# SCIENTIFIC REPORT: A MULTIFACTORIAL INVESTIGATION OF THE ABILITY OF ORAL HEALTHCARE PRODUCTS TO COMBAT ORAL MALODOUR

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## 1. PURPOSE OF INVESTIGATION

In this investigation, the clinical effectiveness of a series of two oral rinse products [Ardox-X™ and Corsodyl oral rinses], each tested against a water placebo treatment, towards oral malodour (halitosis) was determined using a newly-developed, portable gas-chromatographic system with the ability to determine parts-per-billion (ppb) levels of 3 different VSCs in air directly sampled from the oral cavity. These VSC determinations were made before, and at selected diurnal time-points after treatment of participants with each of the oral rinse formulations in the recommended manner, and then compared with corresponding measurements made after they rinsed with a $H_2O$ placebo control in place of the oral rinse formulations.

## 2. MATERIALS AND METHODS

We explored the relative effectiveness and longevity (up to 6 hr. post-administration) of 2 commercially-available mouthrinses, specifically Ardox-X™ halitosis rinse and Corsodyl [the latter serving as a commonly-utilised 'baseline' positive control oral healthcare product and containing 0.20% (w/v) chlorhexidine gluconate] in suppressing oral malodour (VSC levels in air directly sampled from the oral cavity of human participants were determined as a function of time before and also at pre-selected time-points following mouthrinse administration). The VSC-neutralising capacity of each of the mouthrinse products tested was rationalised with special reference to their chemical compositions. In order to achieve this effectively, we employed an OralChroma™ Portable Gas Chromatography Device for the simultaneous and rapid determination of 3 different VSCs (hydrogen sulphide, methyl mercaptan and dimethyl disulphide) in the oral headspace at each sampling time-point.

### 2.1 *Volatile Sulphur Compound (VSC) Determinations*

Measurements of each VSC were made on an OralChroma™ portable gas chromatographic monitoring system. Participants were required to refrain from talking for 5 min. prior to measurement and to breathe through their noses during the collection of oral cavity air samples via a syringe. Results were recorded as parts-per-billion (ppb) VSC concentrations.

2.2 *Patient Population*

This investigation involved 30 non-smoking human volunteers (14 male, 16 female) ranging in age from 22 to 58 years. Once recruited, participants were supplied with a Participant Information Sheet and, if agreeing to take part in the investigation, were subsequently required to sign a University Research Ethics Consent Form. All participants recruited were also required to complete a short questionnaire which requested essential information, including medical history, age, gender, body mass index (BMI), dental treatment history and any current medication that they were receiving.

2.3 *Exclusion Criteria*

Participants were excluded from the investigation if they they were outside the above specified age ranges, and if they had any serious or chronic medical condition such as diabetes, cardiovascular diseases or cancer, or any other condition which precluded their participation in the trial. Subjects receiving any form of medication during the 7 days prior to the first testing day were excluded from the investigation. All participants were also instructed not to receive any form of medication during the three sampling test days of the trial.

2.4 *Evaluations of the Abilities of Oral Rinse Products to Combat Oral Malodour*

Participants were required to rinse with 10 ml volumes of each of the above 2 commercially-available mouthrinse products for a period of 30 s. Each participant also rinsed with an equivalent volume of tap water which served as a placebo control.

VSC levels were determined both prior to (0.00 hr.) and following oral rinsing episodes with each mouthrinse examined (0.30, 1.30, 2.30, 4.00 and 6.00 hr. post-administration). The first (baseline) measurement was made at 09.00 am, and all participants were required to agree to avoid their early morning breakfast meal [and, of course, all further oral activities such as eating, drinking, tooth-brushing, etc.] during the period between awakening in the morning and the first (baseline zero-control) VSC determination on each of the 3 days in which they were involved in the investigation. Administration of the oral rinses to each of the 30 participants was ‘staggered’ throughout time, and the ‘washout’ period between each of the 2 products administered was 3 days. During these

‘washout’ periods, all participants resumed their normal oral health care activities.

2.5 *Experimental Design for the Statistical Analysis of Oral Cavity VSC Concentrations*

For each of the above clinical datasets, we employed an analysis-of-variance (ANOVA)-based experimental design. This procedure was employed to determine the significance of the ‘Between-Treatments’ and ‘Between Study Time-Points’ effects for each of the Oral Healthcare Product (OHCP) groups incorporated into the study, and also the further components-of-variances (CVs) involved, specifically that ‘Between-Participants’, together with those arising from the Treatment x Diurnal Time-Point, Treatment x Participant and Participant x Diurnal Time-Point interactions.

Hence, the experimental design for this investigation was classified as a mixed-model, 3-factor system with treatments (2 OHCPs, together with the water placebo control) and time-points at which the measurements were made being fixed effects at 3 and 6 levels respectively, and participants (n = 30 in total) being a random effect. Hence, this mixed-model component analysis for each VSC determined comprised the 3 main effect factors, their associated first-order interactions, and fundamental error. Data were transformed using the Box-Cox transformation prior to statistical analysis in order to satisfy assumptions of normality and variance homogeneity.

Partial correlations between each of the 3 VSCs determined (similarity/dissimilarity analyses) were also explored using a multiple correlation model system.

