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Original Article

Odor Thresholds and Breathing Changes of Human Volunteers as Consequences of Sulphur Dioxide Exposure Considering Individual Factors

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Objectives: Though sulfur dioxide (SO_2) is used widely at workplaces, itseffects on humans are not known. Thresholds are reportedwithout reference to gender or age and occupational exposure limits are basedon effects on lung functioning,although localized effects in the upper airways can be expected.This study's aim is to determine thresholds with respect to age and gender and suggests a new approach to risk assessment using breathing reflexes presumably triggered by trigeminal receptors in the upper airways.

Methods: Odor thresholds were determined by the ascending method of limits in groups stratified by age and gender.Subjects rated intensities of different olfactory and trigeminal perceptions at different concentrations of SO_2. During the presentation of the concentrations, breathing movements were measured by respiratory inductive plethysmography.

Results: Neither age nor gender effects were observed for odor threshold. Only ratings of nasal irritation were influenced bygender. A benchmark dose analysis on relative respiratory depth revealed a 10%-deviation from baseline at about 25.27 mg/m^3.

Conclusion: The proposed new approach to risk assessment appearsto be sustainable. We discuss whether a 10%-deviation of breathingdepth is relevant.

Key Words: Risk assessment, Respiratory mechanics, Sensory thresholds, Sulfur dioxide

Introduction

Odor threshold of sulphur dioxide

Sulphur dioxide (SO_2) is a colorless gas with a strong, irritating and pungent odor [1]. The gas is used in a variety of workplaces (i.e., in inorganic and petrochemical industries). It serves many functions, such as an antioxidant, bleaching gas, catalyst or disinfectant, and is also used as a preservative in food [2]. Reported odor thresholds of SO_2 range from 0.266 mg/m^3 (0.1 ppm) to 12.5 mg/m^3 (4.7 ppm) [1,3-5]. This variety of different thresholds might be due to differences in the psychophysical procedure for measuring the odor threshold (e.g., number of trials) [3,6,7] or in limited chemical analysis. No information about age and gender of the subjects is given, and this limits generalization to other populations. An experiment was therefore designed, which included the assessment of odor thresholds for SO_2 in different age groups and in female and male subjects.

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Critical remarks regarding occupational exposure limits (OELs) of SO_2

Sensory irritation is considered to be an adverse effect of a chemical and should be avoided by observing OELs. Odor thresholds are usually based on olfactory stimulation, which is transduced via the olfactory nerve, while sensory irritation is detected via trigeminal receptors. For most irritants, sensory irritation is perceived at higher concentrations than odor [8-12]. National OELs for SO_2 range from 1.33 mg/m^3 (0.5 ppm; Denmark, Switzerland) to 13.3 mg/m^3 (5 ppm; Occupational Safety and Health Agency, USA). In Germany, the maximum allowable concentration (MAC)-Commission recommends an OEL of 1.33 mg/m^3. This MAC-value is derived from the effects of SO_2 on lung functions. However, in nasal breathing up to 98% of the SO_2 [13-16] is absorbed in the upper airways and therefore does not reach the lung at all when breathed through the nose [14,16-18].

Sensory irritation by SO_2 is expected to take place in the upper airways, more precisely in the larynx [19] due to the high water solubility of SO_2 (112.7 g/L at 20°C) [20]. Therefore, acute effects of SO_2 can be expected in the upper airways and should also be considered for risk assessment. We suggest a new alternative method for risk assessment in humans using reflexes in breathing as an indicator of adverse effects.

Parameters of breathing regulation are a widely used critical endpoint in the risk assessment of irritants by adopting a 50% decrease in respiratory rate (RD_{50}) in mice [21-23]. The RD_{50} values of sulphur dioxide range from 184 to 1,373 mg/m^3 [22]. To derive a tentative OEL for human risk assessment, the RD_{50} can be multiplied by 0.03; Alarie [24] was able to demonstrate retrospectively that the RD_{50} was in that specific relationship to many OELs. This multiplication serves as a good starting point for further considerations of possible risks. However, it is doubtful whether an approach, based on research in animals, is appropriate for the evaluation of respiratory tract irritation in man [25]. Since it is unreasonable to expect attempts to determine the RD_{50} in humans, an alternative method is needed, one which is more appropriate for humans.

