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Odor and Irritation Thresholds for Ammonia: A Comparison between Static and Dynamic Olfactometry

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Abstract

Odor and lateralization (irritation) thresholds (LTs) for ammonia vapor were measured using static and dynamic olfactometry. The purpose of the study was to explore the test–retest reliability and comparability of dynamic olfactometry methodology, generally used to determine odor thresholds following European Committee for Standardization guidelines in the context of odor regulations to outside emissions, with static olfactometry. Within a 2-week period, odor and LTs for ammonia were obtained twice for each method for 24 females. No significant differences between methods were found: mean odor detection thresholds (ODTs) were 2.6 parts per million (ppm) for either method ($P = 0.96$), and mean LTs were 31.7 and 60.9 ppm for the static and dynamic method, respectively ($P = 0.07$). Test–retest reliability was higher for the dynamic than for the static method ($r = 0.61$ vs. 0.14 for ODTs and $r = 0.86$ vs. 0.45 for LTs). The choice of optimal method for any application, however, depends not only on psychometric factors but also on practical factors such as physicochemical properties of the compound, availability of equipment and expertise, task efficiency, and costs.

Key words: olfaction, perceptual threshold, sensory irritation, trigeminal nerve

Introduction

Airborne volatiles can provoke 2 types of sensations in the nose: the first one being sensations of smell, mediated by the olfactory nerve (CN I), and the second one being sensations of irritancy (typically: burning, tingling, or prickling), mediated primarily by the trigeminal nerve (CN V; Doty et al. 2004). Traditionally, the concentration at which individuals start to perceive an odor has been determined using threshold detection procedures that involve a forced-choice between blanks and odorant stimuli at varying concentrations. More recently, a comparable method was developed for determining the threshold for irritancy in the context of an odor (Cometto-Muñiz and Cain 1998; Wysocki et al. 1997). In this method, individuals are asked to lateralize the stimulus to either the right or left nostril in a forced-choice procedure in which one irritant stimulus and one blank stimulus are presented simultaneously to either nostril. This method is based on the principle that volatiles can be lateralized only after detectable peripheral stimulation of the trigeminal nerve but not after stimulation of only the olfactory nerve (Kobal et al. 1989). When using this method, the irritation threshold is also referred to as the ''lateralization threshold (LT).'' These threshold procedures have been used to determine odor detection thresholds (ODTs) and LTs for a variety of compounds and thus establish ranges of stimulation for the sense of olfaction and irritation (Wysocki et al. 1997; Smeets and Dalton 2002; Cain et al. 2005; Van Thriel et al. 2006). Additionally, because irritation has been identified as one of the adverse effects workers may experience when working with volatile organic compounds (Dick and Ahlers 1998; Paustenbach 2001; Triebig 2002), LT procedures are now finding their way into the field of occupational hygiene, where they have been applied in the context of setting occupational exposure limits for humans (Smeets et al. 2006).

Initially, LTs were determined using a static olfactometry approach, in which each concentration step in a series of stimuli is prepared from successive liquid dilutions of a single chemical compound in an appropriate diluent. The nominal stimulus for nasal stimulation is the gaseous headspace in equilibrium over the liquid, which is actively sniffed from a single closed container (often a bottle with a tight fitting nosepiece: Prah et al. 1995). Static olfactometry can be quite suitable when working with single chemical compounds or simple mixtures. However, if the stimulus of interest is a complex mixture of chemicals, stimuli cannot be so easily prepared in containers in the laboratory. This would be the case for establishing LTs for emissions from outdoor air (but also indoor air, see Brown et al. 1994) such as industrial or agricultural sources, which tend to be complex mixtures and only rarely consist of a single chemical compound. The alternative to static olfactometry would then be dynamic olfactometry, which involves delivering a continuous, well-regulated gas flow that contains odorized air mixed in varying proportions with a carrier gas (typically odorless air or nitrogen: Prah et al. 1995). Here, the starting stimulus from which the dilutions are prepared can be an air sample collected in the field in Teflon or Tedlar bags. This practice has increased the ecological validity of the data as compared with static olfactometry, as it enables the assessment of thresholds for a wide range of actually occurring exposures. ODTs are already frequently obtained for emissions from refineries, pulp mills, and agricultural settings and are then applied in dispersion modeling for the purpose of odor regulation and permit setting (Mahin 2001). Because in those cases the air sample is difficult to characterize in terms of composition and concentration, ODTs are typically expressed in odor units per volume (OU/m^3), in which personal thresholds are calculated from the number of dilution steps necessary to arrive at that person's threshold (CEN 2003; Dalton and Smeets 2004).

