# Efficacy of stabilized chlorine dioxide-based unflavored mouthwash in reducing oral malodor: An 8-week randomized controlled study

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ABSTRACT: **Purpose:** To evaluate the efficacy of a mouthwash containing stabilized chlorine dioxide in reducing oral malodor when used as an adjunct to tooth brushing compared with the use of a placebo mouthwash. **Methods:** This 8-week study in healthy subjects with clinically diagnosed intrinsic oral malodor was a 2-way crossover, double-blind, randomized, and controlled trial design, which was conducted at a single site. Subjects were randomized to receive either unflavored, non-fluoridated, and alcohol-free mouthwash containing 0.1% stabilized chlorine dioxide or a placebo mouthwash with identical bottle packaging. Both groups were provided with the same toothpaste and toothbrush. Subjects consented to the 8-week participation and were instructed to use their allocated treatment twice daily. In Phase I, quantified odor intensity [measured by an organoleptic intensity rating scale of 0-5, with 0=malodor cannot be detected and 5=very strong malodor] was independently evaluated by three calibrated judges at baseline, and after 1, 2, and 3 weeks of treatment. Following a 2-week washout period, Phase II initiated with the redistribution of test products. The subjects' organoleptic scores were assessed by the calibrated judges at baseline, and 6, 7, and 8 weeks of treatment. **Results:** A total of 50 subjects were enrolled and randomized into the two groups. Of these, 47 subjects completed the study. The baseline organoleptic intensity scores for both groups during Phase I and Phase II were not significantly different (P= 0.224, P= 0.071, respectively). At all visits, the organoleptic scores for the placebo rinse group during both Phase I and Phase II were not significantly different. During Phase I, the mean of individual organoleptic change scores from the stabilized chlorine dioxide rinse group were significantly different from the baseline at the last two follow-up visits: Week 1 (P= 0.088), Week 2 (P= 0.001), Week 3 ($P= 0.1\times10^{-3}$). During Phase II, the mean of individual organoleptic change scores from the stabilized chlorine dioxide rinse group were also significantly different from the baseline at the last two follow-up visits: Week 6 (P= 0.120), Week 7 (P= 0.004), Week 8 (P= 0.002). (*Am J Dent* 2018;31:309-312).

CLINICAL SIGNIFICANCE: The results of this study suggest the daily use of a stabilized chlorine dioxide-containing unflavored mouthwash as an adjunct to brushing with fluoride toothpaste provides a clinically relevant reduction in oral malodor after 3 weeks of twice-daily use.

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## Introduction

In high doses, chlorine dioxide is toxic, which is the case with many common substances in dentistry and medicine. However, chlorine dioxide is considered safe in low doses. In fact, 5% of large water-treatment facilities (serving populations over 100,000) in the U.S. use chlorine dioxide at low levels to treat drinking water.[1] An estimated 12 million Americans have exposure to drinking water with chlorine dioxide and chlorite ions.[1] In the 1980s, human chlorine dioxide ingestion studies[2] and long-term rodent studies[3,4] found no evidence of its carcinogenic potential. In the 1990s and 2000s, chlorine dioxide-containing rinse studies in the oral cavity produced evidence of microbicidal effects against *Streptococcus mutans*, Lactobacilli,[5] and malodor reduction properties.[6] Since 2011, in vitro studies of stabilized chlorine dioxide mouthwash have produced data confirming the ability to kill oral pathogens,[7] dissolve biofilms,[8] whiten teeth,[9] and eliminate volatile sulfur compounds (VSCs).[10] By 2015, in vivo interventional studies,[11] including randomized trials,[12-14] produced data showing reduced bacterial counts, tongue coating index, and VSCs associated with oral malodor reduction.

Chronic halitosis is a debilitating oral condition with social-emotional effects. Yet, the number of studies of oral rinses containing stabilized chlorine dioxide on oral malodor, while growing, are modest in contrast to those on fluoride's effect on caries reduction and peroxide's effect on enamel.

This study assessed the efficacy of a buffered stabilized chlorine dioxide (sodium chlorite) containing unflavored oral rinse in human subjects for reducing oral malodor. According to the manufacturer, the buffering compound serves to maintain the bioavailability of the chlorine dioxide from the time of manufacture to the time of use while allowing a safe and efficacious amount of chlorine dioxide to be released when used orally.

## Materials and Methods

This single-center, double-blind (investigators/subjects), crossover assignment, randomized controlled study was designed to evaluate interventional treatment effects on organoleptic intensity score outcomes among patients receiving a 0.1% stabilized chlorine dioxide-containing unflavored oral rinse. The principal investigator screened and reviewed, recruited and enrolled subjects in the study from August 2016 through October 2016. The study was approved and conducted in compliance with the Institutional Review Board (IRB) at Loma Linda University, Loma Linda, California, USA (IRB# 5160255).

