

Day-Long Reduction of Oral Malodor by a Two-Phase Oil:Water Mouthrinse as Compared to Chlorhexidine and Placebo Rinses

Mel Rosenberg,* Ilana Gelemter,[†] Mira Barki,* and Ronit Bar-Ness*

FEW SCIENTIFIC INVESTIGATIONS HAVE ADDRESSED THE ability of mouthrinses to reduce oral malodor for periods longer than 3 hours. In the present report, we have employed simple, recently described techniques to assess the day-long reduction in oral malodor of a novel 2-phase oil:water mouthrinse (TPM), as compared to a corresponding placebo rinse, and to a commercial 0.2% chlorhexidine mouthrinse. Sixty dental students were divided randomly into 3 groups, and instructed to use one of the rinses prior to bedtime and the following morning. Measurements carried out in the late afternoon, about 8 to 10 hours following rinsing, were compared with baseline measurements carried out in the late afternoon of the previous day. Volatile sulphide levels were measured using a portable industrial sulphide monitor. Microbial levels were estimated using a simple rinsing technique employing sterilized milk. These quantitative techniques were corroborated by organoleptic (hedonic) ratings of a single odor judge. Both TPM and chlorhexidine brought about significant decreases in volatile sulphides ($P < 0.05$) as compared to the placebo group. These results were corroborated by the organoleptic data. Similarly, both chlorhexidine and TPM were highly effective in reducing microbial levels as measured by the rinsing technique, in comparison to the placebo group. Chlorhexidine appeared to be more effective than TPM in all measurement categories, although only in the case of microbial activity was there a significant ($P < 0.05$) difference between the two groups. The data showing daylong reduction of malodorous sulphides and microbial levels by TPM extend previous in vitro studies demonstrating its potent microbial desorption properties, and provide the first evidence for its potential clinical efficacy. *J Periodontol* 1992;63:39-43.

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Mouthrinsing is a common oral hygiene practice dating back to ancient times.¹ Although, in recent years, the plaque and gingivitis-reducing properties of various mouthrinses have been emphasized,¹⁻³ one of the major concerns which leads to frequent use of mouthrinses is halitosis (bad breath).² Bad breath usually originates within the oral cavity itself, due to production of gases (primarily volatile sulfur compounds, VSC) by sequestered deposits of microorganisms, generally under anaerobic conditions.^{4,5} Periodontal diseases commonly result in bad breath.^{4,6} Other loci of oral

microbial putrefaction include the tongue dorsum, areas of food impaction, and improper dental restorations.⁴

Much of the evidence for the efficacy of mouthrinses in reducing bad breath is anecdotal⁷ and there are very few publications in the scientific literature on this subject. Long-term malodor reduction has rarely, if ever, been addressed. For example, in the mouthrinse studies of Pitts et al.,⁸ Schmidt and Tarbet,⁹ and Tonzetich,¹⁰ data on malodor reduction were given for up to 3 hours following use.

We have recently shown that 2-phase, oil:water formulations containing low concentrations of cationic surfactants such as cetylpyridinium chloride efficiently bind¹¹ and desorb^{12,13} oral microorganisms. In the present study, we have compared the long-term effect of one such 2-phase

*Laboratory of Oral Microbiology, The Maurice and Gabriela Goldschleger School of Dental Medicine, and the Department of Human Microbiology, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

[†]Statistical Laboratory, School of Mathematical Sciences, Sackler Faculty of Exact Sciences.

40 EFFECT OF MOUTHRINSES ON ORAL MALODOR

Pre-Rinsing Measurements (16:00-18:00)	Bedtime Rinsing (-23:00)	Morning Rinsing (-08:00)	Post-Rinsing Measurements (16:00-18:00)
Day 1		Day 2	

Figure 1. Summary of experimental design.

mouthwash (TPM)² formulation in reducing malodor and microbial levels with a placebo rinse² and an antiseptic mouthrinse containing 0.2% chlorhexidine gluconate (Corsodyl) (CHM).⁵ A portable, industrial sulphide monitor¹⁴ was employed to measure volatile sulphides; these measurements were corroborated by organoleptic (hedonic) measurements by a single judge. The effect of the mouthrinses on microbial levels was assessed using the Oratest.^{15,16} The results demonstrate that mouthrinsing with appropriate formulations can result in day-long reduction in bad breath-associated parameters.

