

Intra- and extra-oral halitosis: finding of a new form of extra-oral blood-borne halitosis caused by dimethyl sulphide

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Abstract

Aim: The aim of this study was to unravel the origin and cause of intra-oral and extra-oral halitosis.

Material and Methods: We studied 58 patients complaining of halitosis, using gas chromatography of volatile sulphur compounds (VSCs) in mouth and nose breath, organoleptic scoring of mouth and nose breath, Halimeter[®] readings of mouth air and tongue-coating inspection. Subjects had no presence or history of periodontitis.

Result: Of 58 patients, 47 patients had halitosis of oral origin, six had halitosis of extra-oral origin and five had no halitosis (halitophobia). A strong correlation was found between the degree of intra-oral halitosis as measured by organoleptic scoring of mouth breath and the concentration of the VSCs hydrogen sulphide (H₂S) and methyl mercaptan (CH₃SH) in mouth breath. Taking into account the much larger odour index of CH₃SH, it was concluded that CH₃SH is the main contributor to intra-oral halitosis. In all six cases of extra-oral halitosis, halitosis was caused by the presence of elevated levels of dimethyl sulphide (CH₃SCH₃) in mouth and nose breath.

Conclusion: Our study provides evidence that the VSC, CH₃SH and to a lesser extent H₂S are the main contributors to intra-oral halitosis and that CH₃SCH₃ is the main contributor to extra-oral or blood-borne halitosis, due to a hitherto unknown metabolic disorder.

Key words: dimethyl sulphide; extra-oral halitosis; hydrogen sulphide; methyl mercaptan; oral halitosis

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Halitosis is a concern to millions of people. Most adults suffer from occasional bad breath with an estimated 10–30% of the United States (Meskin 1996) or Chinese (Liu et al. 2006) population experiencing halitosis on a regular basis. This can lead to personal discomfort, embarrassment and social isolation.

Despite these unfortunate consequences, halitosis is unquestionably one of the biggest taboos in our society, yet it has received more attention in the popular literature than in the scientific one. The general population along with many physicians and dentists are still poorly informed about the causes and treatment of halitosis. Many people still believe that halitosis originates in the stomach, which is seldom the case. The oesophagus, which connects the stomach with the mouth, is not an open tube and is normally collapsed (Attia & Marshall 1982), thereby preventing odorous gases to escape from the stomach to the

mouth. Moreover, no evidence exists for the formation of odorous substances in the stomach. Halitosis is rarely a gastrointestinal condition. Regrettably, many patients with halitosis have undergone an unnecessary gastroscopy before visiting a breath clinic. Recently, halitosis received much more scientific attention, especially through the development of the Halimeter[®] (Interscan Corp., Chatsworth, CA, USA), an apparatus to measure odorous volatile sulphur compounds (VSCs) (Rosenberg et al. 1991). Most reports now agree that the most frequent sources of halitosis (80–90%) exist within the oral cavity

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and include bacterial reservoirs such as the dorsum of the tongue, saliva and periodontal pockets, where anaerobic bacteria degrade sulphur-containing amino acids to produce the foul smelling VSCs (Tonzetich 1977, Attia & Marshall 1982, Rosenberg 1996). These VSCs are the predominant elements of oral malodour, although some do believe that other odorous volatiles, such as certain amines and fatty acids, may play a role (Rosenberg & McCulloch 1992, Goldberg et al. 1994, Rosenberg 1996, Greenman et al. 2004). Oral malodour can now be treated effectively (Tonzetich 1977, Richter 1996, Rosenberg 1996, Tangerman 2002, Yaegaki et al. 2002) especially by the use of a tongue scraper and certain mouthrinses (Winkel et al. 2003). In contrast to oral malodour, less attention has been paid to extra-oral halitosis (Attia & Marshall 1982, Preti et al. 1992, Durham et al. 1993, Richter 1996, Rosenberg 1996, Tangerman 2002). Extra-oral halitosis, covering about 10% of all cases of halitosis, might be a manifestation of a serious disease for which treatment is much more complicated. It is of utmost importance to differentiate between intra-oral and extra-oral halitosis. This can be easily carried out by comparing mouth breath with nose breath (Durham et al. 1993, Richter 1996, Rosenberg 1996). Among the host of patients with extra-oral halitosis, only a few have been investigated using analytical techniques to identify the volatile odorous compounds associated with the odour (Tangerman 2002). This is highly important, in order to diagnose the cause and to find a possible treatment. In the literature (Yaegaki & Coil 2000), the term *oral malodour* has been reserved for bad breath originating from the mouth. To clearly differentiate between halitosis of intra- or extra-oral origin, we used the term *intra-oral halitosis* instead of *oral malodour* throughout this article.

Patients and Methods

This study was carried out with the approval of the medical ethical committee of the University Medical Centre Groningen.

Study population

A total of 75 subjects participated in this study. As a reference group, 17 persons

with no complaints of halitosis were selected from the staff (mean age of 38.1 years; range 26–58). The other 58 subjects all had complaints of halitosis and were referred to the Clinic for Periodontology Amsterdam for diagnosis and treatment of halitosis. After a screening session, these patients were consecutively selected on the basis of the following criteria:

- (1) an organoleptic score (OLS) ≥ 1 , using a 0–5 scale (Rosenberg et al. 1991, Yaegaki & Coil 2000);
- (2) a level of VSC > 110 parts per billion (p.p.b.) determined with a portable sulphide monitor (Halimeter[®]).

