Chem. Senses 35: 523–530, 2010
doi:10.1093/chemse/bjq046
Advance Access publication May 27, 2010

Use of a Calibration Gas Generator for Irritation Threshold Assessment and As Supplement of Dynamic Dilution Olfactometry

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Accepted April 20, 2010

Abstract

Human odor and mucosal membrane irritation thresholds are used as criteria for assessing air quality in occupational and environmental settings. Unfortunately, reported threshold values still differ by several orders of magnitude rendering most standard compilations of little practical utility. Thus, in view of the need to repeat odor threshold measurements with a reliable methodology, a new technical approach based on original equipment manufacturer integrated solutions is presented. To test applicability, a calibration gas generator was used to continuously generate a fixed odor vapor concentration. Different dilution steps were realized by coupling to a purchasable olfactometer. Comparison with the "standard," that is, odor stimulus supply via sample bags revealed good correspondence. As a second step, the calibration gas generator was used to generate rapid changes in stimulus concentration between consecutive trials. Irritation thresholds were measured with an ascending series of ammonia concentrations generated from an aqueous solution. The obtained thresholds lay within the range previously reported. The introduced technology enables quick and reliable odor stimulus generation and provides flexibility in choosing the optimal start concentration, the step-size between dilutions, and the range of stimulus concentrations. Errors from usage of rotameters or sample bags can be avoided.

Key words: calibration gas generator, mucosal membrane irritation, odor threshold, olfactometry

Introduction

Odor perception consists of 2 sensations, olfaction and mucosal membrane irritation, for example, burning, tingling, or prickling. At sufficient quality and intensity, odors can be perceived as hedonically unpleasant and annoying and cause emotional or somatic complaints, activity impairment, or complaint filing (Dalton 2003; van Thriel et al. 2006, 2008; Sucker et al. 2008). In order to prevent such adverse effects, odor detection or irritation thresholds are used as endpoints in a variety of settings, including occupational and environmental hygiene, indoor air quality, and control of consumer products.

Unfortunately, reported threshold values still differ by several orders of magnitude rendering most standard compilations of little practical utility (van Gemert 2003). Recent developments in olfactometric measurement techniques (Cain and Schmidt 2009; Schmidt and Cain 2010) and international standardization of odor measurement practices (CEN 2003; ASTM 2004) reveal that methodological shortcomings rather than interindividual differences in sensitivity or considerable day-to-day fluctuations within the same individuals are the major cause for the observed variations.

Today, a dynamic dilution olfactometer presenting ascending odorant concentrations seems to become the standard instrumentation for precise odor threshold measurement. Particularly, if odorants with high vapor pressures are tested, techniques employing static headspace dilution have difficulties in securing a stable and reliable stimulus delivery (Cain et al. 1992; Cometto-Muñiz et al. 2003). Testing ammonia, Smeets et al. (2007) obtained more reliable and repeatable odor detection thresholds as well as irritation thresholds using dynamic compared with static olfactometry. The substantial loss of stimulus strength in the 250-mL glass bottles used for static olfactometry was supposed to be one of the main reasons for poorer reliability. By testing vapor concentrations in the same bottle at various times throughout the day changes in both intercept and slope were noticed. More vapors were lost from bottles containing lower concentrations and vapor concentration inside the bottle did not return back to its original value by the time the next subject was tested (Smeets et al. 2007).

Due to substantial improvements in dynamic olfactometry, odor thresholds show lower and lower variation. As older studies found individual differences up to 5 orders of magnitude (Cain and Schmidt 2009), recent studies report individual differences to be in the range of 1 or 2 orders of magnitude, depending on the number of trials per concentration step and selection of subjects (Cometto-Muñiz and Abraham 2008; Cometto-Muñiz et al. 2008). Quick and efficient measurement of odor detection or irritation thresholds is possible, as up to 8 subjects can be tested simultaneously (Cain et al. 2007; Smeets et al. 2007). Reliability of measurements is assured by checking accuracy and reproducibility of stimulus presentation at concentrations of threshold and suprathreshold levels via gas chromatography before and during testing (Cain et al. 2007). Another approach to ensure reliability of measurements is to comply with instrumental accuracy and repeatability performance criteria as required by standards (CEN 2003; ASTM 2004).