Although odor from outside exposures can be hedonically unpleasant and annoying, and thus adverse, there is also a need for a more ''objective'' endpoint of adversity from experiencing exposure to outside air emissions. In analogy to the indoor air field, irritancy experienced from stimulation of the trigeminal nerve in the nose is an important endpoint for characterizing industrial or agricultural emissions. Consequently, LTs could be collected in addition to ODTs for outside emissions using dynamic olfactometry to arrive at 2 independent endpoints of adversity. To date, LTs for ambient emissions air have rarely been collected: Schiffman et al. (2001) presented ambient air collected outside of 4 subjects' homes located 427 m downwind from a swine facility to one nostril and charcoal-filtered air to the other nostril and found that all 4 subjects were able to lateralize emissions of swine waste to the nostril to which it was presented. This outcome suggested that the compounds in the air surrounding their homes reached levels capable of stimulating nasal irritation.

The present study collected LTs using dynamic olfactometry equipment typically used to determine ODTs to outside emissions. The aim of the study was to assess the test–retest reliability and the comparability of this procedure to static olfactometry. In order to enable the collection of LTs, the olfactometer was adjusted to allow the presentation of separate airstreams to the separate nostrils (see Materials and Methods). To determine the validity of the procedure, both odor detection and LTs were collected using dynamic and static olfactometry in the same subjects, and the results were compared. Test–retest reliability was assessed by obtaining each threshold type 4 times, twice per method.

To allow for a comparison between the 2 types of olfactometry, we used the single chemical compound ammonia (NH_3), dilutions of which can also be prepared in bottles. We selected ammonia for the following reasons: firstly, because it is a compound that is present in many industrial and agricultural emissions. For example, 30% of the N_2 input of pig production in Europe is emitted as ammonia, both from animal housing and manure application (IPPC 2003; in Ogink and Aarnink 2003). Secondly, there have been few publications stating ODTs for ammonia and even fewer stating LTs. In the Devos et al. (1990) compilation of olfactory thresholds, the mean ODT for ammonia is 5 parts per million (ppm); Michaels (1999) refers the range of ODTs to be between 0.04 and 57 ppm; and Van Thriel et al. (2006) reported a median of 0.05 ppm. LTs of around 37–67 ppm (Wise et al. 2005) have been reported, although in Table 3 of the Van Thriel et al. study, the median LT for ammonia was higher at 314 ppm. Ruth (1986) listed an irritation threshold (not determined with the lateralization method) for ammonia of 72.00 mg/m^3, which is equivalent to 101.4 ppm at 20 °C and 1013.25 hPa. Thus, in view of the scarcity of human odor and irritation detection studies using ammonia, this compound seemed appropriate for the present purposes.

Materials and Methods

Subjects

The medical ethics committee of the University Medical Center Utrecht (UMCU) approved the protocol prior to the start of this study. All research was performed in accordance with the Declaration of Helsinki. Female subjects between 18 and 45 years of age who did not smoke, did not have asthma, were not pregnant, had a normal sense of smell, and who could attend all sessions were recruited by a local employment agency and invited to attend a screening session at the UMCU. At the start of this session, all subjects signed informed consent. They thereupon received a rhinoscopic examination performed by one of the coauthors who is an ENT physician. Next, they were screened for allergies, chemical sensitivities, respiratory disease, general health, and prior chemical exposures by personal interview. The last portion of the session involved a quick screening with butanol

using a subset of the Sniffin' Sticks (Hummel et al. 1997), to ensure they had a normal sense of smell for this compound, followed by a quick screening with several of the ammonia stimuli expected to be around the odor and irritation threshold to make certain that they would be able to detect the stimulus of interest in this study. Only females without major obstructions of the nasal airway or severe congestion of the nasal mucosa, without (serious) allergies, chemical sensitivities, and having no frequent prior exposure to chemicals, most notably ammonia, but with the ability to detect the presented olfactory and trigeminal stimuli were included in the study. Of 26 female subjects who attended the screening session, 2 were excluded from participation in the study for health reasons. The mean age of the sample was 29.9 years, standard deviation (SD) = 8.9. Most females' primary occupation was a student or homemaker. Of the 24 subjects accepted in the study, 2 subjects did not attend 1 of the 4 test sessions due to illness. All subjects received financial remuneration for their participation.

Static olfactometry (preparation and chemical analysis of stimuli)

All odor and blank stimuli were kept in 250-ml glass bottles from Scott, Duran, with a 3.5-cm wide opening. Every bottle contained 10 ml of liquid and was outfitted with a custom-made Teflon nosepiece inserted in the cap.

In order to prepare chemically stable stimuli, stoichiometric amounts (volumes) of ammonium chloride (NH_4Cl, purity ≥ 99.5%, Sigma, Steinheim, Germany) and sodium hydroxide (NaOH, purity ≥ 98%, SigmaUltra, Steinheim, Germany) were mixed. To achieve different stimuli concentrations by means of this procedure, 5 different dilutions of both compounds were prepared. Different volumes of these solutions were mixed with ultrapure water (UPW, Millipore filtered) until the final volume of 10 ml was reached.