Probing pocket depths did not exceed 4 mm with the possible exception of distal sites of second molars and pockets around wisdom teeth if present.

Exclusion criteria

- (1) Systemic diseases, pregnancy and systemic medication.
- (2) Systemic antibiotic therapy one month before the study.
- (3) History of periodontal therapy.

Patients were classified in the group of oral halitosis when they had an OLS in mouth breath ≥ 1 and in nose breath < 1 and/or when they had a Halimeter[®] reading > 110 p.p.b., in the group of extra-oral halitosis when they had an OLS in mouth as well as nose breath ≥ 1 and a Halimeter[®] reading < 110 p.p.b., and in the group of halitophobia when they had an OLS in mouth as well as nose breath < 1 and a Halimeter[®] reading < 110 p.p.b.

Screening visit

The purpose of the study was fully explained to the patients who demonstrated their willingness to participate by signing the appropriate informed consent form. During this visit extensive periodontal and halitosis examinations were performed in order to determine whether patients fulfilled the entrance criteria. Patients who met with the inclusion criteria and were willing to participate in the study received written instructions and a detailed medical and halitosis questionnaire. In these instructions subjects were asked not to: (1) consume food containing onions, garlic or hot spices 48 h before the baseline mea-

surements, because of their persistent smell (Tangerman 2002), (2) drink alcohol or smoke in the previous 12 h, (3) perform oral hygiene, including tooth brushing, interdental and tongue cleaning and not to use mouthrinses the morning of the examination, (4) eat and drink in the previous 8 h (drinking water up to 3 h before examinations was allowed) and (5) use scented cosmetics or after-shave lotions in the morning of the examination.

Clinical evaluation

One trained and calibrated examiner (E. G. W.) was responsible for all the clinical measurements, except for organoleptic odour assessments, that was carried out by two trained examiners (A. T. and E. G. W.). The following clinical variables were assessed in order of performance:

1. full mouth and nose organoleptic odour assessments, using the mentioned 0–5 scale (0 = no odour present, 1 = barely noticeable odour, 2 = slight but clearly noticeable odour, 3 = moderate odour, 4 = strong offensive odour, 5 = extremely foul odour). The mean of the scores of the two judges were recorded. Good agreement between the two scores was observed ($\rho = 0.595$, $p < 0.001$),
2. levels of total VSCs scored by means of the Halimeter[®],
3. Winkel tongue coating index (WTCl) (Winkel et al. 2003).

Organoleptic measurements

For the organoleptic evaluation, participants were instructed to close their mouth for 1 min., then to slowly exhale air out of the mouth, at a distance of approximately 10 cm from the nose of the examiner. For evaluation of extra-oral halitosis, patients were also asked to slowly exhale air out of the nose, also at a distance of approximately 10 cm from the nose of the examiner.

VSC measurement

The methods used were gas chromatography and a Halimeter[®]. For gas chromatography, mouth and nose breath was collected in polypropylene-coated sampling balloons. After breathing for 1 min. through the nose, patients were

asked to breath out 500 ml of mouth breath in the balloon. Nose breath was collected by placing a cap over the nose. This cap was connected to the sampling balloon. Patients were then instructed to breath out 500 ml of nose breath via the cap into the balloon, while closing the mouth. Gas chromatography of these samples was performed within 12 h after collection. The concentrations of the VSCs hydrogen sulphide (H₂S), methyl mercaptan (MM) and dimethyl sulphide (DMS) within the sampling balloons remained constant for at least 24 h. This was tested by adding known concentrations of the various VSCs to 500 ml of breath in the balloons. The concentrations of the VSCs inside the balloons were monitored during 24 h. The balloons could be reused after flushing with nitrogen. Gas chromatography of the breath samples was performed as described previously (Tangerman et al. 1983, Tangerman 1986). In short, 100 ml of breath was collected out of the balloons into a large sampling syringe and pre-concentrated onto a Tenax trap tube at -196°C (liquid nitrogen). The trap tube was then inserted in the injection port of the gas chromatograph where the adsorbed sulphur compounds were thermally liberated directly into the carrier gas stream and transferred to the gas chromatographic column (glass column 2 m × 4 mm i.d., packed with 20% SE-30 on Chromosorb P, Chrompack, Middelburg, the Netherlands; 60–80 mesh, column temperature: 80°C). A Packard gas chromatograph, type 429, equipped with a sulphur-specific detector (flame photometric detector; Model 906, Packard Becker, Delft, the Netherlands) was used for analysis of the individual VSCs.

DMS in blood was measured as described previously (Tangerman et al. 1985). In short, 2 ml of venous blood was injected, immediately after sampling, into a stoppered evacuated 15 ml glass vial. The DMS in the headspace was quantitatively concentrated onto a Tenax trap tube and measured by gas chromatography as described above.

The Halimeter[®] measures the level of total VSCs in mouth air. The Halimeter[®] was calibrated to zero on ambient air before each measurement. The patient was asked to close the mouth for 1 min. after which the mouth was opened and the tongue protruded. A disposable straw was placed at the dorsal posterior mid part of the tongue and fixed until the maximum peak value of VSC was recorded. Peak VSC level was registered in p.p.b.

