Article

Additivity between Key Odorants in Pig House Air

Michael Jørgen Hansen [1,*], **Anders Peter S. Adamsen** [1,2], **Chuandong Wu** [3] **and Anders Feilberg** [1]

[1] Department of Biological and Chemical Engineering, Aarhus University, 8000 Aarhus, Denmark; apa@bce.au.dk (A.P.S.A.); af@bce.au.dk (A.F.)

[2] SEGES, Danish Pig Research Centre, 1609 Copenhagen, Denmark

[3] School of Chemistry and Biological Engineering, University of Science and Technology, Beijing 100083, China; wuchuandong@ustb.edu.cn

[*] Correspondence: michaelj.hansen@bce.au.dk; Tel.: +45-21622710

Abstract: The verification of odor abatement technologies for livestock production based on chemical odorants requires a method for conversion into an odor value that reflects the significance of the individual odorants. The aim of the present study was to compare the SOAV method (Sum of Odor Activity Values) with the odor detection threshold measured by olfactometry and to investigate the assumption of additivity. Synthetic pig house air with odorants at realistic concentration levels was used in the study (hydrogen sulfide, methanethiol, trimethylamine, butanoic acid, and 4-methylphenol). An olfactometer with only PTFE in contact with sample air was used to estimate odor threshold values (OTVs) and the odor detection threshold for samples with two to five odorants. The results show a good correlation ($R^2 = 0.88$) between SOAV estimated based on the OTVs for panelists in the present study and values found in the literature. For the majority of the samples, the ratio between the odor detection threshold and SOAV was not significantly different from one, which indicates that the OAV for individual odorants in a mixture can be considered additive. In conclusion, the assumption of additivity between odorants measured in pig house air seems reasonable, but the strength of the method is determined by the OTV data used.

Keywords: odor; odorants; SOAV; OTV

1. Introduction

The verification of odor abatement technologies for animal houses is normally based on dynamic olfactometry, where samples are collected in bags and transported to a laboratory for threshold measurements by human panelists within 30 h [1]. This method has some drawbacks, such as the impaired storage stability of odorants in bags [2–5], the low recovery of odorants in olfactometers [6,7] and high variability between panelists [8,9]. The chemical measurement of odorants is an alternative method with a lower variability and less influence on the odorants from the sampling equipment, and if on-line methods are applied, a high time resolution is achieved [10–12]. However, the chemical measurement of odorants requires a conversion into a theoretical odor concentration that accounts for the contributions of the individual odorants in a mixture. The sum of odor activity values (SOAV), defined as the summation of concentration/odor threshold ratios, is often used to convert concentrations of odorants into a theoretical odor concentration. This approach assumes that the OAV for the individual odorants in a mixture is additive. Some previous studies have compared the SOAV method with the odor detection threshold and have demonstrated additivity between odorants [13–15]. Odor threshold values (OTVs) have been applied in a few studies related to livestock production to evaluate the significance of odorants found in the air matrix [16–18], but the assumption of additivity has to our knowledge not been investigated for this type of air matrix. There are also more advanced conversion methods such as the sums of odor intensity (SOI) or the equivalent odor concentration (EOC), where both the odor threshold and the sensitivity of the odor perception

defined as the slope of the Weber–Fechner law are included [19]. However, Weber–Fechner law data required for these methods are relatively scarce. OTV data are available in the literature, and although there is variation between measured OTVs [20–26], the SOAV is a simple method that can be applied in the short term, and it is therefore relevant to investigate the assumption of additivity.

Air from livestock houses contains a large number of odorants, but only a limited number of odorants may account for the majority of the odor perception [10]. Odorants in air from pig houses were used as the case in the present study. Based on previous studies concerned with concentration levels of odorants [10–12] and a recent evaluation of OTVs for pig house air [23], the five most dominant odorants were selected: hydrogen sulfide (H2S), methanethiol (MT), trimethylamine (TMA), butanoic acid (BA) and 4-methylphenol (4MP). A synthetic odor mixture containing these five odorants at realistic concentration levels was used in the study. The aim of the present study was to compare the SOAV method (Sum of Odor Activity Values) with the odor detection threshold measured by olfactometry and to investigate the assumption of additivity. It was concluded that the assumption of additivity for the five odorants included in the present study is reasonable, but the strength of the method is dependent on the OTV input data.