The different aqueous solutions of the 2 chemical compounds were as follows: starting with a stock solution of 0.8 mol NaOH/l (0.8 N NaOH), 4 sodium hydroxide solutions were prepared (0.16, 0.016, 0.0016, and 0.00016 mol/l). The stock solution of ammonium chloride (C1) had a concentration of 84.66 g/l (1.58 mol/l). Four further solutions of NH_4Cl were prepared in water with concentrations of 0.158 mol/l (C10), 0.0158 mol/l (C100), 0.00158 mol/l (C1000), and 0.000158 mol/l (C10 000). The specific volumes of the solutions of NH_4Cl and NaOH and the additional H_2O to produce the dilution series are given in Table 1. UPW was used as the blank. Ten blanks were prepared for every series of 20 bottles.

Ammonia headspace concentrations in the static dilution series were measured using the MiniRae 2000 (Rae Systems Inc., San Jose, CA), a pumped handheld Volatile Organic Compound monitor with a photoionization detector (PID) with a 10.6-eV lamp. For every series of stimulus bottles, ±5 bottles that were within or close to the MiniRae's most sensitive range of 0–100 ppm, which is also the range of the expected ODT and LT for ammonia, were sampled by inserting the pump into the bottle through the Teflon enclosure for 5 s. Every stimulus series was assessed typically 3 times, that is, in the morning, afternoon, and at the end of every testing day. The MiniRae was calibrated daily with zero air and

Table 1 Pipetting scheme for the preparation of the NH_3 concentration range in glass bottles for static olfactometry and results of chemical analysis by PID

Dilution step # (bottle)	NH_4Cl	NaOH	H_2O	NH_3 vapor concentration (ppm[a])
C1, 0.8 mol/l				
1	1.6 ml	3.2 ml	5.2 ml	3367.38
2	0.8 ml	1.6 ml	7.6 ml	1073.06
3	0.4 ml	0.8 ml	8.8 ml	341.95
4	160 µl	320 µl	9.5 ml	108.97
C10, 0.16 mol/l				
5	0.8 ml	1.6 ml	7.6 ml	34.72
6	0.4 ml	0.8 ml	8.8 ml	11.07
7	160 µl	320 µl	9.5 ml	3.53
C100, 0.016 mol/l				
8	0.8 ml	1.6 ml	7.6 ml	1.12
9	0.4 ml	0.8 ml	8.8 ml	0.36
10	160 µl	320 µl	9.5 ml	0.11
C1000, 0.0016 mol/l				
11	0.8 ml	1.6 ml	7.6 ml	0.04
12	0.4 ml	0.8 ml	8.8 ml	0.01
13	160 µl	320 µl	9.5 ml	0.004
14	80 µl	160 µl	9.8 ml	0.001
C10 000, 0.00016 mol/l				
15	0.4 ml	0.8 ml	8.8 ml	0.0004
16	160 µl	320 µl	9.5 ml	0.0001
17	80 µl	160 µl	9.8 ml	3.81×10^{-5}
18	40 µl	80 µl	10 ml	1.21×10^{-5}
19	16 µl	32 µl	10 ml	3.87×10^{-6}
20	8 µl	16 µl	10 ml	1.23×10^{-6}

[a]Based on $y = 4.03\ (\pm 0.41) - 0.50\ (\pm 0.14)x$, where y = log ppm concentration and x = dilution step # (=bottle #). The equation is the average of 48 equations fitted for every individual and week ($24 \times 2 = 48$), based on multiple measurements (approximately 3 per day over 8 days, ±5 bottles per measurement) and extrapolations from equations for threshold assessments conducted between measurements. Please note that dilution steps 1 and 2 were never offered to subjects for safety reasons. Measured concentrations from these bottles were not used to fit equations. Vapor concentration in ppm (5th column), were calculated from y values of the aforementioned equation ($10^{\log \text{value}}$).

a 300 ppm ammonia cylinder (Rae Systems Inc.). Typically, 5-point regression curves were generated from MiniRae readings and corresponding bottle numbers, which were then used to convert the dilution steps of the bottle stimuli into concentration units (ppm by volume, see Table 1). With very few exceptions, the coefficient of determination (r^2) for each curve was satisfactory and (well) above 0.90.

Except for the first week of testing, in which a single series of 20 bottles were used, 2 series of freshly prepared stimuli were used each week with 12 subjects (6 per series) to prevent loss of stimulus concentration during testing as much as possible. As can be seen in Table 1, the NH_3 vapor concentration of the headspaces showed a tertiary spacing, whereas we intended to prepare a binary series of stimuli. There are 2 possible reasons explaining this effect. Firstly, throughout testing, we observed that the stimulus value per bottle became depleted throughout the day. We considered that effect by measuring the headspace concentrations via PID 3 times per day (morning, noon, and late afternoon). For example, on one test day, based on these 3 measurements, and over the course of testing 5 subjects, the dilution series started out as approximately binary, with every next bottle containing 2.3 times less the concentration of the previous bottle, and ended as an almost tertiary series, with every next bottle containing 2.8 times less than the previous one. On other days, the decay throughout the course of the day was more substantial. Thus, on average (calculated across times per day and all test days), we arrived at a regression equation (see Table 1) that is approximately tertiary. Secondly, the stoichiometric mixing of ammonium chloride and sodium hydroxide might have been suboptimal especially when small volumes were used. We will return to this phenomenon in the Discussion.