WTCI (Winkel et al. 2003)

The dorsum of the tongue was visually divided into six areas, i.e. three in the posterior and three in the anterior part of the tongue. The tongue-coating in each sextant was scored as 0 = no coating, 1 = light coating and 2 = severe coating. The tongue coating value was obtained by addition of all six scores, range 0–12 (WTCI).

Blood samples

Blood sampling was not a standard procedure in this study. Venous blood samples were only taken from those patients diagnosed as having extra-oral halitosis with elevated levels of DMS, with the main goal of finding out if this extra-oral halitosis is a form of blood-borne halitosis.

Data analysis

Statistical analysis was carried out using SPSS 13.0.1 UK software (SPSS Inc., Chicago, IL, USA). Spearman's rank correlation coefficients (ρ) were calculated to determine the association between individual VSCs from blood, nosebreath and mouthbreath and Halimeter[®] values, OLSs and the WTCI. The non-parametric Spearman test was used because most of the data did not conform to a normal distribution, as shown by a modified Kolmogorov–Smirnov (Lilliefors) test. For testing whether there was a significant difference between patients with intra-oral halitosis, patients with extra-oral halitosis, patients with halitophobia and normal volunteers for the three sulphur gases, the Median test was used.

Results

Of the 58 patients visiting our clinic because of complaints of bad breath, 47 (81%) had oral halitosis, six (10%) had

extra-oral halitosis and five (9%) had no measurable halitosis at all (pseudo-halitosis/halitophobia). As shown in Tables 1 and 2, marked differences were seen in the VSC concentrations between intra- and extra-oral halitosis. Compared with normals (Table 3), both H₂S and MM were significantly elevated ($p < 0.01$) in mouth breath of patients with intra-oral halitosis, reaching values that became objectionable. H₂S and MM were not present in nose-breath, indicating that the origin of these VSCs lies within the oral cavity. The DMS concentrations in intra-oral halitosis were not different from normal and were almost the same in mouth and nose breath, indicating that the origin of DMS lies outside the oral cavity. DMS in oral halitosis did not reach odorous concentrations. Patients with oral halitosis had only bad breath from the mouth, as shown by the elevated OLS for mouth breath. The OLS for nose breath in these patients was normal (below 1).

In contrast to oral halitosis, H₂S and MM concentrations in mouth breath of patients with extra-oral halitosis were low and not different from normal and had no odorous concentrations. Again these VSCs were absent in nose breath. The DMS concentrations in both mouth and nose breath of patients with extra-oral halitosis were significantly elevated compared with normals ($p < 0.001$). Both mouth and nose breath had the distinct smell of DMS. Both odour judges confirmed that simulated gas mixtures with the same concentrations of DMS had an identical odour. In extra-oral halitosis a marked correlation was found between the elevated concentrations of DMS in mouth and nose breath and the OLS of mouth and nose breath (Table 4). Just as was seen in oral halitosis, the DMS concentration in the mouth breath of patients with extra-oral halitosis was nearly identical to that in nose breath with a perfect correlation of

Table 1. Concentration of VSCs (nmol/l)* and OLS in mouth and nose breath of 47 patients (17♂–30♀) with oral halitosis

	Mouth breath		Nose breath	
	mean ± SD	range	mean ± SD	range
H ₂ S	0.86 ± 0.96	0.10–5.75	0.0	–
MM	0.48 ± 0.33	0.06–1.60	0.0	–
DMS	0.30 ± 0.13	0.08–0.57	0.25 ± 0.12	0.05–0.48
OLS	2.11 ± 0.98	1.0–4.0	0.20 ± 0.35	0–0.75

*1 nmol/l = 24.0 p.p.b. at 20°C and 760 mmHg.

VSC, volatile sulphur compound; OLS, organoleptic score; H₂S, hydrogen sulphide; MM, methyl mercaptan; DMS, dimethyl sulphide.

Table 2. Concentration of VSCs (nmol/l) and OLS in mouth and nose breath of six patients (2♂–4♀) with extra-oral halitosis

	Mouth breath		Nose breath	
	mean ± SD	range	mean ± SD	range
H ₂ S	0.11 ± 0.13	0–0.41	0.0	–
MM	0.07 ± 0.04	0–0.22	0.0	–
DMS	1.16 ± 0.72	0.51–2.50	1.19 ± 0.74	0.50–2.52
OLS	2.00 ± 0.63	1.0–3.0	2.00 ± 0.63	1.0–3.0

VSC, volatile sulphur compound; OLS, organoleptic score; H₂S, hydrogen sulphide; MM, methyl mercaptan; DMS, dimethyl sulphide.

Table 3. Concentration of VSCs (nmol/l) in mouth and nose breath of 17 normal volunteers (7♂–10♀) without any complaints of halitosis

	Mouth breath		Nose breath	
	mean ± SD	range	mean ± SD	range
H ₂ S	0.14 ± 0.14	0–0.38	0.0	–
MM	0.15 ± 0.14	0–0.45	0.0	–
DMS	0.21 ± 0.12	0.08–0.47	0.20 ± 0.11	0.07–0.45

VSC, volatile sulphur compound; H₂S, hydrogen sulphide; MM, methyl mercaptan; DMS, dimethyl sulphide.