*Study population* - The study sample consisted of 50 participants (21-65 years old) recruited by IRB approved notifications placed in the Loma Linda University, Loma Linda,

Table 1. Oral odor judge calibration.

| | Intraclass correlation | Lower bound | Upper bound | F test with true value 0 | | | |
|---|---|---|---|---|---|---|---|
| | | | | Value | *df*1 | *df*2 | Significance |
| Single measures | 0.757 | 0.468 | 0.925 | 11.5 | 9 | 18 | <0.001 |
| Average measures | 0.903 | 0.725 | 0.974 | 11.5 | 9 | 18 | <0.001 |

Table 2. Organoleptic intensity rating scale.*

| Rating | Odor intensity |
|---|---|
| 0 | Malodor cannot be detected |
| 1 | Questionable malodor, barely detectable |
| 2 | Slight malodor, exceeds the threshold of malodor recognition |
| 3 | Malodor is definitely detected |
| 4 | Strong malodor |
| 5 | Very strong malodor |

*Adapted from Rosenberg & McCulloch[15,16] and Miyazaki et al.[17]

California, newsletter, and in the local community. Enrolled participants examined at the Center for Dental Research, School of Dentistry, Loma Linda University, had slight to strong intrinsic oral malodor scores, as determined by three calibrated oral odor judges (Table 1). A 6-level organoleptic scoring of 0-5 was used (Table 2);[15-17] with 0 indicating malodor cannot be detected and 5 indicating malodor is very strong.

*Inclusion criteria* - Eligibility requirements included a completed informed consent, good general health, and an average organoleptic intensity rating of more than 2.6 but less than 4.5 on an intensity scale of 0-5 following 12 hours without performing oral hygiene care.

*Exclusion criteria* - Exclusion criteria included xerostomia; oral piercing; oral appliances; excessive gingival recession; advanced periodontal disease; heavy deposits of calculus; fixed or removable oral appliance; mucosal inflammation; visible oral disease; or unwillingness to abstain from other oral hygiene products during the study.

*Masked oral rinse therapy* - Subjects were randomly assigned to one of two groups:

Group A: 0.1% stabilized chlorine dioxide-containing oral rinse (ClōSYS[a] alcohol-free unflavored oral rinse) serving as the test group;

Group B: Placebo oral rinse devoid of stabilized chlorine dioxide and containing the same other ingredients as those in the test oral rinse provided by Rowpar Pharmaceuticals, Inc. serving as the control group.

Mouthwash bottle packaging was identical for the two groups and each 16-ounce white bottle was identified with only numeric coding assignment, and concealed throughout the study. The placebo rinse did not contain active ingredients such as stabilized chlorine dioxide but in appearance and taste closely matched the treatment rinse. The assignment of each subject to a group was not known to subjects, principal investigator, and odor judges. Subject identities were also masked and were identified solely by their subject number throughout the study. A study coordinator allocated study subjects using randomized computer-generated assigned treatment groups. The coordinator was not involved with clinical assessment nor an odor judge.

Table 3. Gender and age distribution of Group A (test) and Group B (placebo) at conclusion of Phase I and II.*

| Group | Gender | | Age (year) | |
|---|---|---|---|---|
| | Male | Female | Range | Mean ± SD† |
| PHASE I | | | | |
| A | 10 | 13 | 22 - 65 | 45.6 ± 13.5 |
| B | 8 | 17 | 21 - 65 | 45.7 ± 13.9 |
| PHASE II | | | | |
| A | 7 | 17 | 23 - 65 | 44.7 ± 14.4 |
| B | 10 | 13 | 22 - 65 | 45.6 ± 13.5 |

*Demographics as of final examination.
†SD: Standard deviation. SD values for the two groups are not significantly different (P= 0.831) as determined using the Mann-Whitney U statistic.

Table 4. Ethnicity distribution of Group A (test) and Group B (placebo) at conclusion of Phase I and II.*

| | Group (No. and %) | |
|---|---|---|
| | A | B |
| PHASE I | | |
| Ethnicity | | |
| Hispanic | 11 (47.8) | 9 (36.0) |
| Caucasian | 5 (21.7) | 9 (36.0) |
| African-American | 2 (8.7) | 4 (16.0) |
| Asian | 3 (13.0) | 2 (8.0) |
| Other/Mixed | 2 (8.7) | 1 (4.0) |
| Total | 23 (100) | 25 (100) |
| PHASE II | | |
| Hispanic | 9 (37.5) | 11 (47.8) |
| Caucasian | 8 (33.3) | 5 (21.7) |
| African-American | 4 (16.7) | 2 (8.7) |
| Asian | 2 (8.3) | 3 (13.0) |
| Other/Mixed | 1 (4.2) | 2 (8.7) |
| Total | 24 (100) | 23 (100) |

*Demographics as of final examination.

