

Relevance of *n*-butanol as a reference gas for odorants and complex odors

Anders Feilberg, Michael J. Hansen, Ole Pontoppidan, Arne Oxbøl and Kristoffer Jonassen

ABSTRACT

Odor analysis by olfactometry relies on the use of *n*-butanol as a reference compound for standardizing the selection of human panelists. This requires that human sensitivity towards *n*-butanol is correlated to sensitivity towards other odorants as well as complex odor mixtures. However, there is limited evidence in the literature of such correlations. In this work, datasets from three odor laboratories were investigated in order to clarify this. All panels routinely analyzed *n*-butanol and H₂S samples. Two of the laboratories analyzed samples from pig production or industry, whereas one laboratory determined odor threshold values for typical pig production odorants. Non-significant correlations were observed in most cases and odor threshold values for structurally related compounds were not well correlated. The work presented strongly indicates that the sensitivity of odor panelists towards *n*-butanol is not well transferred to other odorants or odor samples. Furthermore, minimization of variance by using *n*-butanol is not transferable to other odorants or environmental samples. Thus, the harmonization of human panelists for odor analysis based on *n*-butanol does not appear to result in harmonization with respect to other odorants or odor samples.

Key words | inter-laboratory variation, odor threshold, olfactometry, quality assurance

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INTRODUCTION

Measurement of odor by dynamic dilution-to-threshold olfactometry based on human panelists is used throughout the world to regulate odor emissions (Clanton *et al.* 1999; Nicell 2009; Laor *et al.* 2014). In Europe for example, olfactometry is carried out by accredited laboratories in compliance with the European standard EN 13725 (CEN 2003), whereas in other parts of the world similar standards are used to harmonize odor measurements. EN 13725 describes a range of quality criteria, such as accuracy and precision (repeatability). In order to standardize the measurements, minimize variability, and achieve quantitative measurements, the sensory panel (the detector in this case) needs to be calibrated to ensure that sensitivity is within an acceptable range. With the specification of EN 13725 in 2004, *n*-butanol was selected as the only reference gas to be used for this purpose. According to EN 13725, *n*-butanol is used to test with respect to quality criteria for single laboratories and thereby document compliance to

the precision and accuracy within a laboratory that is requested by the standard. Known traceable concentrations of *n*-butanol are used for this purpose. It is assumed that the quality criteria for *n*-butanol are transferable to other odorants and to complex odorant mixtures, but as discussed by Klarenbeek *et al.* (2014) there is limited evidence to support this assumption. In addition to the variance within one laboratory, there is also a contribution to variance from the use of different laboratories. However, this variance is not included in EN 13725 as a quality parameter. Thus, only the use of *n*-butanol across laboratories is applied as a reference to compare results from different odor laboratories. For odor laboratories to provide comparable results, however, requires that sensitivity of the odor panels to environmental odor samples is to some degree correlated to sensitivity to *n*-butanol. As discussed by Klarenbeek *et al.* (2014), there is little experimental evidence to support this. The work of Zernecke *et al.* (2011) demonstrated that panelist sensitivity

towards different odorants may not be correlated for compounds with different chemical structures if the panelists are normosmic (having a normal olfactory sensation). Other studies also indicate that sensitivity towards *n*-butanol is not well correlated to H₂S samples (McGinley & McGinley 2010) or to complex samples from a cattle feedyard (Parker *et al.* 2005).

This means that, in principle, two odor laboratories with identical average *n*-butanol thresholds can give quite different results for an environmental odor sample in which one or more compounds other than *n*-butanol are the predominant odorants. In recent years, it has been observed that results from different odor laboratories can differ substantially not only in absolute levels, but also in abatement efficiencies measured for environmental technologies (Bokowa & Beukes 2012; Jonassen *et al.* 2012). This may be ascribed to the lack of useful measures of interlaboratory reproducibility.

In this work, data from three different odor laboratories were investigated with respect to (1) correlation between panelist sensitivity towards *n*-butanol and other odorants or environmental samples and (2) variability in panelist detection thresholds for *n*-butanol and odorants/samples. The objective was to test if the *n*-butanol performance (of olfactory panelists) is transferable to other odorants and if possible to identify other suitable reference odorants. In this study, the focus was on samples from intensive livestock production and livestock waste management and odorants known to be of importance from these sources (Wright *et al.* 2005; Kim *et al.* 2007; Feilberg *et al.* 2011; Trabue *et al.* 2011, 2016; Hansen *et al.* 2012).