MATERIALS AND METHODS

Experimental Design

The experimental design of the present study is summarized in Figure 1. The study took place on March 15 and 16, 1989 at Tel-Aviv University. Sixty dental student volunteers were randomly divided into 3 groups: one group (n = 22) received TPM; the second (n = 19) placebo rinse, and the third (n = 19) CHM.⁵ No exclusion criteria were employed. The 2-phase mouthwash formulation consisted of an aqueous phase containing cetylpyridinium chloride (0.05%), food color which also serves as a biological stain (FD&C blue No. 1),¹⁶ and artificial sweetener, overlaid with a discrete oil phase containing a mixture of olive oil and essential oils. The placebo rinse contained the same concentrations of food color and sweetener, but without the CPC and oil phase. All mouthrinses were provided in standard brown glass bottles. Volunteers rinsed for two consecutive 30-second periods, directly before bedtime on day 1, and again early the next morning of day 2. Volunteers were instructed to shake the bottles prior to use, and to refrain from rinsing with water, eating, or drinking for at least 30 minutes following rinsing. No other changes regarding oral hygiene were instructed. Pre-rinsing measurements were performed in the late afternoon of day 1. Post-rinsing measurements were performed in the late afternoon of day 2; i.e., about 8 to 10 hours following the previous morning rinse.

Measurements

Quantitative measurements of volatile sulphides were carried out using the Interscan 1170 monitor,¹⁴ 1 ppm full-scale, connected to a pen recorder. Measurement was car-

ried out essentially as previously reported,¹⁴ except that disposable straws, rather than Teflon tubing, were employed. This instrument is based on an enclosed liquid electrochemical cell, through which the gas sample passes at a constant flow rate, and is sensitive to both hydrogen sulphide and mercaptans (M. Shaw, personal communication Oct 28, 1991.). Volunteers were asked to refrain from talking for 5 minutes prior to measurement. The monitor was zeroed on ambient air, and measurement performed by inserting a disposable 1/4" plastic straw approximately 4 cm into the partially opened oral cavity. The volunteer was asked to breathe through his/her nose during measurement. Results were recorded as peak ppb sulphide equivalents.

Organoleptic estimations were carried out by a single judge, following monitor measurements, as previously described.¹⁴ For organoleptic rating, volunteers were instructed to exhale briefly through the mouth, at a distance of approximately 10 cm from the nose of the judge. Organoleptic results were rated on a scale of 0 to 5 as follows: 0 = no appreciable odor; 1 = barely noticeable odor; 2 = slight, but clearly noticeable odor; 3 = moderate odor; 4 = strong odor; and 5 = extremely foul odor. All measurements were conducted in a blind fashion.

Microbial levels were estimated using the Oratest, a technique which measures the rate of oxygen depletion in expectorated milk samples. The technique has been previously described and shown to correlate with microbial counts and clinical parameters.^{15,16} Briefly, volunteers rinsed for 30 seconds with 10 ml of sterile milk. Following expectoration, 3 ml of the sample were added to test tubes containing 0.12 ml of methylene blue solution (0.1%), and the time required for a blue-to-white color change over a 6 mm diameter at the bottom of the test tube was recorded. The test was performed directly following the volatile sulphide measurements.

Statistical Analyses

One way analysis of variance (ANOVA) was used to determine significant effects among the three groups. Pairwise comparisons between groups were made using the Scheffe procedure, at the $P = 0.05$ significance level. Spearman correlation coefficients were employed to test for correlations among the results of the different measurement techniques.