Static olfactometry (procedure of threshold assessment)

ODTs and LTs were collected using a forced-choice, 2-alternative, up-and-down staircase with a 5-reversal criterion (Wetherill and Levitt 1965). Reversals were defined as follows: proceeding from low to high concentrations, the first bottle concentration at which the subjects correctly detected the stimulus repeatedly was taken as the reversal. For the first reversal, 4 correct detections of the same dilution were required; for reversals 3 and 5, 2 consecutive correct detections were considered adequate. Proceeding from high to low concentrations (reversals 2 and 4), the first bottle concentration at which the subject failed to detect the stimulus was taken as the reversal. The threshold was calculated as the mean of the last 4 reversals after ignoring the first one.

For the assessment of ODTs, subjects were always presented with 2 pairs of bottles. One pair (either the first or the last) contained the odor stimulus, the other pair contained just blanks. In the stimulus pair, the stimulus was presented to one nostril (monorhinally), and nostril was randomized across presentations. Subjects were allowed to take one sniff from each pair. They were instructed to indicate from which pair they smelled the odor of ammonia. For the assessment of LTs, subjects were presented with only one pair of bottles. One bottle contained the stimulus, the other one the blank. They were instructed to indicate from which bottle they detected the ammonia. In this case, detection was defined as a feeling of burn or irritation. The duration of the sniff was no more than 2 s. There was a 30- to 60-s break between each set of stimuli.

Dynamic olfactometry (olfactometer and dilution process)

Dynamic olfactometry was conducted using an olfactometer built by PRA/Odournet (Amsterdam, the Netherlands) that is generally used for the collection of odor thresholds according to the European standard EN 13725 (CEN 2003). The olfactometer seated a panel of 6 people who were tested simultaneously. (A diagram of the dynamic olfactometer is displayed in Figure 1, upper panel. The lower panel of Figure 1 provides an overview of the possible nose configurations for each of the threshold assessments. Figure 2 shows one set of sniffing ports.)

Ammonia, metered from a cylinder, was diluted with clean air in a 15-step dilution series that was approximately binary. Actual dilution steps were based on an annual calibration following EN 13725 (CEN 2003) using CO as the tracer gas (see Table 2, 2nd column). Thus, dilutions varied

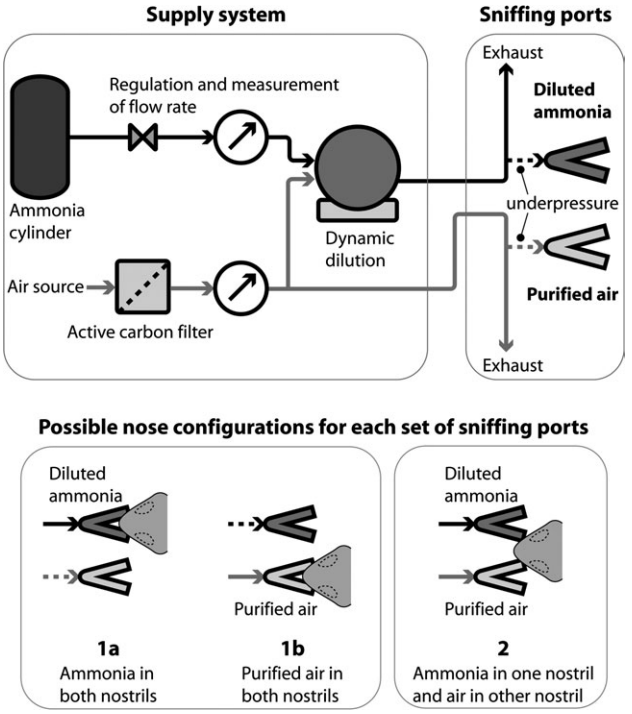

Figure 1 Upper panel: a diagram of the supply system and sniffing port configuration of the dynamic olfactometer used in this study. Lower panel: possible nose configurations for each set of ports during odor threshold detection (1a and 1b) and LT detection (2).