Table 4. Spearman's rank correlations between the concentrations of DMS in mouth and nose breath, and of DMS in blood and OLS in six patients with extra-oral halitosis

	DMS (nose)	DMS (blood)	OLS [†]
DMS (mouth)	1.000***	0.943**	0.676*
DMS (nose)		0.943**	0.676*

p* < 0.05, *p* < 0.01, ****p* < 0.001.

[†]The OLS in mouth breath was identical to that in nose breath.

OLS, organoleptic score; DMS, dimethyl sulphide.

Table 5. Spearman's rank correlations between the individual VSCs, Halimeter[®] values, organoleptic score (OLS) and Winkel tongue-coating index (WTCl) in 47 patients with oral halitosis

	H ₂ S (mouth)	MM (mouth)	DMS (mouth)	DMS (nose)	Halimeter [®]	OLS (mouth)	WTCl
H ₂ S (mouth)		0.565***	0.340*	0.093	0.527***	0.449**	0.089
MM (mouth)	0.565***		0.364*	0.123	0.544***	0.435**	0.211
DMS (mouth)	0.340*	0.364*		0.853***	0.213	0.371*	0.115
DMS (nose)	0.093	0.123	0.853***		0.028	0.296	–0.017
Halimeter [®]	0.527***	0.544***	0.213	0.028		0.498**	0.277
OLS (mouth)	0.449**	0.435**	0.371*	0.296	0.498**		0.333*
WTCl	0.089	0.211	0.115	–0.017	0.277	0.333*	

p* < 0.05, *p* < 0.01, ****p* < 0.001.

VSC, volatile sulphur compound; H₂S, hydrogen sulphide; MM, methyl mercaptan; DMS, dimethyl sulphide.

1.000 (Table 4), indicating an origin of DMS outside the mouth. As shown in Table 4, a perfect correlation was also found between the elevated DMS in mouth and nose breath and the highly elevated DMS concentration measured

in venous blood [40 ± 25 (mean ± SD), range 10–80 nmol/l; normals: < 7 nmol/l (Tangerman et al. 1985)]. This points to blood-borne halitosis. Patients with extra-oral halitosis had bad breath from both the mouth and the nose, as shown

by the elevated OLS for mouth and nose breath (Table 2). Three patients were examined several times for 2 years with nearly the same outcome, pointing to a chronic form of extra-oral halitosis. The Halimeter[®] values in patients with oral halitosis amounted to 551 ± 560 p.p.b. (range 110–3000) and the WTCl to 6.35 ± 2.97 (range 2–12). Table 5 shows the Spearman rank correlations between the individual VSCs and between the individual VSCs and the other breath parameters in oral halitosis. A highly significant correlation between H₂S and MM in mouth breath means that both VSCs are often simultaneously elevated. No correlation was found between these VSCs and DMS. The highly significant correlation between H₂S and MM and the OLS of mouth breath might indicate a causal relationship between these parameters. The Halimeter[®] readings showed a significant correlation with H₂S and MM but not with DMS. The correlations of the Halimeter[®] readings with OLS of mouth breath and with WTCl were similar to those found for H₂S and MM with these parameters. The presence of tongue coating is one of the main causes of oral halitosis. Only a weak correlation was found between the VSCs H₂S and MM and OLS (mouth) on one hand and the degree of tongue coating as represented by the WTCl on the other hand.

Figure 1 shows some characteristic gas chromatographic spectra of mouth and nose breath of patients with oral- and extra-oral halitosis. Patients with oral halitosis have only elevated concentrations of H₂S and MM in mouth breath. Patients with pure extra-oral halitosis have elevated concentrations of DMS in mouth and nose breath.

The five patients with severe complaints of halitosis but without any detectable halitosis [low, normal values of VSCs (Table 6), Halimeter[®] readings, OLS and WTCl] have been classified in the group of patients with halitophobia.

Discussion

So far, in all reports on oral halitosis, mouth air was sampled for detection of VSCs (Tonzetich 1977, Rosenberg et al. 1991, Yaegaki & Sanada 1992). Subjects were asked to close their mouth for 1 min. while breathing through the nose, resulting in elevated levels of VSCs

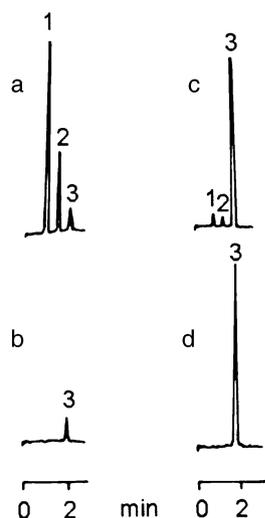


Fig. 1. Gas chromatograms of the mouth breath (a) and nose breath (b) of a patient with oral halitosis, and of the mouth breath (c) and nose breath (d) of a patient with extra-oral halitosis. Peak assignment: 1, H₂S; 2, MM; 3, DMS.

within the oral cavity. After that minute, mouth air was sampled by means of a syringe out of the mouth for gas chromatographic measurement of the separate VSCs or by means of a straw for Halimeter[®] measurements of total VSCs. It was found (Furne et al. 2002) that the volume of gas in the oral cavity averaged 27 ml. To mimic more closely the real situation in patients with oral halitosis, where patients continuously suffer from halitosis in their breath, we used mouth breath instead of mouth air for gas chromatographic detection of VSCs. After breathing for 1 min. through the nose, patients were asked to breath out 500 ml of mouth breath in a polypropylene sampling bag. This procedure leads to VSC concentrations, which are about 19 times lower than those of mouth air, assuming that the amounts of VSCs in this 500 ml of mouth breath are about the same as those in the 27 ml of mouth air. This explains the much lower concentrations of H₂S and MM in mouth breath in this study when compared with those in mouth air from other studies. It must be stressed in this context that the sampled 500 ml of mouth breath consists of more than 90% of alveolar air and for a small part of mouth air. Thanks to our technique of breath pre-concentration before gas chromatography (Tangerman et al. 1983, Tangerman 1986), we were able to measure such lower concentrations.