RESULTS

Comparisons of the effects of oral rinsing with the placebo mouthwash, TPM, and CHM, as measured by the sulphide monitor, the Oratest, and organoleptic assessment, are shown in Tables 1 through 3, respectively. Initially, the CHM group gave slightly higher sulphide and organoleptic scores, as well as shorter Oratest times; these baseline differences, however, were not significant (ANOVA). Similarly, there were no significant differences among the three groups in the number of hours which had passed since last eating or drinking, prior to the tests. The results of the sulphide mea-

²AGIS, Ltd. Tel-Aviv, Israel.⁵Corsodyl, ICI, Imperial Chemical Industry, PLC, Macclesfield, UK.

Tiberias Medical Center, Tiberias, Israel.

¹⁴Interscan Corp, Chatsworth, CA.

Table 1. Sulphide Monitor Measurements*

	Placebo Rinse (n = 19)	TPM (n = 22)	CHM (n = 19)	P(ANOVA)
Before rinsing	1.691 ± 0.372	1.664 ± 0.232	1.717 ± 0.306	0.8595
Change	0.041 ± 0.316	-0.205 ± 0.225*	-0.308 ± 0.292*	0.0009

*Results are presented as mean log ppb sulphide equivalents ± standard deviation.
*Significantly different from placebo at $P < 0.05$ (Scheffe procedure).

Table 2. Oratest Measurements*

	Placebo Rinse (n = 19)	TPM (n = 22)	CHM (n = 19)	P(ANOVA)
Before rinsing	1.936 ± 0.499	1.943 ± 0.420	1.708 ± 0.251	0.1166
Change	-0.218 ± 0.367	0.456 ± 0.572*	1.009 ± 0.489*	0.0001

*Results are expressed as mean log minutes required for color change ± standard deviation.
*Significantly different from placebo at $P < 0.05$ (Scheffe procedure).
*Significantly different from both placebo and TPM at $P < 0.05$ (Scheffe procedure).

Table 3. Organoleptic Scores*

	Placebo Rinse (n = 19)	TPM (n = 22)	CHM (n = 19)	P(ANOVA)
Before rinsing	1.316 ± 1.293	1.500 ± 1.058	1.737 ± 1.240	0.5556
Change	0.053 ± 1.129	-0.500 ± 1.535	-1.316 ± 1.250*	0.0091

*Scores were recorded on a scale of 0 to 5 as described in Materials and Methods. Results are presented as mean scores ± standard deviation.
*Significantly different from placebo at $P < 0.05$ (Scheffe procedure).

Table 4. Correlations of Baseline Measurements Among the Techniques Employed*

	Oratest	Organoleptic Rating
Sulphide measurement	$r = -.0977$ $P = 0.229$	0.5954 $P < 0.001$
Oratest		-0.1164 $P = 0.188$

*Spearman correlations among the baseline data are presented for all 60 participants. The levels of significance are presented in italics.

Table 5. Correlations Between Individual Changes (post-rinse vs. pre-rinse) Among the Techniques Employed*

	Oratest	Organoleptic Rating
Sulphide measurement	$r = -0.5449$ $P < 0.001$	0.4238 $P < 0.001$
Oratest		-0.3541 $P = 0.003$

*Spearman correlations among the individual changes (post-rinse vs. pre-rinse) among the three techniques are presented for all 60 participants. The levels of significance are presented in italics.

measurements and Oratest were transformed into base 10 logarithmic values to obtain a normal distribution.¹⁴⁻¹⁶

Comparison of the three groups, based on sulphide measurements, is summarized in Table 1. The overall decrease in sulphide levels following rinsing was statistically significant in both TPM and CHM groups as compared to the

placebo group. No significant difference was found in the reductions in the TPM versus CHM groups.