The detection of irritants by trigeminal receptors leads to protective reflexes, such as for example, sneezing and apnea [26]. Trigeminal nerves have been shown to be the basis of respiratory reflexes (changes in breathing pattern) in animal studies [27,28]. In a short-time challenge, a reduction of respiratory volume can be observed at lower concentrations than those evoking a decrease in respiratory rate [29-32]. Grunstein et al. [30] found that cats react to SO_2 challenge with a decrease of tidal volume and an increase in respiratory rate. A reduction of respiratory volume, as a trigeminally mediated reflex response to the detection of sensory irritation [29,33], could be used to identify critical endpoints in a dose-effect relationship for human subjects. The benchmark dose (BMD) approach is a method for risk assessment in humans recommended by the U.S. Environmental Protection Agency. This method takes dose-response information into account by fitting a mathematical model to dose-response data [34]. Using this method, differences in sensitivity were considered by predicting the sample's variation. Most studies investigating SO_2 use only one or two concentrations so that an analysis of dose-response relationships is impossible. Moreover, these studies cannot be aggregated as they are not comparable with regard to subjects, design, and critical endpoints.

This study was designed to allow a BMD analysis of the risk of SO_2. Breathing depth as a continuous variable was used as the critical endpoint. For studies with human subjects, a change of 10% from a baseline is recommended to be regarded as an adverse effect [34,35]. Monitoring of breathing depth is a more objective method for measuring sensory irritation than subjective ratings. Nevertheless, both subjective and physiological (reducing respiratory volume as a reflex action) measures of sensory irritation at different concentrations of SO_2 were made on the assumption that this would strengthen the validity of the assessment data.

Materials and Methods

Subjects

The initial sample was composed of 22 male and 22 female non-smoking subjects stratified by age and gender (male/female, young/old). A 'young' group comprised subjects aged 20-44 years, and an 'old' group subjects aged 45-65 years. Due to technical reasons, complete data sets were available for only 39 subjects. Within this final sample, the average ages of the young and old female subject groups were 31 (±8 standard deviation [SD]) and 54 years (±7 SD), respectively. The average ages of the young and old male subjects were 26 (±5 SD) and 56 years (±7 SD), respectively.

The sample was recruited by announcements in local newspapers in Dortmund, Germany. All aspects of the study were performed in accordance with the Declaration of Helsinki. Accordingly, subjects were informed about the substance and possible effects during the psychophysical scaling experiment (e.g., malodor, sensory irritation), and written informed consent was obtained. The study protocol was approved by the local ethics committee. Lung function had been assessed via spirometry (VitaloGraph, Hamburg, Germany) by a physician before the study began, and subjects with impaired lung func-

tion (forced expiratory volume in 1 second/ forced vital capacity < 0.7 regarding age and gender) were excluded.

Determination of odor thresholds

The determination of odor threshold was conducted using the standard procedure of *ascending method of limits* [36-41]. Starting with very low concentrations, increasing concentrations of SO_2 were interspersed with blank samples. In three trials, the lowest of two subsequent correctly identified concentration steps was used as an estimate of reliable olfactory detection. The geometric mean of of these three estimates was calculated and represented the individual odor threshold.

Rating procedure

The rating procedure involved the use of a visual analogue scale presented in the format of a 'labeled magnitude scale' [42]. This is a widely used scale to rate intensity of chemosensory stimuli [43-48], and it mimics the ratio-like properties of magnitude estimation [42]. The descriptors comprised three olfactory sensations (odor intensity, annoyance, and nauseous) and seven trigeminal sensations (burning, tickling, nasal irritation, sneeze, prickling, sharp, and pungent). These trigeminally mediated perceptions were based on those perceived by congenitally anosmic subjects [49]. The rating procedure was performed by means of a PocketPC™ (HP Jornada 540, 240 × 320 pixel , Hewlett-Packard, Palo Alto, USA), which displayed the scale label at the top of the screen (e.g., odor intensity), a slider on the right side, and six categories for rated intensity (range: from barely detectable to strongest imaginable) close to the slider. The ratings were conducted during the one minute breaks after each stimulus presentation. During a pre-test session, preceding the scaling experiments, all subjects were familiarized with the handling of the PocketPC, the use of the slider, and the olfactometer. This rating procedure and device has been used in several studies [50].