Figure 2 One set of sniffing ports. Each vessel terminates into 2 extensions with tightly fitting nosepieces to allow separate airstreams to each nostril. Odorized air bypasses the extensions to the exhaust and enters into the nose only during active sniffing (see text).

between dilution step 1 (maximum concentration, 13 times diluted) and step 15 (minimum concentration, 79 400 diluted). For the assessment of the odor threshold, a cylinder of 2000 ppm ammonia in N_2 was used (Luxfer, Nottingham). Consequently, the maximum concentration that could be reached was 2000/13 = 153.85 ppm and the minimum concentration was 2000/79400 = 0.03 ppm. For the assessment of the LT, an 8000 ppm ammonia in N_2 cylinder was used (Luxfer). Employing the same range of dilution steps, the maximum concentration that could be presented was 615.4 ppm, the minimum 0.10 ppm of NH_3, a range that was assumed to cover the LT. Because estimated values of NH_3 were based on the ISO-certified calibration using a different chemical (CO) as the tracer gas, we also measured exit concentrations of NH_3 at the ports using a Multi-gas Monitor type 1323 (INNOVA, Nærum, Denmark) for the high concentration range (154–2 ppm and 615–15 ppm for the 2000 and 8000 cylinders, respectively) and NOx-monitor with NH_3 convertor (API, San Diego, CA) for the lower concentration ranges from 2 to 15 ppm and below for either cylinder. For the 2000 ppm cylinder, dilution steps 13, 14, and 15 (around or below 0.1 ppm) could not be registered reliably

Table 2 Estimated vapor concentration per dilution step for dynamic olfactometry (ppm value)

Dilution step #	Calibrated dilution[a]	Vapor concentration (ppm) based on 2000 ppm[b]	Vapor concentration (ppm) based on 8000 ppm[c]
1	13.0	153.85	615.38
2	24.6	81.30	325.20
3	45.0	44.44	177.78
4	82.4	24.27	97.08
5	152.0	13.16	52.63
6	283.0	7.07	28.27
7	521.0	3.84	15.36
8	928.0	2.16	8.62
9	1820.0	1.10	4.4
10	3210.0	0.62	2.49
11	6010.0	0.33	1.33
12	11200.0	0.18	0.71
13	20800.0	0.10	0.39
14	38700.0	0.05	0.21
15	79400.0	0.03	0.10

[a]Dilution steps are theoretically binary starting from a dilution of 13 at step # 1. However, estimates of vapor concentration exiting at the sniffing ports have been based on an ISO-certified calibration (CEN 2003) carried out in April 2005 over the full range of dilution steps; these were not exactly binary.

[b]For ODT testing, actual vapor concentrations in ppm for each dilution step were calculated by dividing the starting concentration of volatile NH_3 flowing from the cylinder (2000 ppm) by the calibrated dilution step in column 1.

[c]For LT testing, actual vapor concentrations in ppm for each dilution step were calculated by dividing the stock concentration of volatile NH_3 flowing from the cylinder (8000 ppm) by the calibrated dilution step in column 1.

and were therefore not considered in the analyses. The coefficient of determination r^2 expressing the correspondence between the expected (based on the ISO-certified calibration) and determined values was very high (≥0.99) for the 2000 and 8000 ppm cylinder. Because of the very small difference between the expected and determined values, threshold values in ppm were based on the ISO-certified calibration steps (Table 2).

Dynamic olfactometry (threshold assessment)

Airflow of the ammonia stream or clean air was maintained at 20 l/min. However, a negative pressure was maintained such that the stimulus would only enter the subject's nostril if she sniffed from the nosepieces. Only minimal effort needed to be exerted on part of the subject in order to get the stimulus flowing into the nose. When no sniffing occurred, the air would bypass the extension and flow directly

to an external outlet (see Figure 1). This procedure employing active sniffing is different from that used by other olfactometers in which air is streamed into the nose and the subject is passively exposed to the stimulus. The method of active sniffing was preferred here because of its comparability to static olfactometry that also involves active sniffing. The temperature of the air flowing through the olfactometer was around 26 °C, the relative humidity ± 25%.

Normally, odor and clean air stimuli flow from 2 stainless steel vessels into the air, and the subject is asked to smell from both vessels. Because the assessment of LT required the separate presentation of one airstream to the left nostril and the other to the right nostril, stainless steel extensions ending into snugly fitting Teflon nosepieces were built on each vessel to allow separate presentations of either one airstream to both nostrils (for ODT assessment) or either airstream into separate nostrils. Thus, when ODTs were collected, the subject sniffed first from the 2 extensions protruding from the left vessel and then from the 2 extensions protruding from the right vessel. She was then asked to indicate whether she smelled the odor on the left or on the right port. When LTs were collected, the subject sniffed from both vessels at the same time, by placing one extension from the left vessel into the left nostril and one extension from the right nostril into the right nostril. She was then asked to indicate if she felt the stimulus in the left or right nostril (see Figure 1, lower panel).