METHODS

The data presented include three sets of data collected by three different odor laboratories in Denmark. Details for each laboratory are outlined below.

Dataset 1

Recent data on panelist sensitivity towards *n*-butanol, H₂S and odor samples from pig production facilities obtained by the Danish Technological Institute (DTI) were investigated. The data were measured in compliance with the European standard EN 13725 (CEN 2003) using an Ecoma TO8 olfactometer (Odournet GmbH, Kiel, Germany). The laboratory is accredited by Danish Standards to carry out odor analyses. The olfactometer was used to estimate the

dilution threshold. The olfactometer was based on the yes/no response method and was designed for four panelists.

Four pig production facilities (facilities 1–4) with growing–finishing pigs were used in the study. At facility 1, two identical pig houses were used. One of the pig houses had slurry acidification installed and the other was a control section without acidification. At facility 2, a pig house designed with a partly slatted floor was used. The air outlet from the pig house was divided into pit ventilation, extracting at up to 10% of the maximum ventilation rate, and room ventilation, extracting at up to 90% of the maximum ventilation rate. At facility 3 a pig house was equipped with a biological air cleaner. At facility 4, a typical pig house without environmental technology was used.

Dataset 2

Data from determination of odor threshold values for six compounds (H₂S, methanethiol, dimethyl sulfide, acetic acid, butanoic acid, and 4-methylphenol) were included. These compounds are typical pig production and livestock waste odorants (Feilberg *et al.* 2010), but are also found in other emission sources. Threshold values were measured by a new approach by which proton-transfer-reaction mass spectrometry (PTR-MS) was used to achieve a reliable assessment of the odorant exposure concentration (de Gouw & Warneke 2007; Feilberg *et al.* 2010; Cappellin *et al.* 2012). Measurement of odor threshold values were carried out by Aarhus University (AU) in accordance with EN 13725 using an Ecoma TO8 olfactometer. In the data presented here, data from two panelists for whom the *n*-butanol threshold values are outside the acceptable range (20–80 ppb) are included.

Prior to the experiment, human panelists were selected based on *n*-butanol as described by the European standard for olfactometry (CEN 2003). Due to the previously demonstrated time delay of some odorants in the olfactometer (Hansen *et al.* 2013) the presentation time at each dilution step was set at 2.2 seconds for H₂S, methanethiol, and dimethyl sulfide, and 6.6 seconds for acetic acid, butanoic acid, and 4-methylphenol. The measurements were carried out over 10 days and for each odorant, the odor threshold values were estimated on two different days by each panelist. The resulting odor threshold values and discussions of these are presented in a separate paper (Hansen *et al.* 2018).

H₂S, methanethiol (both from AGA, Copenhagen, Denmark) and dimethyl sulfide (Air Liquide, Horsens, Denmark) were introduced from certified gas cylinders. Acetic acid, butanoic acid, and 4-methylphenol were

introduced from permeations tubes (VICI Metronics, Inc., Houston, TX, USA) using a permeation oven (Dynacalibrator model 150, VICI Metronics, Inc.). The gas release (ng min^{-1}) was measured gravimetrically with at least three repetitions.

A high-sensitivity PTR-MS (Iconon Analytik GmbH, Innsbruck, Austria) was used to estimate the concentration of odorants delivered to the nose cone at each dilution step. The drift tube conditions for the PTR-MS were set at a voltage of 600 V, a pressure of 2.1–2.2 mbar, and a temperature of 75 °C.

Dataset 3

Data on panelist sensitivity to *n*-butanol, H_2S , and odor samples from industrial odor sources were obtained by Force Technology. The data were measured in compliance with EN 13725 using a custom-built glass olfactometer. The laboratory is accredited by Danish Standards to carry out odor analyses. The dataset was limited to three days of measurements by six panelists on each day and should therefore be regarded as tentative. The environmental samples were from three sources: (1) a feed production industry, (2) a household waste composting plant with biofiltration, and (3) a pig production facility with biofiltration.

RESULTS AND DISCUSSION

The sample and odorant threshold values have been treated by least-squares regression analysis in order to identify statistically significant correlations. In addition, the variability has been expressed simply as the standard deviation or relative standard deviation (RSD).