In view of the need to repeat odor thresholds with sound methodology and advance archival databases as suggested by Cain and Schmidt (2009), some improvements are considered necessary in order to utilize the advantages of dynamic olfactometry for quick and reliable and cost-effective threshold measurements. The vapor deliver device (VDD)-8 used in the Cain laboratory (Schmidt and Cain 2010) is a rotameter-based dynamic dilution olfactometer. In the morning of a testing day, a fixed concentration of odor vapor is produced by means of a syringe pump and a heater, mixed with a feed stream of nitrogen, and stored in a vapor capacitor. At each of the 8 delivery stations, the respective dilution step is generated by using rotameters to modify the flow rate of the original odor/nitrogen vapor concentration when mixing with a steady background flow of air provided by a generative blower. Predilution can be achieved within the attenuator using rotameters again. Rotameters are extremely sensitive to downstream pressure variations that may result in errors in rotameter readings of up to 25%. Furthermore, rotameters are set manually by the operator, that is, another source of errors. In order to minimize errors due to changing the sighting of a float differently from one adjustment to another, the operator does not reset rotameters within an experiment. The VDD settings remain the same during a 7- to 8-h test day and even across several days of testing.

Most of the commercially available olfactometers are mass flow controller (MFC) based like the Olfaktomat used in the Netherlands or the Ac'scent olfactometer used in United States. The range of stimulus concentrations and the flexibility in dilution increase between dilution presentation levels is limited, depending on the construction characteristics. Furthermore, the MFC are susceptible to contamination buildup that can alter calibration and result in reduced performance. If the stimulus material is prepared in sample bags (e.g., Tedlar or Nalophan) or purchased in compressed gas cylinders, the generation of vaporous stimulus material from the neat compounds (liquid or gaseous) and the flexibility is limited to start with any form of stimuli. Some studies show that stimulus concentration in the sample bag decreases due to permeation or adsorption effects depending on bag material and storage time (Müller 2002; Trabue et al. 2006). Just as well the use of gas cylinders is not easy because a specific storage position is needed and storing quite a few cylinders can get cost-intensive and logistically complicated. Using *n*-butanol, the reference gas for olfactometry in the context of ambient air quality, steel bottles with a higher pressure than 60 bars and more than 60 ppm are not available. Furthermore, the temperature during transportation or storage of the gas cylinder is important, as *n*-butanol condenses at lower temperatures (<15 °C) at the inner surfaces of the bottle. If condensation happened, it would not be possible to mobilize *n*-butanol into the gas phase again without considerable expenditure. The original concentration cannot be guaranteed any more. Thus, temporal variations while using gas cylinders might interfere with appropriate olfactometric measurements.

Exploring odor thresholds without a clue of the proper concentration range, a reasonable source concentration has to be generated for realization of appropriate dilution steps in the olfactometer. Hence, purchasing a gas cylinder with the right source concentration might not be easy.

The current paper presents a new technical approach based on original equipment manufacturer integrated solutions. A calibration gas generator is used to generate odor stimuli quickly and reliably, to choose the optimal start concentration, and to realize reasonable dilution steps. This device has already been used successfully for the generation of volatile inorganic acids (hydrogen chloride, nitric acid) within the scope of round robin tests (Breuer et al. 2005) or calibration of Fourier transform infrared spectroscopy and other multicomponent infrared analyzer applications (Chikhliwala et al. 2009; Vautz and Schmäh 2009). In order to handle highly volatile odorants, aqueous solutions with known concentrations bottled in an elastic transfusion bag are used instead of pure odorants. Exemplarily, the use of the calibration gas generator is shown at first coupled to a dynamic dilution device and then in combination with an irritation threshold measurement device.