Table 6. Concentration of VSCs in mouth and nose breath of five patients (1♂–4♀) with halitophobia

	Mouth breath		Nose breath	
	mean ± SD	range	mean ± SD	range
H ₂ S	0.09 ± 0.10	0–0.24	0.0	–
MM	0.08 ± 0.11	0–0.25	0.0	–
DMS	0.22 ± 0.16	0.05–0.47	0.20 ± 0.15	0.07–0.46

VSC, volatile sulphur compound; H₂S, hydrogen sulphide; MM, methyl mercaptan; DMS, dimethyl sulphide.

Disorders of the oral cavity cause 80–90% of all cases of halitosis, whereas about 10% has extra-oral causes (Rosenberg 1996, Tangerman 2002). The percentages found in this study lie in the same range. Extra-oral halitosis might be caused by a serious disease, e.g. liver disease (Chen et al. 1970, Tangerman et al. 1994). Differentiation between intra- and extra-oral halitosis is therefore of utmost importance, also in order to find a suited therapy. These two forms of halitosis can be easily differentiated by inspection of mouth and nose breath. Patients with oral halitosis only have a bad breath in their mouth breath but not in their nose breath. This study shows that the majority of patients with extra-oral halitosis have blood-borne halitosis. Malodorous volatile substances can be absorbed from anywhere in the body into the bloodstream and later transferred to the pulmonary alveoli. Excretion of these volatiles into the alveolar air then causes bad breath in mouth as well as nose breath. Earlier reports (Rosenberg 1996) claimed that nose infection is one of the major causes of extra-oral halitosis. Such patients have only a bad breath in nose breath but not in mouth breath. However, this form of transient halitosis is probably rare. We never saw such patients in our clinic. This form is probably more common in very young children who often insert foreign bodies into their nostrils, which might lead to an offensive odour that comes from the nose (Rosenberg 1996). Besides intra- and extra-oral halitosis, 9% of the patients were found to have no halitosis at all. This condition is known as halitophobia or pseudo-halitosis (Attia & Marshall 1982, Richter 1996, Rosenberg 1996, Yaegaki & Coil 2000, Porter & Scully 2006) and is a recognized psychiatric condition.

Concerning intra-oral halitosis, most reports now agree that the VSCs H₂S and MM and to a lesser extent DMS are the major causative factors in oral halitosis (Tonzetich 1977, Attia & Marshall

1982, Richter 1996, Rosenberg 1996, Yaegaki et al. 2002). Some reports still suggest that other volatile non-sulphur compounds may influence oral halitosis (Rosenberg & McCulloch 1992, Goldberg et al. 1994, Greenman et al. 2004, 2005, Porter & Scully 2006) but this has not been confirmed *in vivo*. In this study we found a strong correlation between the VSCs H₂S and MM and organoleptic scoring, indicating a possible causative relationship between these parameters. Definite proof of such a causative relation was given by inspection of the odour parameters of the VSCs as depicted in Table 7. The VSCs have a very high odour index (OI) and thus a high odour potential. The VSCs have very low threshold values, meaning that these volatiles produce an odour at very low concentrations. In halitosis research the threshold of objectionability is very important. These thresholds as determined by us for H₂S (4 nmol/l) and MM (0.5 nmol/l) were close to those found earlier by Tonzetich (1977) (4.4 nmol/l for H₂S and 1 nmol/l for MM). The concentration of MM in the mouth breath of patients with oral halitosis reached objectionable levels in most patients. For H₂S this was observed in only a few patients. The concentrations of H₂S were generally almost twice as high as those of MM. However, the OI of MM is about three times higher than that of H₂S, the recognition threshold of MM is about 1/30 that of H₂S and the threshold of objectionability is about 1/8 that of H₂S. These values show that MM has a much higher odour potential than H₂S, indicating that MM causes odour problems at much lower concentrations than H₂S. These results suggest that MM is the predominant causative factor in oral halitosis, which is in accordance with earlier findings (Tangerman 2002, Awano et al. 2004). Our own perception was in line with this conclusion. Oral halitosis had more similarity with the pungent smell of MM than with the

Table 7. Odour characteristics of VSCs (Verschueren et al. 1983)

VSC	Odour qualification	Odour index*	100% odour recognition threshold† (nmol/l) (p.p.b.)	Threshold of objectionability‡ (nmol/l) (p.p.b.)
H ₂ S	Rotten eggs	17,000,000	42 (1000)	4 (95)
MM	Pungent, rotten cabbage	53,300,000	1.5 (35)	0.5 (12)
DMS	Unpleasantly sweet	2,760,000	4.2 (100)	1 (24)

*The odour index (OI) represents the ratio of the vapour pressure and the 100% odour recognition threshold, in other words the ratio of the driving force to introduce an odourant into the air *versus* the ability of an odourant to create a recognized response.