In contrast to the odor threshold assessment, nine concentrations were presented in random order.

Measurement of breathing depth

Breathing depth was measured by means of respiratory inductive plethysmography (RIP) during stimulus delivery for the ratings of olfactory and trigeminal perceptions. RIP is suitable for monitoring breathing movements during natural breathing [51]. A flexible breast belt was fitted around the subject's chest, and, within this flexible belt, a forked wire is used as a coil. Technically, the movement of the chest changes the inductance of this 'coil', and a biosignal recorder converts these changes into electronic signals, which were digitized and stored on CF-cards

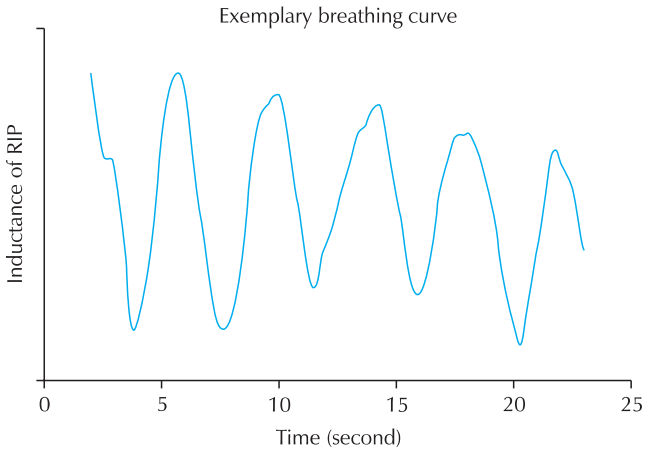

Fig. 1. Exemplary oscillating breathing curve; inductance change of the respiratory inductive plethysmography in time. RIP: respiratory inductive plethysmography.

(biosignal recorder "Varioport-B", Variograf-Software; Becker Meditec, Karlsruhe, Germany). The signals for inhalation and exhalation of the olfactometer were additionally stored on the CF-cards so that movements and signals could be matched to separate the inhalations of SO_2 and the inhalations of blanks. The mean individual breathing depth (average amplitude of inhalation and exhalation) was calculated using the recorded oscillating breathing curve (Fig. 1).

To be able to compare breathing depth inter-individually, the single breathing depth at each concentration was normalized by the mean breathing depth at the lowest concentration. Such an approach allows for the detection of intra-individual changes in breathing. For the quantification of breathing volume, a more sophisticated assessment is necessary.

Stimulus delivery (flow-olfactometry)

Determination of odor thresholds

SO_2 was delivered to the subjects' noses by means of a flow-olfactometer (TO7, ECOMA GmbH, Kiel, Germany). Anatomically shaped sniffing ports delivered the stimulus to four subjects simultaneously. SO_2 was injected into 25 L Tedlar-bags filled with nitrogen. The substance was homogenized within the bag by heating and rotation. By means of the dilution unit of the TO7 olfactometer, nine different concentrations of SO_2 could be generated. SO_2 concentration was presented in a geometric series starting with a dilution of 1/2.5. Since nine concentrations were used, the lowest dilution was 1/640. The 'real' concentration was measured by a SO_2 analyzer (UV fluorescent sulphur dioxide analyzer AF21M; ANSYCO, Karlsruhe, Germany) 10 times at four concentrations (1/640, 1/160, 1/40, and 1/20). The other concentrations were extrapolated from

Table 1. Values of the investigated concentrations for odor threshold

Dilution	Desired value SO_2, mg/m^3	Mean value SO_2, mg/m^3	Deviation, %
1/640	.0798	.0851 (.012)	5.9
1/320	.1596	.1569*	-0.6
1/160	.3165	.3112 (.017)	-1.8
1/80	.6331	.6298*	-2.2
1/40	1.266	1.133 (.048)	-10.6
1/20	2.532	2.248 (.047)	-11.3
1/10	5.065	4.299*	-15.1
1/5	10.13	8.565*	-15.4
1/2.5	18.76	15.58*	-17.0

Measured values (ten repetitions per concentration) at dilutions of 1/640, 1/160, 1/40, and 1/20 with standard deviations in brackets. *extrapolated values.