Materials and methods

Apparatus and stimulus material

To generate precise odor vapor mixtures, a calibration gas generator (HovaCal 321/2-SP, IAS GmbH) was used (Figure 1). Odor vapor can be generated from neat material as well as aqueous solutions. The calibration gas generator works either with 2 MFC which steer the required amounts of odor vapor and carrier gas or with an additional evaporator unit which transfers liquid media with 2 computerized high precision syringe pumps and mass flow controlled compressed air in the gas phase. The 2 independent syringe pumps deliver liquids in a precise, steady flow without variation. Although one syringe doses the liquid, the second syringe sucks in liquid. A continuous crossing of one syringe to the other is achieved by positively controlled rotary slide valves. The capacity of the 250-μL syringe pumps is in the range of 8.0 μL/min to 1.5 mL/min. Using other syringe sizes, a greater dosage range from 1 μL/min to ~10 mL/min can be covered. The evaporator allows the use of MFCs with a maximum flow rate of 10 L/min (MFC No. 1 for carrier gas). The additionally build in MFC works with a flow rate of 2.5–50.0 L/min (MFC No. 2). Possible temperatures of the evaporator are in the range of 20 °C (room temperature) to 200 °C. The medium gets into contact with Teflon, stainless steel, polychlorotrifluoroethylene (Kel-F), and glass. With the Hova-Cal 321/2-SP, a concentration range of 1:1000 ppm is possible. Every inert solvent with a boiling point below 200 °C is suitable for use in the calibration gas generator. Standard solutions in a wide concentration range are available for components like ammonia for example. The reagents have nearly infinite stability over time, and no gas cylinders are necessary.

To generate an ammonia concentration of 500 ppm, a 2 molar aqueous solution bottled in a transfusion bag (IAS GmbH) was used in order to avoid concentration loss by degassing. The ammonia solution was continuously pumped (flow rate: 0.0558 mL/min) into the evaporator, vaporized at 130 °C, and mixed with compressed air at a flow rate of 5 L/min. Compressed air, which served as carrier gas as well as clean air (odorless stimulus), was generated from an oil-free air compressor (DE 50/254, FIAC Air Compressors). Water vapor was eliminated by an integrated absorption dryer. Furthermore, active coal and a fine (1 μm of pore width) as well as a finest filter (0.01 μm of pore width) were used to eliminate odorous contaminants and particles.

Steady odor stimulus generation

In order to show the possibilities that emerges from using the calibration gas generator as supplement of dynamic dilution olfactometry, the calibration gas generator was coupled to an air dilution olfactometer (TO8, ECOMA GmbH) built according to the European Standard EN13725 (CEN 2003) for the evaluation of odor samples by dynamic olfactometry. It specifies that the airflow rate emanating from individual panelist sniff ports must be at least 20 L/min and that the air velocity at the opening must be between 0.2 m/s and 0.5 m/s. In principle, the olfactometer is a dilution system, where a sample of odorous air is diluted with clean air. The dilution ratios span a range of 1:2.5–1:64 000. Two gas jet pumps are operating with clean air, for example, synthetic air from steel cylinders via pressure reducing valve or with compressed oil-free air processed in a filter unit. Usually, the odor sample is sucked from a sample bag through the dilution system. A stainless steel valve fitted with nozzles with different diameters adjusts the concentration of the odorous air. In the gas jet pumps, the odorous air is mixed intensively thoroughly with the clean air. The mixture flows via the rotary slide valve to the sniffing ports. Up to 4 panelists judge the diluted sample at the same time. A binary (2-fold) dilution series of ascending stimuli can be presented. Actual dilution steps are based on an annual calibration using propane in nitrogen as tracer gas.

In our experimental setup, the odorant sample was sucked into the olfactometer from a sample bag or directly from the calibration gas generator (Figure 2).