2. Materials and Methods

2.1. Gas Dilution System

H2S, MT, and TMA were introduced to the gas dilution system from pressurized gas cylinders (AGA, Copenhagen, Denmark). BA was generated from a liquid calibration unit (Ionicon Analytik GmbH, Innsbruck, Austria) and 4MP was generated from a permeation tube (VICI Metronics, Inc., Houston, TX, USA) using a permeation oven (Dynacalibrator model 150, VICI Metronics Inc.), see Figure 1. The odorant mixtures were diluted with atmospheric air purified by a Supelpure HC filter (Supelco, Bellefonte, PA, USA). Mass flow controllers (Bronkhorst, The Netherlands) controlled the flow of the dilution air and odorants. The gas dilution system (reduction valve/permeation oven, mass flow controllers, and tubing) was allowed to equilibrate for at least two hours before the measurements were carried out. All tubes and fittings in the gas dilution system were made of PTFE.
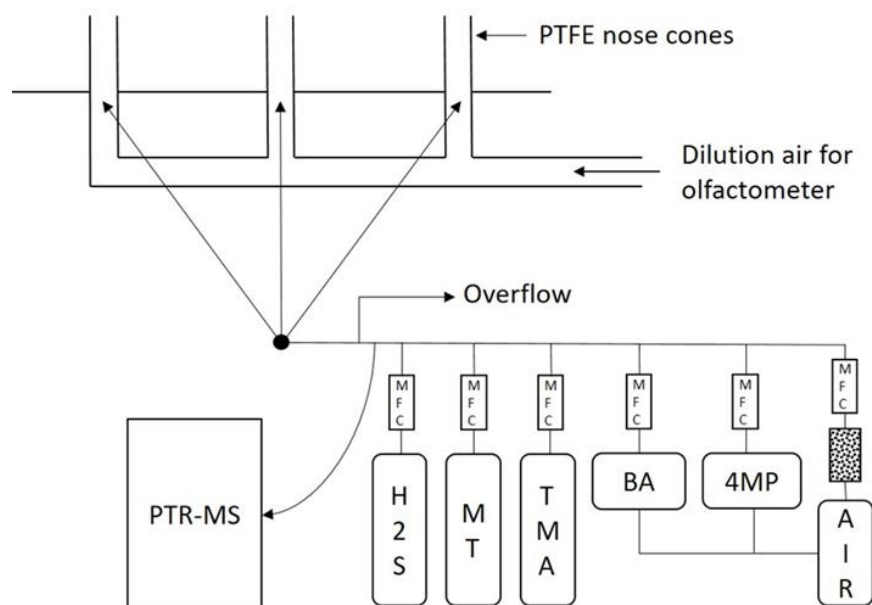

Figure 1. Schematic drawing of gas dilution system and connection to the olfactometer. H2S: Hydrogen sulfide; MT: methanethiol; TMA: trimethylamine; BA: butanoic acid; 4MP: 4-methylphenol; PTR-MS: Proton-Transfer-Reaction Mass Spectrometry; MFC: mass flow controller.

2.2. Analytical Methods

A custom-made glass olfactometer (Eurofins Denmark, Galten, Denmark) was used to estimate the dilution to threshold. The olfactometer was based on the two-alternative forced-choice method (2AFC) and was designed for three panelists at a time. The olfactometer provided a constant flow of ca. 20 L min^{-1} in each nose cone and the presentation time at each dilution step was set at maximum 15 s. The nose cones in the olfactometer were made of PTFE, since it has previously been shown that PTFE only has a very limited effect on the recovery of odorants in an olfactometer [7]. Each nose cone was connected to the gas dilution system with 1/8″ PTFE tubes. A needle valve made of PTFE (Bohlender GmbH, Grünsfeld, Germany) was used to adjust the required flow of the gas mixture to the nose cones and the flow was measured prior to each presentation using a calibrated flowmeter (Agilent Technologies, Ballerup, Denmark). The odorant mixtures were diluted in descending order from ca. 18.000 to 40 times dilution with a step factor of 1.5.