Table 2. Values of the investigated concentrations for rating and measurement of breathing depth

Dilution	Desired value SO_2, mg/m^3	Mean value SO_2, mg/m^3	Deviation, %
1/640	.1596	.1702 (.014)	6.6
1/320	.3165	.3298*	3.8
1/160	.6331	.6331 (.029)	-0.1
1/80	1.269	1.256*	-1.1
1/40	2.538	2.290 (.052)	-9.8
1/20	5.075	4.501 (.051)	-11.3
1/10	10.15	8.592*	-15.3
1/5	20.30	17.07*	-15.9
1/2.5	37.59	33.58*	-10.7

Measured values (ten repetitions per concentration) at dilutions of 1/640, 1/160, 1/40, and 1/20 with standard deviations in brackets. *extrapolated values.

the measured values. The concentrations for the determination of odor thresholds are shown in Table 1.

There is an increasing difference between estimated and measured/extrapolated concentrations. This might be due to flow loss for technical reasons. Subsequent analyses are based on the measured/extrapolated concentrations. Subjects were instructed to breathe according to a fixed pattern. Two flashing light-emitting diodes (LEDs) on the top of the olfactometer indicated inhalation and exhalation. By this means, the breathing pattern of the participants was synchronized with stimulus delivery. If the LEDs flashed on, subjects inhaled through the nose for two seconds. When the LEDs turned off, subjects were instructed to exhale through the mouth. Subjects were trained in this synchronized breathing before the experiment started.

Supra-threshold assessment of olfactory and trigeminal perceptions (psychophysical functions) and measurement of breathing depth

For the evaluation of sensations and the measurement of breathing depth, higher concentrations were delivered by the olfactometer than those used for the determination of odor thresholds (Table 2).

At the start, two blank intakes were administered, so that the subjects could regulate their breathing to the given breathing rhythm. After that, SO_2 was presented to the subjects for two seconds during every second inhalation (inhalation phase), and each concentration step was repeated five times [5 × (2 seconds inhalation + 2 seconds exhalation) = 20 seconds]. Each stimulus presentation lasted 40 seconds (alternating five blank and five SO_2 inhalations and exhalations), and the total stimulus duration was 10 seconds (five inhalations). After one stimulus presentation, the respective concentration step (e.g., 4.5 mg/m^3 SO_2) was evaluated with respect to the intensity of olfactory and trigeminal perceptions. The order of concentrations was quasi-random to avoid adaptation effects, which could affect the evaluation of perceptions. For the same reason, there was a break of at least one minute between subsequent concentration steps.

A dynamic flow olfactometer is an adequate device to deliver stimuli of different concentrations to subjects in (nasal) breathing situations, in which individual regulation of breath intake could take place.

Study protocol

The determination of odor thresholds lasted approximately 35 minutes. Fifteen minutes after the threshold assessment, odor evaluation and measuring of breathing depth took place, lasting approximately 40 minutes (every concentration was presented twice).

Statistics

Determination of odor thresholds

A repeated measures ANOVA on odor thresholds with age and gender as between-subjects factors was conducted to identify gender or age differences.

Subjective ratings

A repeated measures MANOVA on subjective ratings (10 perceptions) with age and gender as between subjects factors and concentration as a within-subjects factor was conducted to identify gender or age differences in subjective ratings. Using planned comparisons of the subjective ratings made at a certain concentration step of SO_2, the initial effect concentration was defined as the first significant deviation from the preceding concentrations (*initial effect level*, IEL). Non-linear regression analyses were used to fit psychophysical functions according to Stevens' power law [52]: $\Psi(I) = kI^a$. Ψ represents the perceived intensity of a physical stimulus, I stands for the intensity of the physical stimulus (in this case, the concentration of SO_2), k is a proportional constant, and the exponent a determines the slope of psychophysical function. The higher the exponent a, the steeper is the psychophysical function. Only this parameter is analysed.