The odor stimulus was passed from the calibration gas generator into a vapor capacitor (1-L glass bottle) in excess. The gas excess was transported in an exhaust duct with a large tube (20-mm outer diameter), keeping the backpressure in the bottle negligibly small. All screw connections of the glass bottle were provided with Teflon fittings. Teflon tubes (6-mm outer, 4-mm inner diameter) connected the vapor capacitor with the calibration gas generator and the olfactometer. The olfactometer sucked the required amount of the odor stimulus out of the vapor capacitor. Adsorption effects were compensated by continuous gas production, resulting in a steady state of the odor concentration with no significant changes over time. In order to verify this, we measured exit concentrations of ammonia at the sniffing ports of the olfactometer using a calibrated photoacoustic detector (Field Gas-Monitor 1412, Innova AirTech Instruments). The photoacoustic detector measured gas concentrations by recording sound waves that were emitted by stimulation of

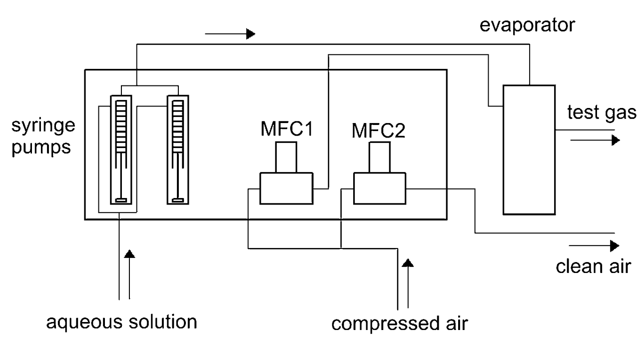

Figure 1 Sketch of the functional principle of the calibration gas generator.

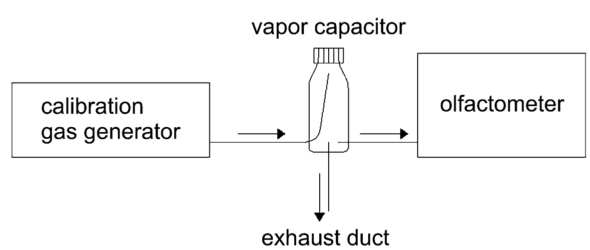

Figure 2 Sketch of the connection between calibration gas generator and olfactometer.

modulated infrared radiation of the analyte. A gas cylinder with 500 ppm ammonia in nitrogen (Westfalen AG) was used to calibrate the detector, taking into account water vapor. Measurement uncertainty was in the range of 1.5% at 100 ppm. To compare the use of the calibration gas generator and the use of a sample bag connected to the olfactometer, a sample bag with 500 ppm ammonia was prepared using a 10-L Tedlar bag with a stainless steel fitting (SKC Inc.) and a gas cylinder with 500 ppm ammonia in nitrogen (Westfalen AG).

Continuous odor stimulus generation

To use the calibration gas generator in connection with a device for odor irritation threshold assessment, different ammonia concentrations have to be generated quickly and reliably. Therefore, different amounts of ammonia of the 2 molar aqueous solution were evaporated in the heating block at a temperature of 130 °C and mixed with compressed air as carrier gas, taking into account water vapor volumes. The odor irritation threshold assessment method used here is based on the principle that humans cannot identify to which side of the nose a pure olfactory stimulus (e.g., phenyl ethyl alcohol, vanillin, and hydrogen sulfide) is presented but can do so readily when the stimulus elicits a trigeminal response (Kobal et al. 1989). Therefore, this irritation threshold is also referred to as the lateralization threshold. Because the assessment of the lateralization threshold requires the separate presentation of one airstream to the left nostril and the other to the right, the calibration gas generator produced 2 mass flow controlled gas streams in parallel, one with ammonia and one with clean air as odorless stimulus (Figure 3).

Two gas capacitors (250-mL glass bottles) were used to present sufficient amounts of ammonia and clean air to the panelist's nostrils. The calibration gas generator and the flasks were connected by Teflon tubes (6-mm outer, 4-mm inner diameter). Each of the flasks was provided with a Teflon tube (30-cm long, 4-mm outer, and 2-mm inner diameter) ending into a closely fitting Teflon nosepiece (12-mm diameter). Although no sniffing was performed, the gas excess was transported in an exhaust duct with a large tube (8-mm outer diameter) in order to prevent large stimulus amounts emitting through the nosepieces.