Dataset 1

In Figure 1, the log-transformed mean centered threshold values for a large number of samples have been compared to the corresponding log-transformed *n*-butanol threshold values. The data in Figure 1 are based on the average ($n = 3$) response of individual panelists exposed to samples from intensive pig production and their average *n*-butanol threshold values. Thus, the y- and x-axes represent the relative sensitivity of the panelists towards the specific environmental odor samples and *n*-butanol, respectively. The overall average *n*-butanol threshold for the whole set was 43 ppb, whereas the average odor concentration was $736 \text{ OU}_E \text{ m}^{-3}$. As can be seen from Figure 1, there is no

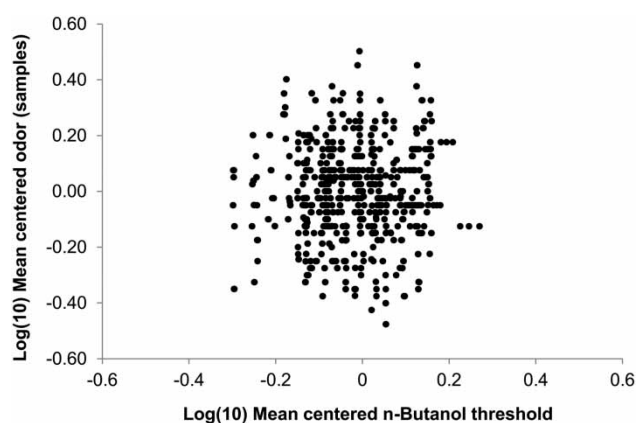


Figure 1 | Mean centered odor results (\log_{10} base) for samples from pig production facilities as a function of the corresponding mean centered *n*-butanol thresholds (\log_{10} base) determined on the same day as the samples ($n = 1468$).

correlation between the sensitivity towards pig odor samples and *n*-butanol. A similar result was obtained by Parker *et al.* (2005) for a set of complex agricultural odor samples. Furthermore, it observed that the variance is larger for pig odor samples compared to *n*-butanol. The relative standard deviation of pig odor results is 127% whereas the relative standard deviation of *n*-butanol thresholds is only 23%. The standardization of the panelists by *n*-butanol is therefore not transferable to the environmental samples.

The odor laboratory responsible for dataset 1 has also routinely measured the odor threshold values of the panelists towards H_2S in parallel to *n*-butanol. Thus, it can be assessed if H_2S would be a better reference material than *n*-butanol. As seen in Figure 2, however, there is no correlation between sensitivity towards pig odor samples and H_2S either. Using H_2S as a reference odorant by including

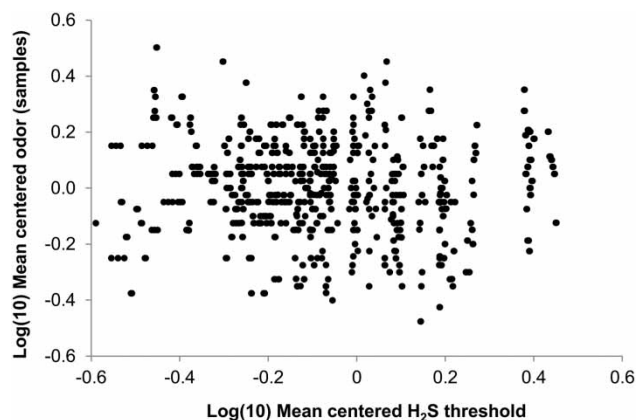


Figure 2 | Mean centered odor results (\log_{10} base) for samples from pig production facilities as a function of the corresponding mean centered H_2S thresholds (\log_{10} base) determined on the same day as the samples ($n = 1468$).

only data of panelists within a factor of 2 above or below the average odor threshold value (similar to the use of *n*-butanol) also did not reduce the relative standard deviation of the pig odor samples (RSD = 130%). The average threshold of H₂S was 1.1 ppb with a relative standard deviation of 47% and thus the attempted standardization by *n*-butanol is not transferrable to H₂S either.

The dataset also allows for a comparison of panelist sensitivities towards H₂S and *n*-butanol as shown in Figure 3. Clearly, there is no correlation, which is in agreement with a previous study (McGinley & McGinley 2010). Another observation from this dataset is that the variations in panelist responses are larger for environmental samples and H₂S compared to *n*-butanol, as shown in Figure 4. This confirms that harmonization of the panelists based on *n*-butanol