Measurement of breathing depth

A BMD-analysis for continuous variables with non-linear and linear regression was conducted on breathing depth with a 10%-deviation from baseline as the critical value.

To test the impact of odor sensitivity on breathing depth, the individual odor threshold was correlated with breathing depth at every concentration using Spearman's rho.

Results

An ANOVA on age with gender and age group as between-subjects factors revealed no interaction between age group and sex ($p > .10$).

Odor threshold assessment

The median odor threshold was around 2.77 mg/m^3 (25th percentile: 1.78 mg/m^3; 75th percentile: 5.32 mg/m^3). The odor thresholds for the different groups of subjects are shown in Table 3.

Table 3. Odor thresholds for each investigated group

		Median	25th percentile	75th percentile
Young	Female	1.766	1.434	2.865
	Male	3.556	1.790	8.172
Old	Female	3.575	2.487	6.052
	Male	2.790	2.221	8.307

Median, interquartile range in mg/m^3.

The ANOVA on odor thresholds showed no significant main or interaction effects for sex or age ($p > .10$), although the median threshold for young women seems to be lower than that for the other groups.

Supra-threshold assessment

The repeated measures MANOVA on subjective ratings revealed that the concentration of SO_2 has a highly significant effect on all of the ratings ($p < .001$) with higher ratings at high concentrations. For the ratings of all perceptions, power func-

Table 4. Parameters of fitted power function with goodness-of-fit measure (adjusted R^2)

Perception	a	Adjusted R^2	Perception	a	Adjusted R^2
			Odor intensity	.45	.95
Olfactory	.48	.96	Disgust	.45	.93
			Annoyance	.50	.95
			Tickling	.25	.88
			Prickling	.29	.91
			Nasal irritation	.36	.88
Trigeminal	.40	.95	Pungent	.41	.95
			Sneeze	.44	.78
			Burning	.44	.90
			Sharp	.51	.96

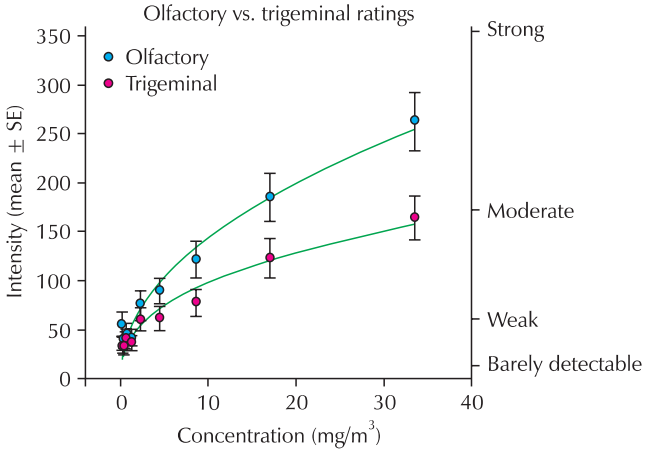

Fig. 2. Mean ratings of olfactory and trigeminal perceptions (±SE) at different concentrations of SO_2 and fitted power curves. SE: standard error.

Table 5. Initial effect levels and perceptual range of each investigated perception

Perception	Mean initial effect level		Initial effect level
Olfactory	3.027	Odor intensity	2.290
		Disgust	2.290
		Annoyance	4.501
Trigeminal	7.419	Nasal irritation	2.290
		Pungent	2.290
		Sharp	4.500
		Sneeze	8.592
		Tickling	8.592
		Prickling	8.592
		Burning	17.07

tions [$\Psi(I) = kI^a$] were fitted to the observed data. Goodness of fit and estimates for a are given in Table 4.