Table 2 shows the comparison among the three groups, based on Oratest scores. Higher Oratest values indicate lower microbial levels,¹⁵ and correspond to lower plaque and gingival scores.¹⁶ No differences were found among the groups in the pre-rinsing scores (ANOVA). However, the overall changes in Oratest scores of both TPM and CHM groups were significantly increased with respect to the placebo group. The increase in Oratest scores was significantly greater in the CHM, as opposed to TPM, group.

Comparison of the organoleptic assessments of the three groups is presented in Table 3. Large variations in scores were obtained in all groups. Nevertheless, the decrease in scores in the CHM group was significant with respect to the placebo (but not TPM) group.

Correlations among the measurement techniques themselves are summarized in Tables 4 and 5. Correlations among the baseline (pre-rinse) measurements are shown in Table 4. Correlations among the individual changes (post-rinsing vs. pre-rinsing) recorded by the various measurement techniques are provided in Table 5. As expected, sulphide monitor scores and organoleptic ratings provided highly significant correlations, both in baseline results (Table 4), as well as in the individual changes recorded by the two techniques (Table 5). Baseline correlations between Oratest scores and the other two measurements were not significant (Table 4). However, as shown in Table 5, the changes recorded by

the Oratest correlated significantly with those obtained using the sulphide monitor ($r = 0.5490$, $P < 0.001$) and organoleptic assessment ($r = 0.3302$, $P = 0.005$).

DISCUSSION

In the present manuscript, we have assessed day-long reductions in malodor-associated variables, following rinsing with two active mouthwashes, as opposed to a placebo rinse. Sixty volunteers, randomly divided into three groups, rinsed before bedtime and the following morning with one of the mouthwashes. Measurements carried out some 8 to 10 hours later (i.e., in the late afternoon) were compared with baseline measurements carried out at about the same time on the previous day. Measurements were carried out in a blind fashion using two recently developed techniques, measurement of oral sulphides using a portable monitor,¹⁴ and measurement of oral microbial levels, based on the rate of oxygen consumption in expectorated milk samples.^{15,16} These measurements were corroborated by organoleptic (hedonic) ratings of a single odor judge (RB) who participated in previous investigations.¹⁴

The two-phase oil:water mouthrinse (TPM) formulation employed in the present study is based on previous research showing that: 1) a high proportion of oral microorganisms adhere to oil droplets;¹⁷ 2) this adhesion can be further enhanced by appropriate concentrations of cetylpyridinium chloride;¹¹ and 3) in a comparison of desorption of a model bacterial film by the formulation, as compared to 10 commercial mouthrinses, TPM formulations proved highly effective.¹² Furthermore, TPM formulations reduced malodor levels in pilot studies (data not shown). In the present study, the 2-phase mouthwash was compared with a placebo rinse, containing the same color and sweetening agent as TPM, but without the active agents; i.e., cetylpyridinium chloride and the oil phase. As a positive control, Corsodyl was selected, because of previous reports of malodor reduction by chlorhexidine-containing sprays⁷ and dentifrices¹⁸ and the excellent substantive antibacterial properties reported for chlorhexidine formulations.¹⁹

The results showed that TPM significantly reduced oral sulphides, as compared with the placebo rinse. CHM appeared to be somewhat more effective than TPM in reducing sulphide levels, although the two groups were not significantly different following the rinsing regimen. The reduction in sulphides was corroborated by the decrease in organoleptic scores, which was significant in the case of CHM. The effect of CHM in reducing organoleptic ratings appeared to be more pronounced than that of TPM, although the difference between the two groups was not significant.

Both mouthrinses brought about highly significant, dramatic decreases in the level of oral microorganisms, as determined by the Oratest. Initial studies have shown that this test is extremely sensitive to reductions in oral microbiota following mouthrinsing.²⁰ Indeed, the drastic decreases brought about by the chlorhexidine rinse were

significantly different than both TPM and placebo results. Similar reductions in levels of aerobic oral microbiota up to 5 hours following rinsing with a 0.12% chlorhexidine mouthrinse were recently demonstrated by Kayrouz et al.¹⁹ However, in an in vitro desorption experiment, Corsodyl removed only 15% of the microbial film, as opposed to 98% for TPM.¹² These data, taken together, suggest that the dramatic reductions in malodor observed with the chlorhexidine are due primarily to its substantive, long-term antibacterial effect, and relatively high concentration (0.2%), rather than its microbial desorption ability.