High sensitivity Proton-Transfer-Reaction Mass Spectrometry (HS-PTR-MS Ionicon Analytik GmbH, Innsbruck, Austria) was used to measure concentrations of odorants in the gas dilution system. PTR-MS is based on chemical ionization of compounds with protonated water (H_3O^+) and detection in a quadrupole mass spectrometer. The principle of PTR-MS has been described in detail in a previous study [27]. Standard drift tube conditions were applied with a pressure between 2.1 and 2.2 mbar, a voltage of 600 V and a temperature of 75 °C. The inlet temperature was set at 75 °C. PTR-MS was operated in single ion mode with a dwell time at one second. The mass-specific transmission factors were checked before the measurements with a mixture of eight compounds between *m/z* 79–237 (AGA, Copenhagen, Denmark). Reaction rate constants between odorants and protonated water were applied for MT, TMA, BA, and 4MP [28] and the humidity dependency of H2S was corrected with a calibration gas (AGA, Copenhagen, Denmark) according to a previously described method [10].

2.3. Experimental Setup

A group of eight panelists from the odor laboratory at Eurofins Denmark was used in the study. All panelists were selected based on the criteria of the European standard for olfactometry [1]. Based on previous studies with odorants in air from pig houses [10–12], a gas mixture was designed with similar concentration levels (see Table 1). The odor detection threshold was measured for individual odorants and for all combinations, in total 26 combinations containing two to five odorants. Each sample was measured twice for all panelists. During each measurement, the actual odorant concentrations were measured in the gas mixture prior to dilution in the olfactometer. Four of the samples including BA were not successfully measured due to technical problems with the liquid calibration unit. Furthermore, the threshold measurements for the sample with only 4MP and the mixture with all five odorants were repeated twice to confirm the results.

Table 1. Average odorant concentration in gas mixture, mean ± standard deviation (SD).

Compound [1]	H2S [2]	MT	TMA	BA	4MP
m/z	35	49	60	89 + 71	109
Reaction rate constant [28], cm^3 molecule^{-1} s^{-1}	-	1.9×10^{-9}	1.58×10^{-9}	2.11×10^{-9}	2.32×10^{-9}
Concentration, ppb$_v$	319 ± 21	14 ± 1	33 ± 1	88 ± 9	11 ± 2

[1] H2S: Hydrogen sulfide; MT: methanethiol; TMA: trimethylamine; BA: butanoic acid; 4MP: 4-methylphenol.
[2] Humidity dependency of H2S was corrected with a calibration gas.

2.4. Data Analysis

The OTVs for individual odorants were estimated as the geometric mean of the individual responses by the panelists. SOAV was estimated as the summation of odor-

ant concentration relative to the odor threshold value (OTV) for individual odorants (Equation (1)).

$$SOAV = \sum [odorant]/OTV \qquad (1)$$

The test for additivity was based on the ratio between the measured odor detection threshold for the individual panelists and SOAV (Equation (2)). A ratio at one indicates that the interaction is additive, a value below one indicates that there is a smaller degree of cooperation between odorants, and a value above 1 indicates that there is a higher degree of cooperation. A *t*-test was used to investigate if the average ratio for the panelists was significantly different from one. The level of significance was defined as a *p*-value below 0.05. A qq-plot was used to check the assumption of a normal distribution, and for all measurements a normal distribution was applicable.

$$Ratio = odor\ detection\ threshold/SOAV \qquad (2)$$

The odorant threshold level for samples with two to five odorants was estimated as the odorant concentration at the odor detection threshold level divided by the OTV (Equation (3)). A value below one means that the odorant concentration is at a subthreshold level, and a value above one means that it is at a suprathreshold level.