†The concentration at which 100% of an odour panel defined the odour as being representative of the studied odourant.

‡The lowest concentration of an odourant producing an objectionable smell, as experimentally determined by us.

VSC, volatile sulphur compound; H₂S, hydrogen sulphide; MM, methyl mercaptan; DMS, dimethyl sulphide.

rotten eggs smell of H₂S. The concentration of DMS in mouth breath of patients with oral halitosis is far below the threshold of objectionability, showing that DMS is not an important factor in causing oral halitosis. The concentration of DMS in nose breath of these patients was similar to that in mouth breath, which means that the bulk of DMS originates outside the mouth. In patients with severe oral halitosis, DMS in mouth breath still tended to be somewhat higher than that in nose breath, although this difference was not statistically significant. A small fraction of DMS might still be formed within the oral cavity, probably by methylation (Weisiger & Jakoby 1981) of MM.

Formation of VSCs by anaerobic bacteria in tongue coating is one of the major causes of oral halitosis. Nearly all patients in our study with oral halitosis had a moderate to severe tongue coating. However, only a weak correlation was found between the degree of tongue coating as measured by the WTCI on the one hand and the VSCs H₂S and MM and OLS on the other hand. This suggests that the presence and the quality of tongue coating is more important in producing oral halitosis than the degree of tongue coating. Besides tongue coating, periodontal disease might also produce oral halitosis (Yaegaki & Sanada 1992), in particular due to elevated levels of MM. These latter patients were excluded from this study.

Previously, we described several known causes of extra-oral halitosis (Tangerman 2002), such as some systemic diseases, some metabolic disorders and the use of certain medications or certain foods such as garlic and onions. All were examples of blood-

borne halitosis. The most important and completely new finding in this study was that in the group of patients visiting our clinic for reasons of bad breath, about 10% were found to have blood-borne extra-oral halitosis of hitherto unknown metabolic origin. These patients were healthy, had no systemic disease and no known metabolic disorder like hypermethioninemia (Mudd et al. 1995) and did not use any medication or food like garlic or onions during our investigation. In all six patients DMS was found to be the only causative factor of this extra-oral halitosis. A perfect correlation between DMS in mouth and nose breath and OLS of mouth and nose breath points to such a causative relationship. Definite proof was provided by simulating breath samples by producing experimental gas mixtures with the same DMS concentrations as found in the breath samples. These experimental gas mixtures had exactly the same unpleasantly sweet smell of DMS as the mouth and nose breath of these patients.

Mouth and nose breath of these patients, containing the same elevated DMS concentrations, had exactly the same typical smell of DMS. These patients had pure extra-oral halitosis without contaminating oral halitosis. The concentrations of H₂S and MM in mouth breath of these patients were very low and below the odorous thresholds. Of course, patients might be seen with both oral and extra-oral halitosis. The odour of mouth and nose breath of such patients will differ, because mouth breath will contain odorous elevated levels of H₂S and MM due to the oral component and of DMS due to the extra-oral component whereas

nose breath will only contain odorous levels of DMS due to the extra-oral component.

The DMS in patients with extra-oral halitosis originates from the blood as proved by the perfect correlation between elevated breath DMS and blood DMS values. Such a correlation was also found in the past for cirrhotic patients (Tangerman et al. 1985). The nature of the VSC DMS (CH₃SCH₃) in extra-oral halitosis differs from that of the VSCs H₂S and MM (CH₃SH) in oral halitosis. The thiols H₂S and MM cannot be found in blood-borne extra-oral halitosis because these thiols are highly unstable in blood. In vitro experiments have shown (Blom & Tangerman 1988) that the thiol MM, containing a free-SH group, immediately reacts with whole blood within seconds, resulting in irreversible binding and oxidation to sulphate, thereby preventing transportation of MM from the blood into alveolar air and thus into breath. The same holds for H₂S. This is also the reason why H₂S and MM were absent in nose breath of all subjects. Nose breath mainly contains volatiles originating from blood. In contrast to the "oral" volatiles H₂S and MM, DMS is a neutral compound, which is stable in blood and can be transported from blood into alveolar air and breath. The neutral nature of DMS is also the main reason for the difficulty of removing DMS from the breath, which in contrast with the very reactive thiols, MM and H₂S, found in oral malodour. The latter thiols can be effectively removed from the breath, for example by binding with zinc salts (Yaegaki et al. 2002, Winkel et al. 2003) or by oxidation (Richter 1996, Greenstein et al. 1997). To develop a future therapy for the extra-oral halitosis found in this study, it is important to know where the elevated DMS is formed in the body and to investigate the reason of the elevated DMS. This will be the subject of further studies. The presented extra-oral halitosis is the predominant form of extra-oral halitosis and is probably caused by a hitherto unknown metabolic disorder. The patients do suffer all the time from this form of blood-borne halitosis. Examples of other known metabolic disorders presenting with bad breath are isolated persistent hypermethioninemia (Mudd et al. 1995) where the bad breath is also caused by elevated DMS and the fish odour syndrome (Mitchell & Smith 2001) where the bad breath is caused by

elevated trimethylamine. These latter metabolic disorders are rare, which contrasts with the one presented here. Assuming that about 10–30% of the general population suffers from bad breath on a regular basis (Meskin 1996, Liu et al. 2006) and that 5–10% of this halitosis population has extra-oral halitosis, one calculates a percentage of 0.5–3 of the general population having some form of extra-oral halitosis. Assuming that the here-presented form of extra-oral halitosis is the predominant one, one might expect a percentage between 0.25 and 1.5 of the general population to have this type of extra-oral halitosis.