Of additional interest are the correlations between the various measurement techniques employed (Tables 4 and 5). We have recently demonstrated that the results obtained with the sulphide monitor are highly significantly related to the average scores of an organoleptic panel of seven judges.¹² Similar correlations were found here between sulphide levels and organoleptic ratings, both in baseline measurements, as well as in the changes measured by either technique. Recently, in a study of 41 subjects complaining of bad breath, among the malodor and periodontal-related tests employed, sulphide monitor results were the most reproducible.²¹ These data, taken together, support the use of sulphide monitors in chairside measurement, and clinical investigation.

The correlation of Oratest scores to the results of the other two techniques appears to be more complex. Whereas baseline correlations provided non-significant correlations between Oratest and both organoleptic as well as sulphide scores (Table 4), the individual changes measured by the Oratest provided highly significant correlations to the changes in both organoleptic and sulphide scores (Table 5). Previous studies have shown that the Oratest yields highly significant correlations with oral microbial counts,¹⁵ as well as plaque and gingival index;¹⁶ plaque index was significantly correlated with malodor in a 1988 study.² Moreover, in an initial study encompassing a population of 40 patients complaining of bad breath, modest but significant correlations were observed between Oratest and sulphide monitor levels ($r = -0.454$; $P < 0.01$) as well as with organoleptic scores ($r = 0.459$; $P < 0.01$) (Rosenberg and Barki, unpublished data). Finally, since both malodor and the Oratest appear to be related to oxygen depletion in the oral cavity,^{9,15} a correlation between the two parameters might be anticipated. One possibility for the poor baseline correlation found here may be the population studied; i.e., a relatively homogeneous, non-symptomatic group of dental students. Indeed, one of the problems associated with clinical malodor studies is subject populations.⁴ The above notwithstanding, the changes measured by the Oratest appear to be highly correlated with changes measured by the other two techniques (Table 5). Thus, while the extent to which the Oratest measures a function which is physiologically related to sulphide or organoleptic scores is unclear from the data presented here, the changes in scores of all three techniques, appear to be related (Table 5).

In summary, the data suggest that both the mouthrinses tested brought about day-long, statistically significant reductions in volatile sulphide and microbial levels, as compared to the placebo rinse control. Both mouthrinses brought about corresponding decreases in organoleptic scores, but only in the case of CHM was the decrease significant. Whereas only in the Oratest category were the results of CHM significantly different from those of TPM, the chlorhexidine rinse appeared more effective in all techniques tested. To our knowledge, the data presented are the first demonstrating day-long reduction of malodor by mouthrinses. Chlorhexidine rinses have several undesirable side effects (discoloration, changes in taste perception, occasional irritation, and desquamation) and are usually not advised for continuous, long-term usage.¹ However, a short-term chlorhexidine rinsing regimen may be of potential diagnostic benefit in helping to determine whether the present malodor is of an oral etiology.²¹

Two-phase oil:water combinations such as that studied here have several possible advantages over single-phase mouthwashes: 1) their relative effectiveness in binding and desorbing oral microorganisms;^{11,12} 2) since the bacteria and oral debris are stained blue by the food color, they can be macroscopically and microscopically observed to adhere to the oil droplets following rinsing, affording direct observation and self-assessment;¹³ and 3) the TPM formulations do not contain alcohol, a substance which has potentially adverse effects: e.g., dehydration, addiction, and irritation of oral and gastrointestinal mucosa. The present study provides the first clinical data supporting the potential use of TPM formulations for reduction of malodor and microbial levels. Further studies on the clinical effects of 2-phase oil:water formulations are in progress.