$$Odorant\ threshold\ level = ([odorant]/odor\ detection\ threshold)/OTV \qquad (3)$$

3. Results

3.1. Odor Threshold Values for Odorants

The measured OTVs for the individual odorants included in the present study and the values from the literature are shown in Table 2. The measured OTVs for H2S, TMA, BA and 4MP were two to four times lower than the reported literature values, whereas the OTV for MT was slightly higher. The measured OTV for 4MP was only based on four of the panelists since the other panelists were unable to detect the odorant within the available dilution steps. The threshold estimate for 4MP was repeated at two different days with the same result. In Figure 2, the correlation between SOAV based on measured OTVs and literature values shows a good correlation ($R^2 = 0.88$).

Table 2. Measured and literature values for odor threshold values (OTVs; ppb$_v$) [min; max].

Compound [1]	H2S	MT	TMA	BA	4MP
Measured OTV	0.2 [0.06; 1]	0.04 [0.01; 0.1]	0.02 [0.01; 0.07]	0.1 [0.02; 0.4]	0.006 [0.003; 0.03]
Literature [23] OTV	0.8 [0.4; 3]	0.03 [0.02; 0.07]	0.08 [0.03; 0.2]	0.2 [0.1; 0.8]	0.02 [0.005; 0.05]

[1] H2S: Hydrogen sulfide; MT: methanethiol; TMA: trimethylamine; BA: butanoic acid; 4MP: 4-methylphenol.

3.2. Additivity between Odorants

The assumption of the SOAV method is that the OAVs for the individual odorants are additive. In order to test this assumption, the ratio between the odor detection threshold measured by olfactometry and SOAV was calculated. In Figure 3, the ratios are shown for the samples with two to five odorants. The results show that, for all samples, the ratio was within 0.6 to 2.3 and with a mean of 1.2. In 17 out of 23 samples, the ratio was not significantly (*p* > 0.05) different from one, which indicates that the effect was additive. For five of the samples, the ratio was significantly (*p* < 0.05) higher than one, which indicates that there was a higher degree of cooperation between the odorants than expected based on SOAV. For one of the samples, the ratio was significantly (*p* < 0.05) lower than one, which indicates that there was a lower degree of cooperation between the odorants than expected based on SOAV. In Figure 4, the odorant threshold level is shown for samples with two to five odorants. Except for 4MP in samples with two and three odorants, the odorant threshold level was below one in all other cases, meaning that the odorants were

at subthreshold level at odor detection. Furthermore, the odorant threshold level and the variation in data decreased as the number of odorants increased.
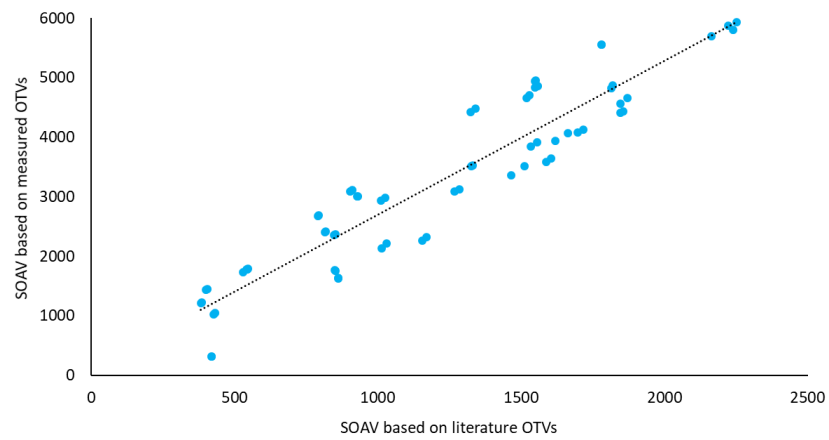