Subjects were allowed one sniff of maximally 2 s per evaluation trial, the interval of which was indicated using a metronome. In addition to selecting either the left or right vessel or nostril as the source of stimulation, they were asked to indicate how certain they were. There were 3 options: guess, doubt, and certain. Subjects did not receive any feedback as to whether or not their answer had been correct after each trial. In this manner, ODTs and LTs were collected using the ascending method of limits, with a maximum of 15 stimuli presented in each series. For reliability, the ascending series was presented 3 times. After the first series, the starting point for each subsequent series began at a concentration determined by the subjects' previous performance (typically 3 dilution steps below the lowest step at which at least one subject had been able to correctly detect the stimulus with certainty). After each stimulus pair, there was a break of at least 1 min to allow the olfactometer to prepare the next dilution step and to allow the subject's nose to recover from any short-term adaptation. For each individual subject, threshold collection was terminated when she had correctly detected 2 concentrations in a row with certainty, by signaling her to move away from the ports and not sniff any more. This prevented any individual from receiving unnecessary additional exposure to the stimulus, as the olfactometer was designed to deliver the same concentration to all 6 ports in an ascending fashion until a threshold was obtained from all subjects. A series of stimuli was ended after the last subject had met the criterion of 2 correct detections with certainty. For each of the 3 series, the individual threshold was determined at the first (of 2) concentration steps at which the subject correctly detected the stimulus with certainty. The 3 thresholds were then averaged to obtain the overall threshold for the session.

During testing, subjects listened to relaxing music on ear phones to prevent them from hearing any sounds from the olfactometer that were associated with switching between airstreams.

Procedure

All test sessions were conducted at the odor laboratory at Agrotechnology and Food Innovations in Wageningen, the Netherlands. After passing the screening, subjects were invited for a 2-h training session. During this session, they were taught to sniff according to the beat of a metronome set at 2 s, in order to prevent individual differences in results between subjects in inhaled volume as much as possible (Cometto-Muñiz and Cain 1984) and to prevent unnecessary exposures to ammonia. In addition, subjects were familiarized with the response procedure. The subjects practiced 2 rounds of ODT and LT detection each while seated at the dynamic olfactometer and listening to the music through headphones.

In the 2 weeks following the training, ODT and LTs for ammonia were collected once a week using both the static and dynamic olfactometry procedure in separate sessions. Hence, there were 4 test sessions in total, 2 per method spread out over 2 weeks. Twenty-four subjects were tested in 4 panels of 6. During each session, the ODT was always collected first. Two panels were tested in September 2005 and 2 in November 2005. One panel was always tested in the morning, the other panel in the afternoon. In September, both panels received static olfactometry during test sessions 1 and 3 and dynamic olfactometry during sessions 2 and 4. For the November panel, this order was reversed.

Safety precautions

In order to assess LTs, we needed to present stimuli of high enough concentration to actually include the LT. In the Netherlands, for ammonia, the maximum allowable concentration (MAC-TGG 8 h) or time-weighted average for an 8-h exposure per day for no more than 40 h per week is 20 ppm, whereas the MAC-TGG 15 min or time-weighted exposure for a 15-min exposure is 50 ppm (Ministerie van Sociale Zaken en Werkgelegenheid 2004). The dose of ammonia that the nose received in our study was only a fraction of that amount, as the durations of actual stimulus exposure were considerably briefer (e.g., only 2 s each). The weighted exposures per time unit received in this study were calculated by conservatively assuming that all 15 concentrations that could be presented during dynamic olfactometry for both the ODT and LT trials would be sniffed for 2 s and that each series would be presented 3 times. This total exposure was then converted to an 8-h MAC-TGG and a 15-min MAC-TGG

and was found to be below 20 and 50 ppm, respectively. Not surprisingly, static olfactometry resulted in less exposure to higher concentrations and fewer presentations than did dynamic olfactometry, due to the difference in test methods (staircase vs. ascending methods). The bottles with higher concentrations were chemically analyzed, and bottles containing concentrations over 600 ppm were not presented to subjects. Stimuli of 300 or 600 ppm were only presented with prior warning to sniff carefully and not too deeply. Duration of sniffing was reduced by adjusting the metronome interval to approximately 1 s. Thus, the time-weighted averages for ammonia as set in the Netherlands were not exceeded in this study.

Results

Threshold means by method

Because ODTs and LTs were not normally distributed, all data points were subjected to logarithmic transformation. Two out of 24 subjects did not participate in one of the dynamic olfactometry sessions. In order not to lose these cases, missing values were replaced by the method of multiple imputation (Schafer and Graham 2002). In multiple imputation, each missing value is replaced by a list of $m > 1$ simulated values, in this case $m = 5$. Each of the 5 data sets was analyzed in the same fashion using SPSS 11.5. Results obtained from the multiple imputation data sets were then combined, and P levels were corrected for uncertainty resulting from missing data, using NORM 2.03 (Schafer and Graham 2002). Separate contrasts were fitted for the main and interaction effects involving the within-subjects factors Method (2 levels: static vs. dynamic olfactometry), Threshold (ODT vs. LT) and Week (Week 1 vs. Week 2). Because the resulting tests (after combining the multiple imputed data sets) had approximately normal distributions, Z values are reported. The reported P values are 2 sided; alpha was set at 0.05.