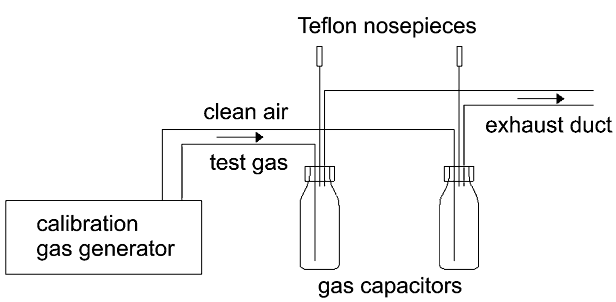

Figure 3 Sketch of the connection between calibration gas generator and irritation threshold measurement device.

To examine the temporal resolution of the stimulus-delivering device, the photoacoustic method was used. The delay for switching between one to the next higher dilution step is due to the volume of air that must be replaced within the vapor capacitor (250 mL). To find the adequate flow rate at which air is replaced, 4 L/min and 7 L/min were compared. We measured the time to switch from 500 ppm ammonia concentration to clean air at the outlet of the vapor capacitor.

Subjects

Four healthy, males, nonasthmatic volunteers, aged between 37 and 50 years, were tested in order to show that the calibration gas generator can be used to produce odor stimuli for the assessment of lateralization thresholds. The Medical Ethics Committee of the Ruhr-University Bochum approved the protocol for the study. Subjects gave written, informed consent to participate.

Procedure

Using the calibration gas generator in connection with the odor irritation threshold assessment device as described above, airflow of the ammonia stream or clean air within the gas capacitors was maintained at 8 L/min. Only minimal effort was needed to inhale the stimulus flowing into the nose. This method of active sniffing was preferred in order to prevent that subjects respond to the flow rate of the stimulus.

During lateralization threshold trials, the subjects sniffed from both nosepieces at the same time. Therefore, the test leader gave one nosepiece to the subject's right hand which had to be placed into the right nostril and the other nosepiece to the subject's left hand which had to be placed into the left nostril. Subjects were blindfolded wearing blackened eyeglasses. Otherwise the subject could identify the nosepiece that contains the stimulus. Subjects were allowed one sniff per evaluation trial with individually chosen duration. They were then asked to indicate if they felt the stimulus in the left or right nostril and in addition how certain they are. Three options were given: 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, lateralization thresholds were collected using the ascending method of limits, with up to 6 stimuli presented in a series. After each stimulus, there was a break of 30 s to allow the calibration gas generator 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 he had correctly detected the nostril where the stimulus was presented for 2 concentrations in a row with certainty. The individual threshold was calculated averaging the first correctly detected and the last not correctly detected concentration (geometric mean). Between 2 series, there was a break of at least

1 min. For reliability, subjects were tested repeatedly on 3 consecutive test days, with 4 stimulus series on every test day. On each of the 3 test days, the first stimulus series started with an ammonia concentration of 100 ppm, followed by the next concentration step increased by 50 ppm. Increase of concentration was maintained at 50 ppm, but the starting points of the next 3 series varied (day #1: 70, 80, 90 ppm; day #2: 60, 70, 80 ppm; day #3: 70, 80, 90 ppm). For every subject, the 12 thresholds were averaged (geometric mean) to obtain the individual irritation threshold.

All test sessions were conducted in the laboratory at IPA in Bochum, Germany. At the beginning, the subjects were familiarized with the measurement procedure while practicing one round of threshold detection.

Results

Comparison of the concentrations of the purchased stimulus gas and stimulus gas generated by calibration gas generator

The photoacoustic detector was calibrated with 500 ppm ammonia in nitrogen (single-point calibration) from a Tedlar bag taking into account the content of water vapor. Previously, the built-in filter for water vapor compensation was calibrated with a steam concentration of 10 000 ppm. This compensation is necessary because vaporization of aqueous ammonia solutions produces large amounts of steam that interferes with the measurement of the ammonia concentration. Ammonia concentration in the sample bag was measured 10 times, revealing concentrations of 503.7 ppm ± 0.1% of the purchased ammonia and 503.9 ppm ± 0.4% of the generated ammonia.