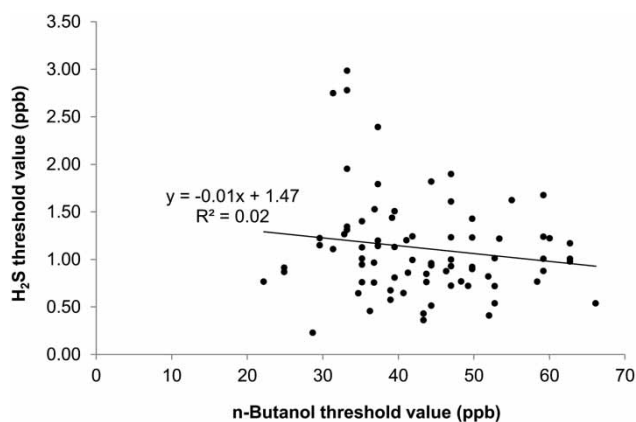


Figure 3 | H₂S odor threshold of individual panelists versus the corresponding *n*-butanol threshold values. Pairs of threshold values were determined on the same day and this was repeated for 16 days.

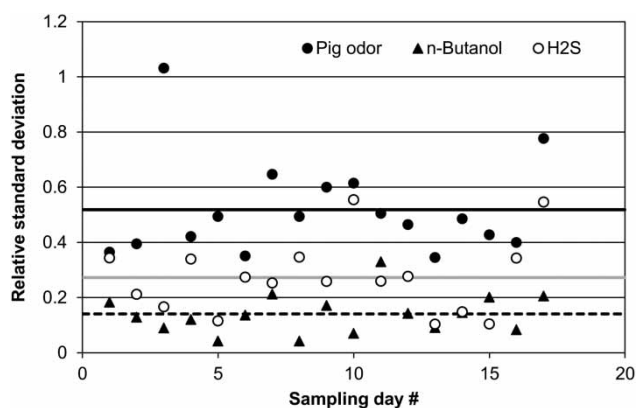


Figure 4 | Relative standard deviations (RSD) for the group of panelists on each measurement day calculated for pig odor samples, H₂S and *n*-butanol. Horizontal lines represent the average RSD for pig odor samples (black line), H₂S (grey line) and *n*-butanol (dashed line).

does not result in an equal harmonization with respect to environmental samples.

Dataset 2

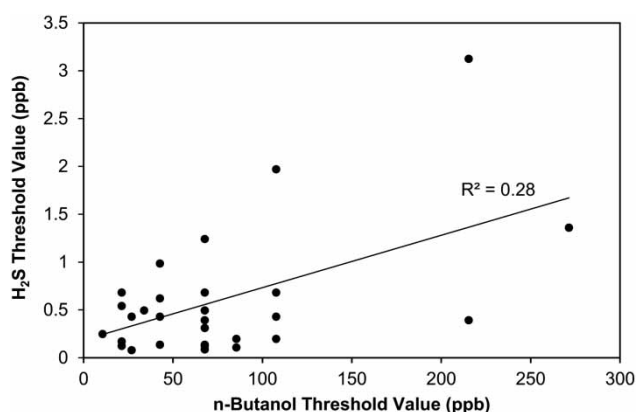
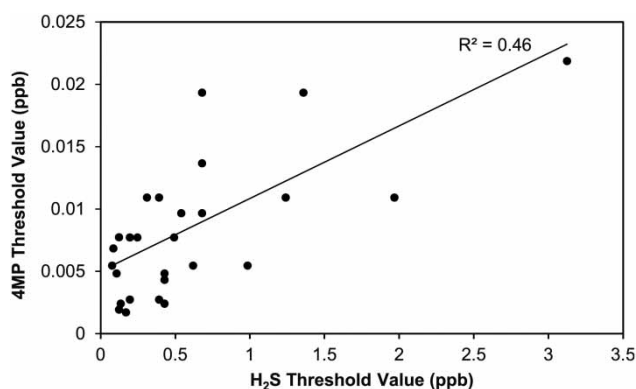
The unique dataset obtained from measuring new odor threshold values of odorants relevant for livestock production allows for an investigation of single odorant correlations with respect to panelist sensitivity. Both *n*-butanol and H₂S are included in these measurements. The threshold of *n*-butanol was determined on each day together with threshold values of one or two odorants. Thus, the inter-correlations of odorants other than *n*-butanol are based on values determined on different days and with varying panelist compositions, which adds additional variation to the correlations. The results are summarized in Table 1 and presented as correlation coefficients (R^2). It can be seen that some correlations are statistically significant despite their low R^2 values, which is due to the relatively large dataset. However, in most cases the correlation coefficients are low and not statistically significant. Compared to dataset 2, a weak but statistically significant correlation between H₂S and *n*-butanol is observed. The data are shown in Figure 5. It was expected that threshold values of compounds with similar molecular structures would be correlated since they may give rise to a response in the same receptors in the olfactory system (Buck & Axel 1991). However, comparing acetic acid with butanoic acid (both containing the -COOH group), H₂S with methanethiol (both containing the -SH group), methanethiol with dimethyl sulfide (both containing CH₃S-) or *n*-butanol with butanoic acid (both containing CH₃CH₂CH₂-) shows that this is not the case. Of these pairs, only for H₂S and methanethiol (containing -SH) is a weak (but significant) correlation observed ($R^2 = 0.24$). The best correlation is observed for H₂S and 4-methylphenol ($R^2 = 0.46$; Figure 6) even though these compounds do not share any structural similarities. Even though some correlations are significant, all R^2 values are below 0.5 and the uncertainties of the slopes are quite high. For H₂S and *n*-butanol ($R^2 = 0.28$) for example, the uncertainty of the slope is 30% (RSD) and for other (poorer) correlations, the slope uncertainties exceed 100%. Thus, the sensitivity of a panel towards one odorant is generally not a reliable predictor of sensitivity towards another odorant.