Figure 2. Correlation between sums of odor activity values (SOAV) based on measured and literature odor threshold values (OTVs) [23]. $SOAV_{measured} = 2.59\ SOAV_{literature} + 107$, $R^2 = 0.88$.
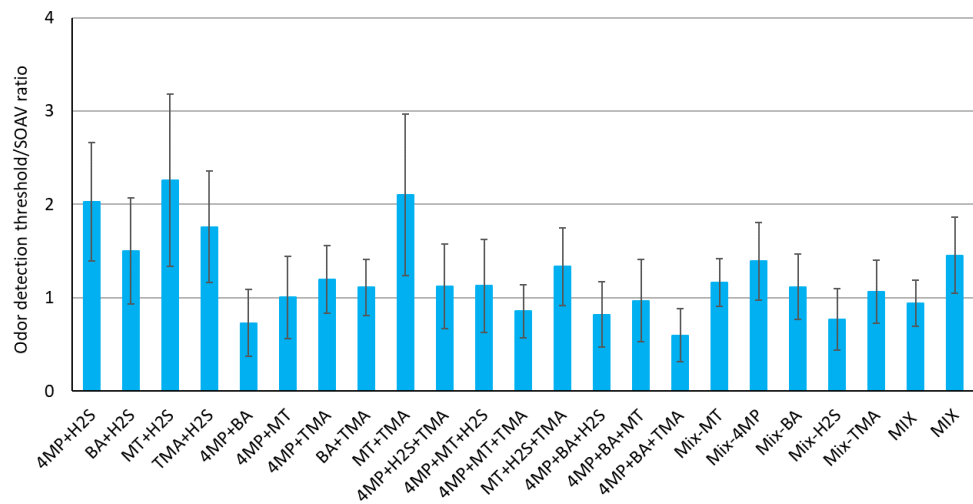

Figure 3. Odor detection threshold/SOAV ratio with 95% confidence intervals. H2S: Hydrogen sulfide; MT: methanethiol; TMA: trimethylamine; BA: butanoic acid; 4MP: 4-methylphenol.
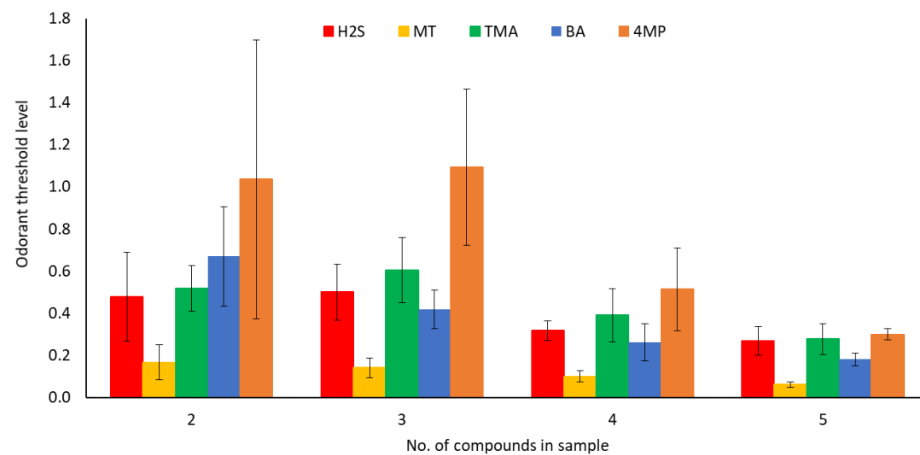

Figure 4. Odorant threshold level of odorants in samples with two to five odorants, mean ± standard deviation (SD). H2S: Hydrogen sulfide; MT: methanethiol; TMA: trimethylamine; BA: butanoic acid; 4MP: 4-methylphenol.