Comparison of sample bag and calibration gas generator connected to an olfactometer

The above described air dilution olfactometer was used to investigate the functionality and validity of the calibration gas generator in combination with a dynamic dilution device for odor detection threshold assessment. The calibration gas generator was compared with the use of sample bags, both providing an ammonia concentration of 500 ppm ammonia. The calibration gas generator produced a continuous flow of ammonia concentration that did not change significantly over time. To verify this, ammonia concentration was measured 3 times at the sniffing ports for every dilution step using the photoacoustic method. The dilutions steps 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128 were examined. Dilution steps lower than 1:128 with ammonia concentrations around 1 ppm and below could not be registered reliably (measurement error around ± 0.5 ppm) and were therefore not taken into consideration. The calculated ammonia concentrations at the sniffing ports are based upon an ISO certified calibration (CEN 2003) carried out in September 2008 over the full range of dilutions steps.

Table 1 shows the results of these measurements. The test gas was propane in nitrogen (20.000 ppm). The specified recovery rates at each dilution step were used to calculate the expected ammonia concentrations at the sniffing ports shown in Table 2.

The standard deviation (SD) for the 3 measurements for each dilution step was in the range of 1 ppm detection limit of the photoacoustic detector. The determination coefficient r^2 expressing the correspondence between the sample bag and the calibration gas generator values is high (≥0.99). The 2 right columns of Table 2 show that the difference between the calculated and the measured ammonia concentration is much higher than between the measured ammonia concentrations delivered by the sample bag and the calibration gas generator. The absolute drifts increase to lower concentrations. A possible reason is an absorption effect of ammonia in the modules of the olfactometer that raises with increasing dilution steps.

Temporal resolution of the irritation threshold measurement device

To examine the temporal resolution of the irritation threshold measurement device, we measured the time required to switch from 500 ppm ammonia concentration to clean air at the outlet of the vapor capacitor. The delay for switching is due to the volume of air that must be replaced within the vapor capacitor (250 mL) and the flow rate at which air is replaced. The first step of the measurement procedure was to flush the vapor capacitor with 500 ppm ammonia for 1 min. Then the injection of the ammonia solution into the evaporator stopped, and from this moment on, the vapor capacitor was flushed with compressed odorless air only. To find the adequate flow rate, 4 L/min and 7 L/min were compared. After different time intervals (10, 15, 20, and 30 s), the compressed airstream stopped and the residual amount of ammonia concentration left in the vapor capacitor was measured with the photoacoustic detector. Within 30 s, the ammonia concentration of 500 ppm in the 250-mL glass bottle was reduced below the detectable limit and replaced with clean air, if airflow was maintained at 7 L/min (Figure 4).

Table 1 Measurement results of the ISO certified calibration with test gas (20.000 ppm propane in nitrogen)

Dilution step	Desired value (ppm)	Actual value (ppm)	Recovery rate (%)[a]
1:4	5000	4247.0	84.9
1:8	2500	2232.5	89.3
1:16	1250	1224.5	98.0
1:32	625	604.5	96.7
1:64	312.5	311.4	99.7
1:128	156.3	157.0	100.5

[a]Recovery rate (%) = 100/desired value × actual value.

Table 2 Measured ammonia concentrations [c] per dilution step for dynamic olfactometry (ppm), drift between odor stimulus supply via sample bag (standard), and calibration gas generator to calculated concentrations

Dilution step	Expected [c]	Calculated [c][a]	Measured [c]		Drift[d]	
			Sample bag[b]	Gas generator[c]	Sample bag[b]	Gas generator[c]
1:4	125.0	106.2	113.8 ± 0.70	110.4 ± 0.67	−7.6	−4.2
1:8	62.5	55.8	57.4 ± 0.15	54.0 ± 0.06	−1.6	1.8
1:16	31.3	30.7	29.1 ± 0.06	27.5 ± 0.20	1.6	3.2
1:32	15.6	15.1	13.6 ± 0.06	12.1 ± 0.11	1.5	3.0
1:64	7.8	7.8	5.7 ± 0.17	4.6 ± 0.15	2.1	3.2
1:128	3.9	3.9	1.3 ± 0.01	1.0 ± 0.10	2.6	2.9

[a]Calculated, based on measurement results of the ISO certified calibration.
[b]Measured, based on 500 ppm from the sample bag.
[c]Measured, based on 500 ppm from the calibration gas generator.
[d]Drift = calculated [c] − measured [c].