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REFERENCES

- Mandel ID. Chemotherapeutic agents for controlling plaque and gingivitis. *J Clin Periodontol* 1988; 15:488-498.
- Wennstrom JL. Mouthrinses in "experimental gingivitis" studies. *J Clin Periodontol* 1988; 15:511-516.
- Overholser CD Jr. Longitudinal clinical studies with antimicrobial mouthrinses. *J Clin Periodontol* 1988; 15:517-519.
- Tonzetich J. Production and origin of oral malodor: a review of mechanisms and methods of analysis. *J Periodontol* 1977; 48:13-20.
- Kostelc JG, Preti G, Zelson PR, Brauner L, Baehni P. Oral odors in early experimental gingivitis. *J Periodont Res* 1984; 19:303-312.
- Kleinberg I, Westbay G. Oral malodor. *Crit Rev Oral Biol Med* 1990; 1:247-260.
- Dever JG. Oral hygiene in mentally handicapped children. A clinical trial using a chlorhexidine spray. *Australian Dent J* 1979; 24:301-305.
- Pitts G, Brogdon C, Hu L, Masurat T, Pianotti R, Schumann P. Mechanism of action of an antiseptic, anti-odor mouthwash. *J Dent Res* 1983; 62:738-742.
- Schmidt NF, Tarbet WJ. The effect of oral rinses on organoleptic mouth odor ratings and levels of volatile sulfur compounds. *Oral Surg Oral Med Oral Path* 1978; 45:876-883.
- Tonzetich J. Oral malodor: an indicator of health status and oral cleanliness. *Int Dent J* 1977; 28:309-319.
- Goldberg S, Konis Y, Rosenberg M. Effect of cervipyrinium chloride on microbial adhesion to hexadecane and polystyrene. *Appl Environ Microbiol* 1990; 56:1678-1682.
- Goldberg S, Rosenberg M. Bacterial desorption by commercial mouthwashes vs. two-phase oil:water formulations. *Biofouling* 1991; 3:193-198.
- Rosenberg M. Bad breath: diagnosis and treatment. *U Toronto Dent J* 1990; 3:7-11.
- Rosenberg M, Septon I, Eli I, et al. Halitosis measurement by an industrial sulphide monitor. *J Periodontol* 1991; 62:487-489.
- Rosenberg M, Barki M, Portnoy S. A simple method for estimating oral microbial levels. *J Microbiol Methods* 1989; 9:253-6, 1989.
- Tal H, Rosenberg M. Estimation of dental plaque levels and gingival inflammation using a simple oral rinse technique. *J Periodontol* 1990; 61:339-342.
- Rosenberg M, Judes H, Weiss E. Cell surface hydrophobicity of dental plaque microorganisms in situ. *Infect Immun* 1983; 42:831-834.
- Niwa M, Saito K, Sugauma N, Watanabe H, Sawamura K, Futakami K. A study on the deodorant effect of dentifrices containing chlorhexidine. *J Dent Health* 1977; 27:49-59.
- Kayrouz GA, Buckner RY, Briner WW. Control of salivary bacteria by dual rinses with 0.12% chlorhexidine. *J Dent Res* 1990; 69(Spec. Issue):240(Abstr. 1053).
- Rosenberg M, Barki M, Goldberg S. The antimicrobial effect of mouthrinsing as measured using the "Oratest." *J Dent Res* 1989; 68(Spec. Issue):661(Abstr. 45).
- Rosenberg M, Kulkarni GV, Bosy A, McCulloch CAG. Reproducibility and sensitivity of oral malodour measurements with a portable sulphide monitor. *J Dent Res* 1991; 70:1436-1440.

Send reprint requests to: Dr. Mel Rosenberg, The Maurice and Gabriela Goldschleger School of Dental Medicine, Tel-Aviv University, Ramat-Aviv, 69978 Tel-Aviv, Israel.

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