The parameter a for the mean olfactory rating is higher than for the mean trigeminal rating, indicating a steeper slope in olfactory ratings. The non-aggregated values of a range from .45 to .50 for olfactory perceptions and from .25 to .51 for trigeminal perceptions. The goodness of fit varies between .78 ('*sneeze*') to .96 ('*sharp*').

Fig. 2 shows the mean olfactory and the mean trigeminal ratings at each concentration and the fitted power curves.

Starting with similar ratings at low concentrations, the slope is steeper for the mean olfactory perception, reaching higher ratings at 2.29 mg/m^3. Planned (difference) contrasts on the non-aggregated perceptions show the IELs (cf. Table 5 for the IELs of all perceptions).

The mean IELs for olfactory and trigeminal ratings differ (3.03 vs. 7.42 mg/m^3). The initial effect is larger for non-aggregated olfactory ratings (cf. Fig. 2). While the non-aggregated IELs of olfactory ratings vary between 2.29 and 4.5 mg/m^3, those of trigeminal perceptions vary between 2.29 and 17.07 mg/m^3.

While there is no significant main effect of age on the ratings, there is a gender effect on *nasal irritation* with male subjects report less *nasal irritation* than female subjects ($p < .05$). Furthermore, there is a significant interaction between concentration and sex for *nasal irritation* ($p < .01$; cf. Fig. 3). Planned comparisons reveal that female subjects made higher ratings than male subjects at the lowest concentration (0.17 mg/m^3) and at the high concentrations (17.07 mg/m^3 and 33.58 mg/m^3).

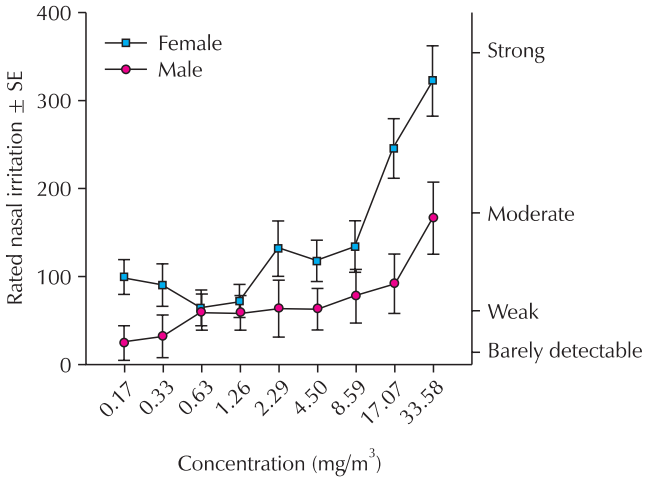

Fig. 3. Gender differences in ratings of *nasal irritation* (±SE) at different concentrations of SO_2 at each concentration. SE: standard error.

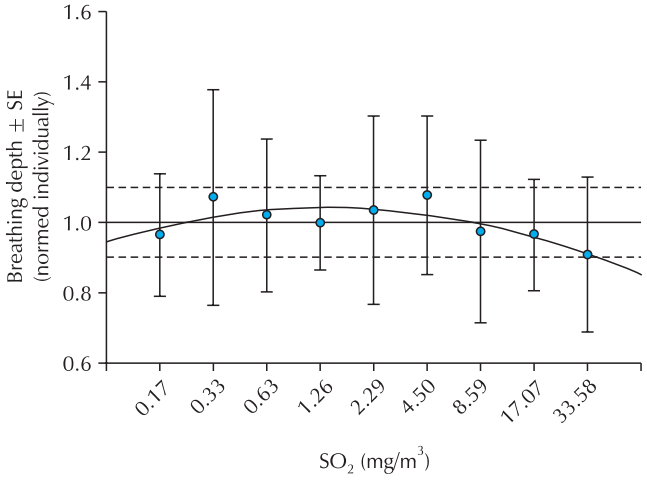

Fig. 4. Individually normed breathing depth in relation to concentration displaying a quadratic trend. SE: standard error.

Breathing depth

The relationship between breathing depth and concentration is displayed in Fig. 4.