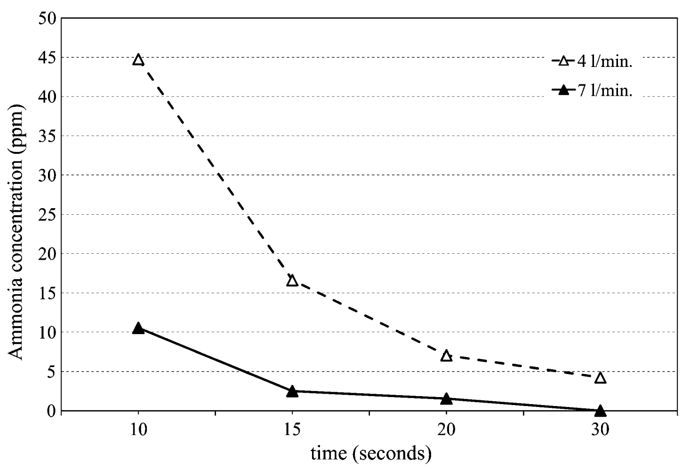

Figure 4 Ammonia concentrations measured at different time intervals, starting with 500 ppm ammonia stored in a 250-mL bottle, comparing carrier gas flow rates maintained at 7 L/min (filled triangle, plain line) versus 4 L/min (empty triangle, dotted line).

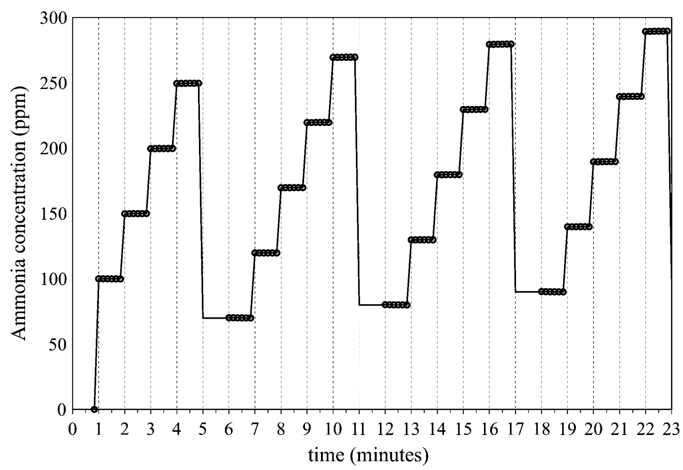

Figure 5 Presentation of 4 series of ascending ammonia concentrations within one test session on test day #1, analytical measurements (6 circles); lateralization trials began 30 s after the generation of the new dilution step started.

Ammonia irritation threshold measurement

In order to test the feasibility of the usage of the calibration gas generator for odor irritation threshold assessment, the lateralization threshold was measured in 4 subjects. Figure 5 shows as examples the presentation of 4 series of ascending ammonia concentrations within one test session on testing day #1.

The mean log-transformed threshold values and SDs as well as geometric mean thresholds of 4 subjects are displayed in Table 3.

Table 3 Log-transformed mean irritation (lateralization) thresholds and SD and corresponding geometric mean (Geo mean) values in ppm

Number of subjects	Mean (SD) log	Geo mean (ppm)
1	2.20 (0.12)	157.1
2	2.22 (0.18)	166.7
3	2.32 (0.08)	207.7
4	2.04 (0.15)	109.1
Total	2.19 (0.13)	155.2

Discussion

Humans tend to believe that their noses will protect them from inhaling dangerous substances, thinking "if I can't smell it, it's not hazardous to me." In a variety of settings, for example, occupational and environmental hygiene or indoor air quality, odor perception is used as an indicator of exposure to chemical substances that might be harmful. In the literature (e.g., van Gemert 2003) or in guidelines/regulations, both odor thresholds and adverse effect levels for many substances can be found. Even though exposure measurements have to be taken and consequences considered, in many cases the odor provides the first information. Hence, compiled odor threshold values matter and, in the main, they appear to be wrong (Cain and Schmidt 2009).

