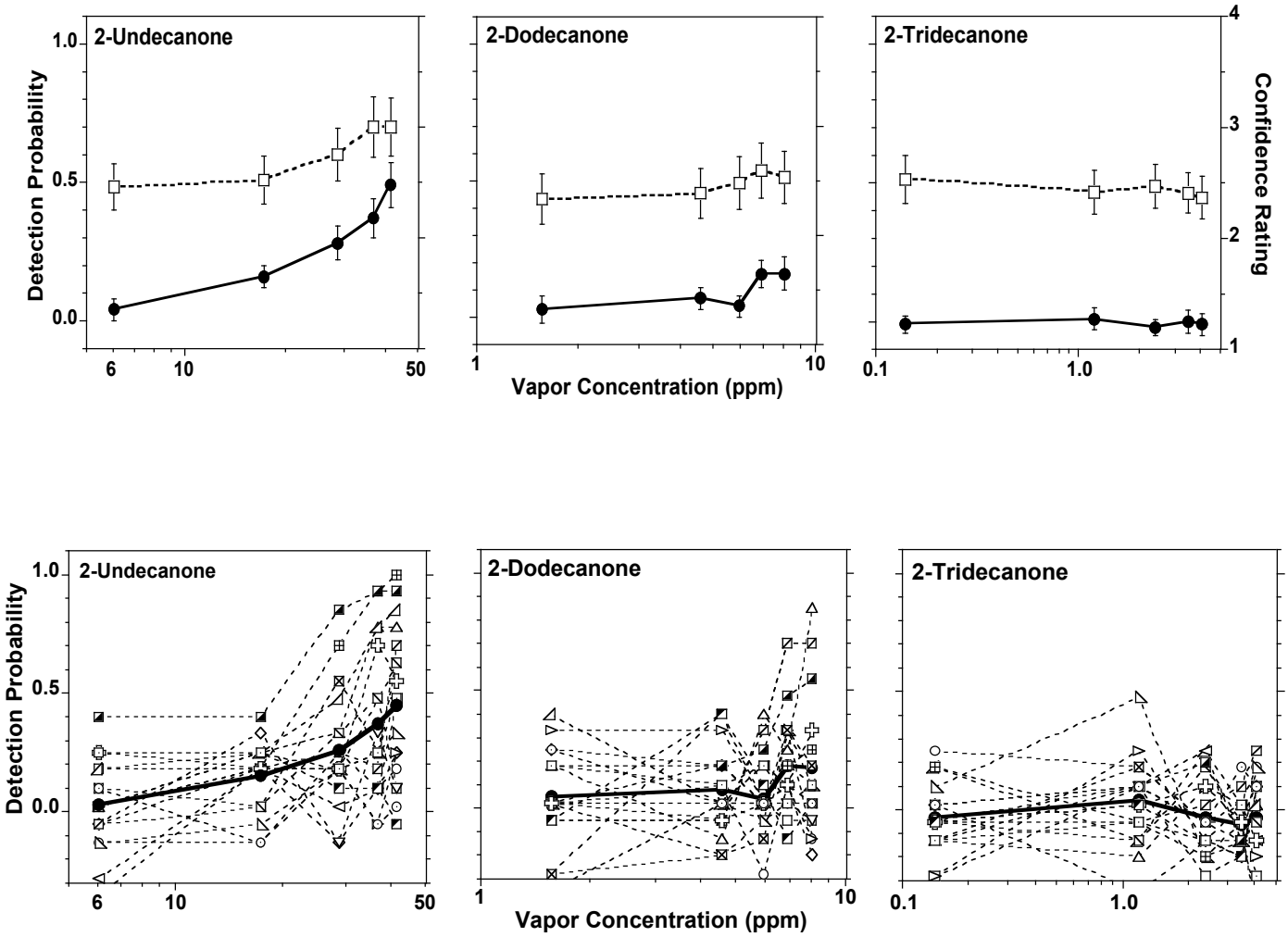

2-Undecanone
2-Dodecanone
2-Tridecanone
Detection Probability
Confidence Rating
Vapor Concentration (ppm)
1.0
0.5
0.0
6
10
50
1
10
0.1
1.0
4
3
2
1