Gas chromatography is the method of choice in halitosis research. It distinguishes between the individual VSCs, which is highly important for the differentiation between intra- and extra-oral halitosis. Without the use of gas chromatography we would never have found the newly discovered extra-oral blood-borne halitosis. Organoleptic scoring has often been postulated as the “gold standard” in halitosis (Rosenberg & McCulloch 1992, Van Steenberghe 1997). The fairly good correlations between the individual VSCs as measured by gas chromatography and OLS in intra-as well as extra-oral halitosis shows that in the clinical setting OLS might be a good substitute for gas chromatography, provided that a trained odour panel of at least two judges is used. The use of the Halimeter[®] has more limitations, especially in the field of extra-oral halitosis. The Halimeter[®] is most sensitive for H₂S, then for MM and the least for DMS. It underestimates MM by about 31% but it markedly underestimates DMS concentrations by some 70% (Furne et al. 2002). In oral halitosis good correlations were observed between the Halimeter[®] values on the one hand and the primary VSCs H₂S and MM, and OLS on the other hand. The Halimeter[®] is therefore a suitable apparatus for clinical studies on oral halitosis. However, the Halimeter[®] values measured in extra-oral halitosis were all below 110 p.p.b. and thus in the “normal” Halimeter[®] range according to the manufacturer (<http://www.halimeter.com/halcal.htm>). Besides underestimation of DMS by the Halimeter[®], the highest DMS concentration in extra-oral halitosis amounted to 2.5 nmol/l (60 p.p.b.), thus far below the Halimeter[®] limit for halitosis of 110 p.p.b., making the Halimeter[®]

totally unsuitable for the detection of extra-oral halitosis.

In conclusion, the present study shows a clear differentiation between oral- and extra-oral halitosis. In oral halitosis, MM (CH₃SH) in mouth breath is the predominant causative factor of oral malodour, more so than H₂S. Gas chromatography of the individual VSCs combined with OLS is the best method to detect oral halitosis. Halimeter[®] measurements and tongue-coating assessments may also be useful methods in the clinical setting. The new finding outlined in this study is that the majority of chronic extra-oral halitosis is caused by a hitherto unknown extra-oral blood-borne halitosis, probably due to a metabolic disorder, resulting in elevated odorous levels of DMS in blood and breath. This blood-borne halitosis could only be detected by the use of gas chromatography and not by Halimeter[®] measurements. Further studies will focus on unravelling the cause of this halitosis in order to find a suited therapy for this form of blood-borne halitosis.