A quadratic function was fitted to the data (adjusted $R^2 = .49$). For lower concentrations (up to 2.29 mg/m^3; below threshold range), breathing depth varies randomly around 1. Beginning at 4.5 mg/m^3 (supra-threshold range), a linear decreasing trend is obvious. A BMD analysis was therefore conducted on the four supra-threshold concentrations. In this analysis, a 10%-deviation from baseline is regarded as a critical value. A linear function provides the best fit. A 10% decrease in breathing depth is observed at about 32 mg/m^3. While the

Table 6. Correlation of breathing depth with odor threshold at each concentration

Breathing depth at	Odor threshold Spearman's rho; p in brackets*
.1702 mg/m^3	-.164 (.34)
.3298 mg/m^3	-.088 (.61)
.6331 mg/m^3	.278 (.10)
1.256 mg/m^3	-.393 (.02)
2.290 mg/m^3	-.213 (.21)
4.501 mg/m^3	.032 (.86)
8.592 mg/m^3	-.124 (.47)
17.07 mg/m^3	.109 (.53)
33.58 mg/m^3	.085 (.62)

*significance: $p < .003$ (Bonferroni corrected).

BMD refers to the central estimates, BMD limit (BMDL) computes the confidence limit (95%) for the BMD based on the asymptotic distribution of the likelihood ratio [53]. The BMDL for breathing depth concerning SO_2 is around 25 mg/m^3.

Table 6 reveals no significant correlations (Bonferroni corrected) between breathing depth and odor threshold at any concentration.

The correlations are both positive and negative, and there is no obvious trend.

Discussion

Age-related differences might be absent because the subjects do not differ substantially enough with respect to age. With regard to odor thresholds, Hummel et al. [54] did find differences in odor thresholds for n-butanol between age groups 16-35 and 36-55 years (9.3 vs. 8.8 on a scale ranging from 1 to 16; every step on this scale represented bisection of concentration [dilution ratio 1:2]) and between age groups 16-35 and > 55 years (9.3 vs. 7.3 with lower numbers representing higher odor thresholds). However, as Hummel et al. [54] only report an analysis of composite scores, it is unclear whether or not these differences in odor thresholds are significant.

Nevertheless, the older group in the present study (45-65 years) comprises subjects from both 'older' age groups.

The age groups in this study were chosen to represent young and old workers. van Thriel et al. [48] investigated age groups similar to those in this study and found no correlation between age and the odor threshold for n-butanol. Furthermore, they found no age effect on the ratings for six other substances (acetic acid, propionic acid, formic acid, ethyl acetate, ethyl formate, cyclohexylamine) in an experiment using a similar procedure.

Men and women seem to be influenced by SO_2 in a comparable manner. Though Hummel et al. [54] found gender differences in odor thresholds for n-butanol, this study revealed no such differences for SO_2. Only the ratings of a trigeminal perception, *nasal irritation*, were affected by gender. van Thriel et al. [48] were also unable to demonstrate gender effects on olfactory perceptions (odor intensity, disgust, annoyance) for the substances mentioned above. The only gender effect found was on the pungency ratings, this was in the same direction as the only gender effect reported in this study: in this particular respect, females appear to be more sensitive than men.

Subjects are able to perceive concentration changes. Subjective ratings reveal a dose-effect relationship as seen in the goodness of fit of power functions. There are IELs at 2.29 mg/m^3 though the odor threshold is higher (2.77 mg/m^3). At 2.29 mg/m^3 a number of subjects are already able to smell SO_2 (40% with odor threshold lower than 2.29 mg/m^3). At lower concentrations, the mean ratings might represent apparently random ratings ('noise').

Surprisingly, no clear difference in initial effect level could be found between olfactory and trigeminal perceptions. Even trigeminal perceptions, like *nasal irritation* or pungency, have an initial effect level below the odor threshold. However, olfactory ratings are steeper, demonstrating a more differentiated olfactory rating at the investigated concentrations. As the 'trigeminal' ratings follow the saturation curve, they might be olfactory-driven. Trigeminal activation, as a mechanism in pain transduction, should show a disproportionate increase in ratings. This is because the perception of pain (for example, pain evoked by electric shocks) follows a power curve with exponents between 2 and 3.5 [55]. Sensory irritation elicited by 'real' trigeminal activation might emerge at even higher concentrations.