Dataset 3

The third dataset includes a smaller number of samples from industries collected on three different days. An all days, odor

Table 1 | Intercorrelation coefficients (R^2) for odorants. Each correlation is based on 32 odor threshold values for each odorant compared. Each panelist threshold value is based on three repetitions

	NB	AC	BA	MT	DMS	H ₂ S
<i>n</i> -butanol (NB)						
Acetic acid (AC)	0.004					
Butanoic acid (BA)	0.08	0.08				
Methanethiol (MT)	0.15*	0.07	0.004			
Dimethyl sulfide (DMS)	0.02	0.07	0.16*	0.07		
Hydrogen sulfide (H ₂ S)	0.28**	0.20*	0.10	0.24**	0.22*	
4-methylphenol (4 MP)	0.06	0.10	0.06	0.22*	0.11	0.46**

*Significant; $P < 0.05$ (t-test).**Significant; $P < 0.01$ (t-test).**Figure 5** | Odor threshold value of H₂S versus odor threshold value of *n*-butanol. The solid line represents a linear regression of the data.**Figure 6** | Odor threshold value of 4-methylphenol (4MP) versus odor threshold value of H₂S. The solid line represents a linear regression of the data.

threshold values of H₂S and *n*-butanol were determined together with the samples. Due to the limited number of comparable repetitions, it is difficult to assess if 1) significant correlations exist and 2) if the variation is transferable from

n-butanol to H₂S and complex samples. Out of the 13 samples analyzed, only in one case was a statistically significant correlation observed between single panelist sensitivity towards samples and *n*-butanol (t-test; $P < 0.05$; $R^2 = 0.79$; data not shown).

Implications

From the discussion above, it is clear that using *n*-butanol as a reference odorant does not result in harmonization of odor analyses by dynamic olfactometry. However, it is not indicated that H₂S would be a more suitable reference odorant for pig odor samples and it remains questionable if a single suitable reference compound can be identified. The results are in agreement with Klarenbeek *et al.* (2014).

In all three datasets, odor threshold values for *n*-butanol and H₂S are included. The average odor threshold values for *n*-butanol were 43 ppb, 46 ppb, and 33 ppb for datasets 1, 2, and 3, respectively. Including two panelists from dataset 2 outside of the acceptable range, gave an odor threshold value of 65 ppb. The corresponding values for H₂S were 1.1 ppb, 0.33 ppb (0.39 ppb incl. all panelists), and 0.33 ppb for datasets 1, 2, and 3, respectively. Thus, it becomes clear that odor panels with very similar sensitivity towards *n*-butanol can have different sensitivity towards other odorants. The results can explain why identical samples analyzed by different laboratories and panels can give very different results (Bokowa & Beukes 2012; Jonassen *et al.* 2012).

Further work should be carried out based on other odor sources and odorants to further investigate the implications of this. In addition, investigations based on human panelists that are not preselected using *n*-butanol would be interesting.

CONCLUSIONS

The work presented in this paper strongly indicates that the sensitivity of odor panelists towards *n*-butanol is not well transferred to other odorants or complex odor samples. Either weak or non-significant correlations are observed and variance of sensitivity is also not transferable. Thus, harmonization of human panelists for odor analysis based on *n*-butanol does not result in harmonization with respect to other odorants or odor samples. The application and revision of international standards for odor measurements (e.g. EN 13725) should take this lack of transferability into consideration.

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