The mean log-transformed threshold values and SDs of greatest interest as well as geometric mean thresholds are displayed in Table 3. There was no main effect of Method ($Z = -1.11$, $P = 0.27$). Separate contrasts were fitted to test differences between methods in ODT versus LT. There was neither a difference in ODT between methods ($Z = -0.05$, $P = 0.96$) nor in LT ($Z = -1.80$, $P = 0.07$).

As expected, there was a main effect of Threshold ($Z = -10.69$, $P < 0.0001$). With the mean ODT being 2.59 ppm (mean log value = 0.41, SD = 0.97, not in Table 2), the mean LT of 43.94 ppm (mean log value = 1.64, SD = 0.60, not in Table 2) was significantly higher. The effect of Week was significant ($Z = 2.06$, $P = 0.04$), with the mean threshold being lower during the second assessment ($M_{W2} = 8.76$, mean log value = 0.94, SD = 1.12, not in Table 2) than during the first ($M_{W1} = 12.96$, mean log value = 1.11, SD = 0.89, not in Table 2). However, this effect was no longer significant after exclusion of one subject who had an extremely low ODT of 0.019 ppb during the second static olfactometry assessment ($Z = 1.74$, $P = 0.08$).

Reliability

Correlations were computed on untransformed data to determine the test–retest reliability of the assessments within and between methods. Within the dynamic olfactometry method, there was a strong correlation from Week 1 to Week 2 between ODTs ($R = 0.61$, $P < 0.005$) as well as LTs ($R = 0.86$, $P < 0.005$). Test–retest reliability was lower for ODTs obtained using the static olfactometry method: $R = 0.14$, $P = 0.52$. For LTs, $R = 0.45$ and $P < 0.05$. Correlations between methods were all low and not significant: for ODTs, $R = -0.14$ at Week 1 and $R = 0.12$ at Week 2, and for LTs, $R = -0.06$ at Week 1 and $R = -0.08$ at Week 2.

Discussion

LTs to irritants have been mainly collected using static olfactometry. More recently, various applications of static

Table 3 Log-transformed mean odor detection (ODT) and LTs and SD and corresponding geometric mean (Geo mean) values in ppm by time point and method ($n = 24$)

	Week 1		Week 2		Total	
	Mean (SD) log	Geo mean (ppm)	Mean (SD) log	Geo mean (ppm)	Mean (SD) log	Geo mean (ppm)
Static olfactometry						
ODT	0.54 (0.95)	3.45	0.28 (1.57)	1.89	0.41 (1.29)	2.56
LT	1.49 (0.53)	30.68	1.51 (0.57)	32.73	1.50 (0.54)	31.69
Total static olfactometry					0.95 (1.13)	9.0
Dynamic olfactometry						
ODT	0.53 (0.54)	3.42	0.30 (0.45)	2.00	0.42 (0.51)	2.62
LT	1.89 (0.45)	78.04	1.68 (0.65)	47.56	1.78 (0.63)	60.92
Total dynamic olfactometry					1.10 (0.89)	12.63

and dynamic olfactometry have been employed, such as in Shusterman et al. (2001, 2003), in which brief puffs of n-propanol vapor and blanks were conveyed from bottles to the nares at the time of sniffing; in Wise et al. (2005), lateralization of ammonia was determined by flowing ammonia into the nose using an olfactometer. In the present study, no significant differences between methods were observed. In case of the ODT, for both static and dynamic olfactometry, the average threshold was 2.6 ppm. In case of the LT, a difference in thresholds (M_{do} = 60.92 ppm vs. M_{so} = 31.69 ppm) did not reach statistical significance (P = 0.07).

Potential discrepancies between methods could arise out of a difference in psychophysical procedure (see Bliss et al. 1996). Whereas an up-and-down staircase procedure, where every next step is tailored to the individual's previous performance, is preferable when testing individuals, it is not viable when testing multiple subjects simultaneously, which is why an ascending method of limits procedure was our method of choice in the dynamic method. Interestingly, this did not lead to significant differences between methods in this study, although it may be partially responsible for the slightly lower LTs obtained with the static method.

Because ODTs, and presumably also LTs, tend to fluctuate over time within individuals due to differences in nasal patency, time of day, health condition, etc, we did not expect very high test–retest within-subject reliability. However, because these fluctuations are assumed to be random, we would expect the average of these assessments to be comparable in a similar group of subjects, thus resulting in comparable mean thresholds at different time points. This is in fact what we found: although test–retest reliability for both types of thresholds varied from low to high, on average there were no differences over time. Test–retest reliability from Week 1 to Week 2 was higher for the dynamic olfactometry method than for the static olfactometry method (e.g., R = 0.86 vs. R = 0.45 for the LTs in either method). Between methods, correlations were very low. In comparison, Shusterman et al. (2001) reported test–retest consistency of R = 0.60 for repeated assessments of LTs to n-propanol using static olfactometry in a study of n = 16, and R = 0.50 in a larger study of n = 60 (Shusterman et al. 2003). Using a static method, Frasnelli and Hummel (2005) reported significant correlations of R = 0.41 and R = 0.48 for LTs using linalool and menthol, respectively, over a period of 6–25 days. Van Thriel et al. (2006) reported reliabilities of R = 0.44 for ODTs and LTs for acetic acid assessed with static olfactometry.