The usability of a calibration gas generator was tested not only in order to repeat odor thresholds but also to assess irritation thresholds with sound methodology. We approached this aim by combining the advantages of dynamic dilution olfactometry with the calibration gas generator. We were able to show that the calibration gas generator can be connected directly to an air dilution olfactometer for assessing odor threshold. Odor stimulus supply via calibration gas generator compared with the "standard," that is, odor stimulus supply via sample bags, revealed good correspondence. In contrast, the correspondence between the calculated and the effectively measured ammonia concentration was less sufficient, even though the measurement error at each dilution step should be less than 5% according to the calibration protocol. This indicates that the use of commercially available olfactometer for the reliable measurement of odor and irritation thresholds in the low concentration range is limited due to the measurement error at the higher dilution steps. Nevertheless, the advantages of the calibration gas generator are evident. The calibration gas generator can be used for the quick and reliable generation of vaporous stimulus material. If the airflow is maintained at a minimum of 7 L/min, the concentration of the next dilution step is established within 30 s. Even the use of highly volatile odorants is possible if aqueous solutions with known concentrations are bottled in an elastic transfusion bag. Moreover, the calibration gas generator provides flexibility in choosing the optimal start concentration, the step-size between dilution presentation levels, and the range of stimulus concentrations presented within one test session. All disadvantages like purchasing and storing test gases in compressed gas cylinders or in sample bags and error sources like surface effects can be avoided.

Connected to an irritation threshold measurement device, the calibration gas generator can be used for assessing irritation thresholds. The suitability was demonstrated by assessing lateralization thresholds for ammonia. The obtained lateralization thresholds in the range of 109–208 ppm lay within the range of irritation thresholds previously reported. Mean irritation threshold values of around 37–67 ppm (Wise et al. 2005) and 32–61 ppm (Smeets et al. 2007) as well as much higher median values at 314 ppm (van Thriel et al. 2006) have been reported. A range of 162–189 ppm was measured in the most recent study on nasal irritation threshold values for ammonia, determined with air dilution olfactometry (Petrova et al. 2008).

Further development is necessary to optimize the proposed methodology. In order to advance cost-effectiveness, the connection between the calibration gas generator and the stimulus-delivering device could be modularly structured, including several exchangeable interfaces for assessing odor or irritation thresholds of more than one subject simultaneously. A procedure should be implemented to monitor the performance in accordance with the CEN (2003) instrumental accuracy and repeatability criteria. For this purpose, a more sensitive analytical instrument with sensitivity to a low ppb level should be used. Furthermore, sensitivity to a low ppb level could be tested with sulfur hexafluoride (SF_6). The concentration of SF_6, often used as tracer gas, can be measured with satisfactory accuracy at very low ppb concentrations, and the Earth's atmosphere has a negligible concentration of SF_6. Heating and humidification of the air are desirable to approach natural intranasal conditions to prevent mechanical stimulation and drying out of the nasal mucosa (Hummel et al. 2003). Furthermore, stimulus duration should be controlled, as irritation threshold decreases with stimulus duration (Wise et al. 2005). Standardization of threshold assessment procedures using a calibration gas generator will allow distinct practitioners to generate nearly identical stimuli, thus enabling better comparison of results across laboratories and across time. Further studies with single substances or mixtures will show how much thresholds vary interindividually as it is now possible to separate this random variability from the systematic variability due to methodological shortcomings.

Acknowledgements

The authors would like to thank all participants and are also grateful to Dr D. Breuer (BGIA—Institute for Occupational Safety and Health, Germany), Dr H.H. Fricke (IGF—Research Institute for Hazardous Substances, Germany), and Dr M. Schmäh (IAS GmbH, Germany) for consulting and technical support.

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