References

- Attia, E. L. & Marshall, K. G. (1982) Halitosis. *Canadian Medical Association Journal* **126**, 128–135.
- Awano, S., Koshimune, S., Kurihara, E., Gohara, K., Sakai, A., Soh, I., Hamasaki, T., Ansai, T. & Takehara, T. (2004) The assessment of methyl mercaptan, an important clinical marker for the diagnosis of oral malodor. *Journal of Dentistry* **32**, 555–559.
- Blom, H. J. & Tangerman, A. (1988) Methanethiol metabolism in whole blood. *Journal of Laboratory and Clinical Medicine* **111**, 606–610.
- Chen, S., Zieve, L. & Mahadevan, V. (1970) Mercaptans and dimethylsulfide in the breath of patients with cirrhosis of the liver. *Journal of Laboratory and Clinical Medicine* **75**, 628–635.
- Durham, T. M., Malloy, T. & Hodges, E. D. (1993) Halitosis: knowing when ‘bad breath’ signals systemic disease. *Geriatrics* **48**, 55–59.
- Furne, J., Majerus, G., Lenton, P., Springfield, J., Levitt, D. G. & Levitt, M. D. (2002) Comparison of volatile sulfur compound concentrations measured with a sulfide detector vs. gas chromatography. *Journal of Dental Research* **81**, 140–143.
- Goldberg, S., Kozlovsky, A., Gordon, D., Gelernter, I., Sintov, A. & Rosenberg, M. (1994) Cadaverine as a putative component of oral malodor. *Journal of Dental Research* **73**, 1168–1172.
- Greenman, J., Duffield, J., Spencer, P., Rosenberg, M., Corry, D., Saad, S., Lenton, P., Majerus, G., Nachnani, S. & El-Maaytah, M. (2004) Study on the organoleptic intensity scale for measuring oral malodor. *Journal of Dental Research* **83**, 81–85.
- Greenman, J., El-Maaytah, M., Duffield, J., Spencer, P., Rosenberg, M., Corry, D., Saad, S., Lenton, P., Majerus, G. & Nachnani, S. (2005) Assessing the relationship between concentrations of malodor compounds and odor scores from judges. *Journal of the American Dental Association* **136**, 749–757.
- Greenstein, R. B., Goldberg, S., Marku-Cohen, S., Sterer, N. & Rosenberg, M. (1997) Reduction of oral malodor by oxidizing lozenges. *Journal of Periodontology* **68**, 1176–1181.
- Liu, X. N., Shinada, K., Chen, X. C., Zhang, B. X., Yaegaki, K. & Kawaguchi, Y. (2006) Oral malodor-related parameters in the Chinese general population. *Journal of Clinical Periodontology* **33**, 31–36.
- Meskin, L. H. (1996) A breath of fresh air. *Journal of the American Dental Association* **127**, 1282, 1284–1286.
- Mitchell, S. C. & Smith, R. L. (2001) Trimethylaminuria: the fish malodor syndrome. *Drug Metabolism and Disposition* **29**, 517–521.
- Mudd, S. H., Levy, H. L., Tangerman, A., Boujet, C., Buist, N., Davidson-Mundt, A., Hudgins, L., Oyanagi, K., Nagao, M. & Wilson, W. G. (1995) Isolated persistent hypermethioninemia. *American Journal of Human Genetics* **57**, 882–892.
- Porter, S. R. & Scully, C. (2006) Oral malodour (halitosis). Clinical review. *British Medical Journal* **333**, 632–635.
- Preti, G., Clark, L., Cowart, B. J., Feldman, R. S., Lowry, L. D., Weber, E. & Youg, I. M. (1992) Non-oral etiologies of oral malodor and altered chemosensation. *Journal of Periodontology* **63**, 790–796.
- Richter, J. L. (1996) Diagnosis and treatment of halitosis. *Compendium of Continuing Education in Dentistry* **17**, 370–388.
- Rosenberg, M. (1996) Clinical assessment of bad breath: current concepts. *Journal of the American Dental Association* **127**, 475–482.
- Rosenberg, M., Kulkarni, G. V., Bosy, A. & McCulloch, C. A. (1991) Reproducibility and sensitivity of oral malodor measurements with a portable sulfide monitor. *Journal of Dental Research* **70**, 1436–1440.
- Rosenberg, M. & McCulloch, C. A. (1992) Measurement of oral malodor: current methods and future prospects. *Journal of Periodontology* **63**, 776–782.
- Tangerman, A. (1986) Determination of volatile sulphur compounds in air at the parts per trillion level by tenax trapping and gas chromatography. *Journal of Chromatographic Science* **366**, 205–216.
- Tangerman, A. (2002) Halitosis in medicine: a review. *International Dental Journal* **52** (Suppl. 3), 201–206.
- Tangerman, A., Meuwese-Arends, M. T. & Jansen, J. B. (1994) Cause and composition of foetor hepaticus. *Lancet* **343**, 483.
- Tangerman, A., Meuwese-Arends, M. T. & Tongeren, J. H. M. (1983) A new sensitive assay for measuring volatile sulphur compounds in human breath by Tenax trapping and gas chromatography and its application

- in liver cirrhosis. *Clinica Chimica Acta* **130**, 103–110.
- Tangerman, A., Meuwese-Arends, M. T. & van Tongeren, J. H. M. (1985) New methods for the release of volatile sulfur compounds from human serum: its determination by Tenax trapping and gas chromatography and its application in liver diseases. *Journal of Laboratory and Clinical Medicine* **106**, 175–182.
- Tonzetich, J. (1977) Production and origin of oral malodor: a review of mechanisms and methods of analysis. *Journal of Periodontology* **48**, 13–20.
- Yaegaki, K. & Coil, J. M. (2000) Examination, classification, and treatment of halitosis: clinical perspectives. *Journal of the Canadian Dental Association* **66**, 257–261.
- Yaegaki, K., Coil, J. M., Kamemizu, T. & Miyazaki, H. (2002) Tongue brushing and mouth rinsing as basic treatment measures for halitosis. *International Dental Journal* **52** (Suppl. 3), 192–196.
- Yaegaki, K. & Sanada, K. (1992) Volatile sulfur compounds in mouth air from clinically healthy subjects and patients with periodontal disease. *Journal of Periodontal Research* **27**, 233–238.
- Van Steenberghe, D. (1997) Breath malodor. *Current Opinion in Periodontology* **4**, 137–143.
- Verschuere, K. (1983) *Handbook of Environmental Data on Organic Chemicals*, 2nd edition. New York: Van Nostrand Reinhold Company, pp. 52–59.
- Weisiger, R. A. & Jakoby, W. B. (1981) Thiol S-methyltransferase. *Methods in Enzymology* **77**, 257–262.
- Winkel, E. G., Roldán, S., Van Winkelhoff, A. J., Herrera, D. & Sanz, M. (2003) Clinical effects of a new mouthrinse containing chlorhexidine, cetylpyridinium chloride and zinc-lactate on oral halitosis. A dual-centre double-blind placebo-controlled study. *Journal of Clinical Periodontology* **30**, 300–306.

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Clinical Relevance

Scientific rationale for the study: In this study we have tried to unravel the origin and cause of intra- and extra-oral halitosis.

Principal findings: MM is the main contributor to intra-oral halitosis.

Presence of elevated levels of DMS in mouth and nose breath was clearly related to extra-oral halitosis. This was due to a hitherto unknown metabolic disorder.

Practical implications: A gaschromatograph is needed to differentiate

between intra- and extra-oral halitosis. This differentiation is of paramount importance for selecting the proper therapy. In practice the Halimeter[®] is only useful to monitor intra-oral halitosis patients.