Subjects received assigned products with verbal and written instructions, and one tube of toothpaste (Crest Cavity Protection[b] toothpaste, regular) and a toothbrush[a] to use for the study. They were also given measuring cups for the mouthwash, and a diary log for recording daily use. Subjects rinsed twice a day, morning and evening, with 15 milliliters of mouthwash for 30 seconds. All were instructed to continue normal oral hygiene practices, omitting products except those provided as study materials. Participants were instructed to abstain from non-study related products such as breath mints, gums, and lozenges during the study. They were informed of their right to withdraw from the study at any time for any reason. If a complication or adverse reaction occurred, the participants were told to stop the treatment protocol. If they chose to stop, a closeout examination and follow-up would be requested.

## Results

*Subjects* - A total of 50 subjects were enrolled into this 8-week clinical trial. Three subjects were lost during the duration of the study. The remaining 47 subjects who completed the study were included in the final results. The distribution of gender, average age, and race were comparable between the two groups (P= 0.831). The subjects completing the study consisted of 17

Table 5. Organoleptic descriptive statistics baseline of Phase I to Week 3 and baseline of Phase II to Week 8.*

| | Baseline | | Week 1 | | | Week 2 | | | Week 3 | | | P-value† |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD‡ | Mean | SD | Δ of Mean§ | Mean | SD | Δ of Mean§ | Mean | SD | Δ of Mean§ | |
| PHASE I | | | | | | | | | | | | |
| Group A | 3.09 | 0.34 | 2.90 | 0.52 | -0.19 | 2.64 | 0.48 | -0.46 | 2.58 | 0.43 | -0.52 | <0.001 |
| Group B | 3.23 | 0.41 | 3.19 | 0.50 | -0.04 | 3.12 | 0.43 | -0.11 | 3.19 | 0.43 | -0.04 | 0.750 |
| | P= 0.224¶ | | P= 0.253# | | | P= 0.007# | | | P= 0.002# | | | |
| | Baseline | | Week 1 | | | Week 2 | | | Week 3 | | | P-value† |
| | Mean | SD‡ | Mean | SD | Δ of Mean§ | Mean | SD | Δ of Mean§ | Mean | SD | Δ of Mean§ | |
| PHASE 2 | | | | | | | | | | | | |
| Group A | 3.14 | 0.46 | 3.00 | 0.41 | -0.14 | 3.10 | 0.50 | -0.04 | 3.09 | 0.43 | -0.06 | 0.810 |
| Group B | 3.44 | 0.50 | 3.18 | 0.61 | -0.26 | 3.01 | 0.59 | -0.43 | 2.96 | 0.49 | -0.48 | 0.006 |
| | P = 0.071¶ | | P = 0.057# | | | P = 0.022# | | | P = 0.003# | | | |

*2-week washout with no test product oral rinses used by subjects occurred after Week 3 and prior to commencing Week 6.
† Friedman test.
‡ SD: Standard deviation.
§ Δ of Mean at respective week – baseline.
¶ Mann-Whitney U test.
# One-sample median test.

Table 6. Organoleptic change scores from baseline for Group A (test) and Group B (placebo).

| Phase I | Delta (Week 1) | | Delta (Week 2) | | Delta (Week 3) | | |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | P-value* |
| Group A | -0.19 | 0.54 | -0.46 | 0.48 | -0.52 | 0.45 | $0.1 \times 10^{-3}$ |
| Group B | -0.04 | 0.44 | -0.11 | 0.45 | -0.04 | 0.34 | 0.512 |
| Phase II | Delta (Week 6) | | Delta (Week 7) | | Delta (Week 8) | | |
| | Mean | SD | Mean | SD | Mean | SD | P-value† |
| Group A | -0.24 | 0.68 | -0.40 | 0.61 | -0.46 | 0.64 | 0.002 |
| Group B | -0.14 | 0.56 | -0.04 | 0.63 | -0.06 | 0.57 | 0.624 |

*P values for delta of mean at Week 3 (bold).
†P values for delta of mean at Week 8 (bold).

men, and 30 women, with a mean age of 45 years (Table 3). The subject population completing the study included 20 Hispanic, 13 Caucasian, six African-American, five Asian, and three other/mixed (Table 4).

*Recruitment* - Subjects were recruited, commenced, and completed the study during the year 2016. Follow-up examinations were not performed.