4. Discussion

The aim of the present study was to compare the SOAV method (Sum of Odor Activity Values) with the odor detection threshold measured by olfactometry and to investigate the assumption of additivity. The SOAV method is often applied for evaluating the significance of odorants found in air from livestock houses [16–18], and for estimating the efficiency of odor abatement technologies. The SOAV method is highly dependent on the OTV for the included odorants. Although the present study shows a high correlation between SOAV based on the OTV from measured and literature values, the differences in the applied threshold values will affect the relative effect of an odor abatement technology. However, assuming that significant odorant concentrations are measured with a relatively high precision and reproducibility, the SOAV method will still be a significant improvement for the evaluation of odor abatement technologies. The advantage of the SOAV method is that all odorants in the air matrix are included in the evaluation and with the same proportions as found at the odor source. For olfactometry used in relation to livestock production, it is merely the effect on volatile sulfur compounds that is evaluated since most of the other odorants are lost during storage [2–5] and analysis in the olfactometer [6,7]. It has previously been shown that olfactometry underestimates the effect of air cleaners that have an effect on most of the odorants in pig house air except for volatile sulfur compounds [29], whereas the effect of slurry acidification is overestimated because the concentration of odorants such as volatile fatty acids is increased and volatile sulfur compounds are decreased. Although the SOAV method is an improvement of the evaluation of odor abatement technologies, the discrepancy between OTVs found in the literature and OTVs measured in the present study underlines that the strength of the SOAV method depends on the OTV data quality. OTVs for odorants found in pig house air have been measured in several studies [20–26], but mostly with a rather limited number of panelists and with different methods. In order to improve the strength of the SOAV method, a larger population study would be preferable to obtain a better estimate of the population mean for different odorants and the effect of factors such as age and gender.

An air matrix from pig houses or other odor sources will be much more complex than the five odorants included in the present study. However, the five odorants that were included in the present study normally account for more than 80% of the odor nuisance from pig houses based on the SOAV method [10–12] and are representative of different types of odorants found in pig house air (e.g., volatile sulfur compounds, volatile fatty acids, amines and phenols). The ratio between the odor detection threshold and SOAV was close to one for most of the samples, which indicates that the OAV for odorants in pig house air can be treated as additive. In one sample, the ratio was below one, which indicates a lower degree of cooperation between the odorants, and in some samples, the ratio was above one, indicating some degree of cooperation between odorants. However, it has to be taken into account that although the odorant stimuli were quite stable, there will be a variation related to the odor detection threshold estimated by the panelists that will influence the result. The odorant threshold level (odorant concentration at threshold level) revealed that for most of the odorants, the concentration was as expected at subthreshold level and that the odorant threshold level decreased as the number of odorants increased, which also indicates that the effect of the odorants is additive. For 4MP, the odorant threshold level was above one in samples with two and three odorants. However, only four of the panelists were able to detect 4MP within the available dilution steps in the present study, which means that the OTV for 4MP is underestimated and the odorant threshold level is overestimated. The additive effect of odorants at subthreshold levels is in accordance with other studies about detection probability for binary mixtures [24,26,30], where it was shown that at subthreshold level the interaction between odorants seems to be additive, whereas at levels close to or above the threshold level the interaction is hypo additive. A possible explanation for the additive effect at subthreshold level is that there will be less competition between the molecules for receptors in the olfactory bulb, whereas at higher concentrations (closer to the threshold level) the competition will be

higher or there might be a blocking of receptors for other odorants [30]. Furthermore, the variation in the odorant threshold level also decreased as the number of odorants in the samples increased, which is in line with previous studies [14,15]. The lower variation in more complex samples also indicates that investigations of interaction between odorants in a given matrix should reflect the complexity of the matrix and not only a simple approach with binary combinations.

In conclusion, comparing the odor detection threshold with the sum of odor activity values (the SOAV method) for five key odorants found in pig house air (hydrogen sulfide, methanethiol, trimethylamine, butanoic acid, and 4-methylphenol) indicates that the assumption of additivity between odorants at subthreshold level is reasonable. The strength of the SOAV method is highly dependent on the applied OTVs and more effort should be put into population studies of OTVs. Although there is opportunity for the improvement of the SOAV method, it will be a significant improvement in relation to reproducibility and precision compared to dynamic olfactometry.

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