As there have been no experiments using lateralization to determine sensory irritation threshold for SO_2 [1], the concentration causing reduction in breath intake volume in this study cannot be compared with this measure. van Thriel et al. [12] considered that methods of static olfactometry (i.e., sniffing bottles) to measure lateralization thresholds are artificial when contrasted with the detection of odors in working environments. Two studies using dynamic olfactometry [12,56] reported perceptions of trigeminal symptoms in the subjects at concentrations far below the lateralization threshold determined in static olfactometry. Whether these perceptions were caused by olfactory stimulation or 'real' trigeminal stimulation is not

clear. However, it is possible that simple sniffs, as used in static olfactometry, could lead to an underestimation of trigeminal stimulation due to temporal integration of *nasal irritation* [57,58]. Further experiments are necessary to clarify the role of olfactory and trigeminal stimulation on perceptual ratings.

This study investigated the determination of odor thresholds using breathing depth as an indicator of sensory irritation. This method is a more realistic approach to threshold measurement because in involves several inhalations during a normal breathing rhythm rather than the single sniff required in static olfactometry.

In all subjects, a decrease in breathing depth at around 25 mg/m^3 (BMDL) can be considered an adverse effect. Breathing depth might not be appropriate for an evaluation of the chronic effects of SO_2, but it does prove to be helpful in assessing acute effects using a BMD method. For risk assessment, the BMD approach is recommended by the U.S. Environmental Protection Agency [34]. The BMD method is reported in several reviews [59-61] to be an alternative and favored approach compared with the 'no-observed-adverse-effect-level' (NOAEL) method. While the NOAEL represents the highest experimental dose for which no adverse health effects could be found, the BMD incorporates dose-response information by fitting a mathematical model to experimental dose-response data [34]. Critical effects in the BMD method are usually defined as a 10% deviation from background level [35]. Breathing depth proved to be a suitable indicator for acute effects of an odorous chemical [29,31]. Whether a 10% deviation from baseline in breathing depth is a relevant effect is not clear. Any deviation from baseline caused by an irritant should be avoided. Therefore, an acute evaluation of this deviation has to take into account deviations in normal breathing. The baseline used in this study is the breathing depth observed at the lowest concentrations of SO_2. The 'real' baseline, which should be measured at blank samples, might be higher. Due to technical reasons (adaptive filters adjusting the mean to 1), detection of the 'real' baseline is not possible with the method used. More research is needed to evaluate this deviation. Nevertheless, the suggested method proved to be capable of detecting reductions of respiratory depth. Using only a breast belt, it is possible that these reductions are due to shifting from breast breathing to abdominal breathing, although such a change is unlikely in sitting subjects without any workload. However, an additional abdominal belt should be used in further experiments. Nevertheless, the results showed a concentration-driven change in breathing relative to the breathing movements at the lowest concentration. In combination with refined hypotheses about deviations and with an additional abdominal belt, this method can be a sensitive, valuable tool in risk assessments of local irritants affecting the upper airways.

If breathing movements are regulated voluntarily on the basis of olfactory information and not, as suggested, by reflexes evoked by trigeminal receptors, it could be argued that a low odor threshold would result in a decrease in breathing depth at lower concentrations than would be the case with a high odor threshold. A positive correlation between odor threshold and breathing depth should therefore be noticed, at least at higher concentrations. This could not be confirmed. Instead, on the basis of this study's results, it is possible to argue that respiratory volume might be regulated via reflexes elicited by trigeminal activation. In animal studies, trigeminal activation has been shown to be the cause of protective respiratory reflexes [26-28]. Therefore, the reduction in respiratory volume in our subjects can presumably be related to trigeminal activation.

This method is not limited to occupationally used substances, but can be generalized to naturally occurring substances or environmental pollutants. However, more studies are needed to further validate the method. Whether a 10% deviation in depth of breathing is suitable for such purposes should be topic for further research. Nevertheless, this study suggests a new approach for risk assessment with human subjects using breathing responses.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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