*Baseline data* - Clinical procedures involved obtaining initial baseline values within 2 weeks of the initial screening visit. Organoleptic scores for each group were compared between baseline and subsequent visits, using the Mann-Whitney U test (Table 5). The average baseline breath intensity scores were not significantly different at the start of each phase; P= 0.224 and P= 0.071 for Phase I and Phase II, respectively. This indicated no carryover effect between phases from therapeutic intervention.

*Numbers analyzed* - Organoleptic change scores from baseline for the subjects in Group A and Group B for both study phases are shown in Table 6.

*Outcomes and estimation* - For Phase I, the mean of individual organoleptic change scores from Group B (placebo) were not significantly different from the baseline at any of the follow-up visits: Week 1 (P= 0.599), Week 2 (P= 0.240), Week 3 (P= 0.512). The mean of individual organoleptic change scores from Group A (test) were significantly different from the baseline at the last two follow-up visits: Week 1 (P= 0.088), Week 2 (P= 0.001), Week 3 ($P= 0.1 \times 10^{-3}$).

For Phase II, the mean of individual organoleptic change scores from Group B (placebo) were not significantly different from the baseline at any of the follow-up visits: Week 6 (P= 0.293), Week 7 (P= 0.698), Week 8 (P= 0.624). The mean of individual organoleptic change scores from Group A (test) were significantly different from the baseline at the last two follow-up visits: Week 6 (P= 0.120), Week 7 (P= 0.004), Week 8 (P= 0.002).

Within-group comparisons of changes in organoleptic scores were also evaluated using the Friedman test (Table 6).

*Adverse events* - Normal oral soft tissue examination findings before and after using Group A and Group B products for 3 weeks during Phase I and Phase II were found and included: soft and hard palate, buccal mucosa, mucogingival fold areas, tongue, sublingual and submandibular areas, salivary glands, and the tonsillar and pharyngeal areas.

## Discussion

Chlorine dioxide is an oxidizing agent with bactericidal, virucidal, and fungicidal properties established in the literature to reduce oral malodor.[12-14] The studies were conducted to determine the efficacy and safety of a buffered stabilized chlorine dioxide containing oral rinse in human subjects for reducing oral malodor, in partial fulfillment of the requirements for recognition by the American Dental Association.

The present study findings of 47 subjects who completed the 8-week investigation found no difference in safety outcomes using the placebo rinse or the stabilized chlorine dioxide-containing unflavored oral rinse from baseline. One of three (6%) subjects who dropped out of the study cited tooth sensitivity during Week 1; the remainder reported logistical issues (transportation, new job) preventing them from finishing the study. In context of the outcomes for the majority of the study population, and not knowing the subject's dental history of tooth sensitivity, the significance of the subject's reported sensitivity is unclear. The subject declined to be evaluated.

The placebo was not shown to have a statistically significant effect on oral malodor reduction. The stabilized chlorine dioxide-containing unflavored oral rinse, however, statistically reduced oral malodor intensity scores. Despite comparable demographics of the two groups, due to sample size, representational generalizability to broader populations is limited. Bias was not likely introduced into the group assignments, as Group A (test) and Group B (placebo) were masked to the examiners and the subjects. A study coordinator who was not involved in the clinical examinations is the only one who knew group assignments and product distribution. Odor judges were trained and calibrated (intraclass correlation coefficient = 0.849). Subject identity was concealed behind a privacy barrier during breath evaluation.[18]

The results of this study suggest that not only is stabilized chlorine dioxide-containing unflavored oral rinse used in the present study effective for reducing oral malodor, but that its effectiveness commences upon use, as evidenced in the Week 1 data. In both phases, up to 3 weeks of twice daily use, stabilized chlorine dioxide oral rinse demonstrated its continued and consistent effectiveness in reducing oral malodor intensity.

In conclusion, the results of this study showed that adverse effects such as harm to oral tissues failed to occur after 3 weeks of twice-daily use of placebo rinse and test rinse. Scores from a panel of trained, calibrated, masked odor judges determined the placebo oral rinse failed to provide statistically significant oral malodor reduction from baseline. The scores from the judges found, however, that the buffered stabilized chlorine dioxide-containing unflavored oral rinse provided statistically significant oral malodor reduction.

a. Rowpar Pharmaceuticals, Inc., Scottsdale, AZ, USA.
b. Procter & Gamble, Cincinnati, OH, USA.

*Disclosure statement:* The authors declared no conflict of interest.

Dr. Lee is Professor and Associate Director; Dr. Suprono is Associate Professor; Ms. Stephens is Professor; Ms. Withers is Associate Professor, Center for Dental Research; and Dr. Li is Professor and Associate Dean for Research, Loma Linda University School of Dentistry, Loma Linda, California, USA.

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