FIGURE 3

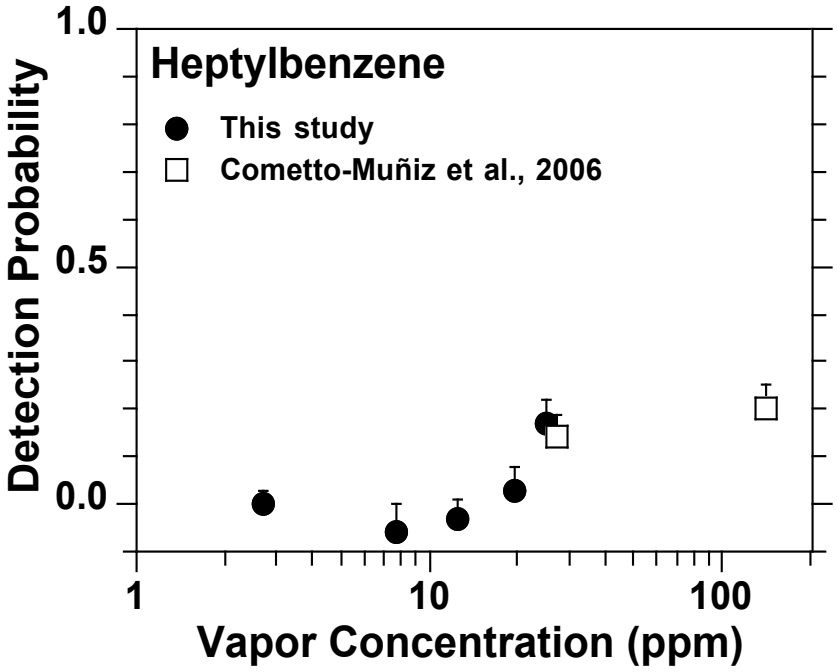
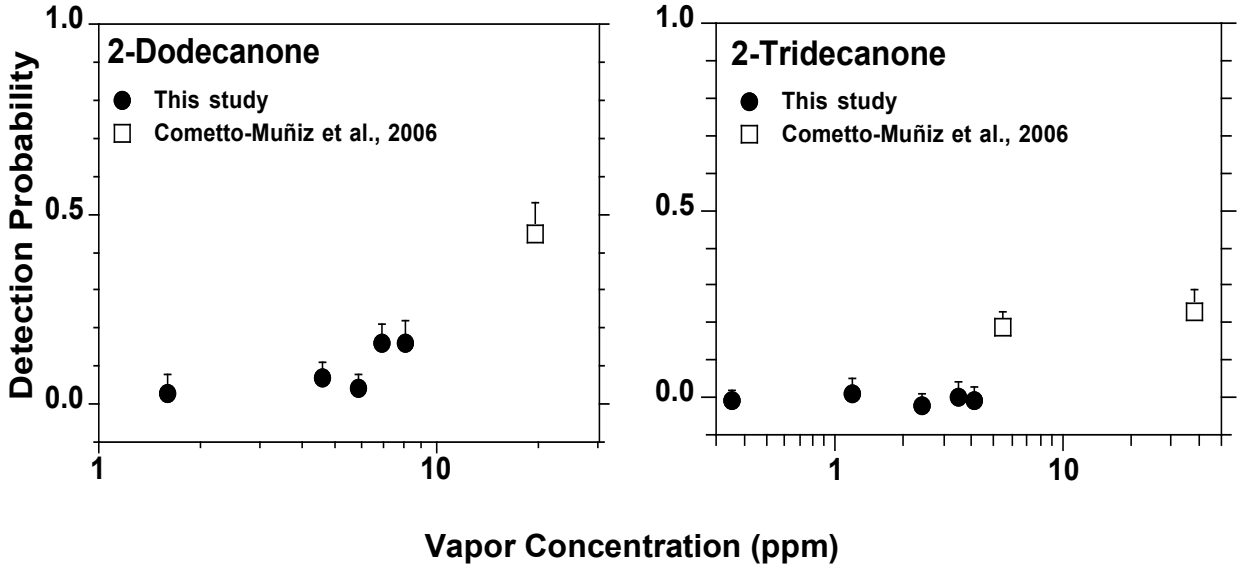


FIGURE 4



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