The olfactometer employed in the present study differs in some important aspects from olfactometers previously described in the literature. The olfactometer employed here is used for determining odor thresholds following an established protocol (CEN 2003) in the context of odor regulations to outside emissions. Air was neither humidified to 80% relative humidity or higher nor was the air temperature heated to body temperature. Heating and humidification of the air are desirable to approach natural intranasal conditions, to prevent drying out of the nasal mucosa and mechanical stimulation (and thus irritation) of the trigeminal nerve by cold air (see Hummel et al. 2003). In our olfactometer, relative humidity and temperature were approximately 25% RH and 26 °C, respectively. By not "blasting" the air containing the stimulus into the nose, but allowing sniffing for only 2 s per trial, and maintaining 1-min breaks between trials, we tried to limit the above-mentioned effects as much as possible. Wise et al. (2004) compared the 2 types of olfactometry with carbon dioxide. Although they suspected that cooling and drying of the mucosa may have played a role when they used the simple olfactometer in which the air was not heated and humidified, they concluded that the agreement between the findings reached with either method was still strong. Our findings seem to support the conclusion that for certain applications, with the proper measures, reliable results can still be obtained.

With respect to the outcomes, the question could be raised whether residents and farmers may actually encounter exposures to ammonia vapor at the levels that were found to produce nasal irritation in the present study. Schiffman et al. (2005) established ammonia levels from a swine operation at diluted levels that could occur downwind from such operations to be 817 ppb, which is too low for most individuals to detect either the smell or irritation from ammonia. On the other hand, average concentrations of ammonia in swine houses (i.e., not downwind from the operation) have been reported to range from 5 to 18 ppm, with maximum concentrations of 43.7 ppm in sow buildings and in finishing barns of 59.8 ppm (Koerkamp et al. 1998, in Schiffman et al. 2005), whereas Zhang et al. (1998) reported a mean level of 26.0 ppm ammonia inside a swine grower/finisher room occupying 144 pigs. Compared with the results reached in this study, some of these exposures could in theory yield irritation in some individuals working in these environments; however, the degree of dilution that occurs once the emissions are mixed with outside air renders it extremely unlikely that residents would encounter concentrations capable of eliciting this effect.

In conclusion, both the static and dynamic method show remarkably similar averages within a population for detection thresholds for the odor and irritancy of ammonia. In comparison to the static method, over time, the dynamic method delivered more reliable and repeatable ODTs. Repeatability of LTs was acceptable to good for both methods. Individual thresholds, however, should not be compared across methods.

Which method is optimal depends on multiple factors. One such factor may be the chemical characteristics of the stimulus compound. For example, odorants with low vapor pressures are well maintained in containers but are more difficult to vaporize and may condense in equipment lines more readily when administered using dynamic olfactometry. Compounds with high vapor pressures, on the other hand, which readily partition into the gas phase under standard

pressure and temperature conditions, such as ammonia, are less suited to static olfactometry because they evaporate easily from the container during use and thus need to be prepared from stock frequently. For example, because we were able to measure vapor concentration in the same bottle at various times throughout the day using a handheld PID, we registered that the vapor concentrations of NH_3 inside the bottles did not return back to their original values by the time the next subject was tested, even though we kept bottles closed as much as possible. This feature alone could account for the lower test–retest reliability of this method when compared with the dynamic method. Loss of stimulus strength was not equal for all bottles, as this would have reflected in changes in intercept only over the course of a day. Rather, changes in both intercept and slope were observed, such that more vapor seemed to be lost from bottles containing lower concentrations than from bottles containing higher concentrations. This finding suggests that it is advisable to establish the decay in stimulus value over testing trials, and use this to correct calculated threshold values, when using highly evaporative compounds in static containers. However, our study revealed that dynamic olfactometry, not subjected to these problems, can be used for the assessment of chemosensory thresholds of highly evaporative stimuli.

Another relevant factor for determining which method is optimal is efficiency. Because 6 subjects can be tested simultaneously using the current setup for the dynamic method, and sample containers do not need to be prepared, testing could proceed faster. Depending on the cost of labor involved in sample preparation and testing, on the other hand, the static method may involve less expense and expertise. If mixtures of odors are to be tested that were sampled in the environment or are difficult to prepare in the laboratory, dynamic olfactometry is clearly to be preferred. If irritation assessment to complex outside emissions become more common, as is the case for indoor emissions, further development of reliable, quick, and cost-effective dynamic procedures are desirable.

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