## 3. RESULTS

The significance of the ‘Between-Products’ effect was manifested by (1) significant or highly significant reductions in oral cavity $H_2S$ levels (relative to those observed with the $H_2O$ control treatment) by both the Ardox-X and Corsodyl products, although no significant differences were found between these two oral healthcare products investigated; and (2) highly significant differences between the mean oral cavity $CH_3SCH_3$ concentrations of the

Ardox-X oral rinse and the $H_2O$ control (i.e. that observed with the former treatment was substantially lower than the latter control one, $p = 0.001$). However, the *p* value observed for the significant difference observed between Corsodyl and the $H_2O$ control was only 0.025. For $CH_3SH$, the Ardox-X oral rinse formulation gave an improved performance over both the Corsodyl product, specifically at the 2.33 and 6.00 hr. time-points, although it should be noted that there was a significant Participant x Treatment Time interaction effect (i.e., the nature of the time-course of response to each product differed significantly between Participants). These results are summarised in Table 1, and plots of mean Box-Cox transformed VSC concentrations versus post-treatment time for the Ardox-X and Corsodyl oral rinse products, and the water placebo (control) are shown in the Figures exhibited (Appendix).

In terms of longevity of the halitosis-neutralising actions of the two products tested, for $H_2S$ both Ardox-X and Corsodyl remained effective at the final 6.00 hr. time-points (mean oral cavity concentrations 28 and 39% respectively of their pre-treatment values, an observation indicating that the former is more effective in neutralising $H_2S$ at this time-point), as indeed they were for $CH_3SH$ (mean oral cavity concentrations 0.7 and 15% respectively of their pre-treatment values respectively). However, for $CH_3SCH_3$, only the Ardox-X product remained effective at the 6.00 hr. post-treatment time-point, its mean oral cavity concentration being 60% of its initial pre-treatment (control) value. Mean time-point-dependent percentage modifications to the mean baseline oral cavity concentrations of $H_2S$, $CH_3SH$ and $CH_3SCH_3$ (i.e., that at the t = 0.00 hr. time-point control) induced by the Ardox-X and Corsodyl oral rinse products and the water placebo treatment are listed in Table 2.

## 4. CONCLUSIONS

For $H_2S$ and $CH_3SCH_3$, both the Ardox-X and Corsodyl oral rinse formulations exerted significant or very highly significant VSC-neutralising activities which were of a much greater magnitude than that observed with the water control rinse; moreover, the Ardox-X product was found to be much more effective than Corsodyl at diminishing oral cavity $CH_3SCH_3$ concentrations. For both $H_2S$ and $CH_3SH$, both the Ardox-X and Corsodyl

products retained their VSC-neutralising actions 6.0 hr. post-administration, but for $CH_3SCH_3$, only the Ardox-X oral rinse retained this activity at this treatment time-point. These results demonstrate that the Ardox-X oral rinse product is at least as effective as (and for $CH_3SCH_3$, more effective than) the Corsodyl formulation at combating oral malodour conditions; previous investigations have revealed that Corsodyl decreases peak VSC levels by only 43%, an observation consistent with the results acquired in this study (Pitts *et. al.*, *J. Dent. Res.* 1983; **62**: 738- 742.). In view of these observations, and the known side-effects associated with the regular and common use of Corsodyl as an oral healthcare product (particularly soreness, swelling and irritation of the mouth, ulceration of mouth surfaces, temporary discolourations of teeth and the tongue, swelling of the salivary glands, taste disturbances or burning sensations, skin irritation, and hypersensitivity reactions including allergic reactions or anaphylaxis), use of the Ardox-X formulation appears to offer major advantages over Corsodyl for the treatment of oral malodour conditions.

| **VSC** | **Product Effectiveness** |
|---|---|
| $H_2S$ | Corsodyl ≥ Ardox-X > $H_2O$ |
| $CH_3SH$ | Ardox-X > Corsodyl ≅ $H_2O$ |
| $CH_3SCH_3$ | Ardox-X > Corsodyl > $H_2O$ |

**Table 1.** Summary of the relative effectiveness of each product tested and the water placebo against each oral cavity volatile sulphur compound (VSC).

**$H_2S$**

| | Time (hr.) | | | | |
|---|---|---|---|---|---|
| **Product** | **0.33** | **1.33** | **2.33** | **4.00** | **6.00** |
| **$H_2O$** | 76.8% | 59.2% | 42.3% | 46.0% | 53.4% |
| **Ardox-X** | 119.2% | 49.1% | 39.0% | 35.3% | 28.1% |
| **Corsodyl** | 56.8% | 39.2% | 65.0% | 35.3% | 39.1% |

**$CH_3SH$**

| | Time (hr.) | | | | |
|---|---|---|---|---|---|
| **Product** | **0.33** | **1.33** | **2.33** | **4.00** | **6.00** |
| **$H_2O$** | 43.7% | 43.7% | 7.5% | 6.2% | 11.9% |
| **Ardox-X** | 123.5% | 9.7% | 5.1% | 5.2% | 0.7% |
| **Corsodyl** | 88.6% | 14.7% | 89.8% | 5.9% | 15.2% |

**$CH_3SCH_3$**

| | Time (hr.) | | | | |
|---|---|---|---|---|---|
| **Product** | **0.33** | **1.33** | **2.33** | **4.00** | **6.00** |
| **$H_2O$** | 103.8% | 74.3% | 41.9% | 50.3% | 64.4% |
| **Ardox-X** | 68.1% | 31.0% | 30.7% | 57.1% | 59.6% |
| **Corsodyl** | 71.1% | 45.1% | 78.2% | 34.2% | 87.0% |

**Table 2.** Mean time-point dependent percentage modifications to the mean baseline oral cavity concentrations of $H_2S$, $CH_3SH$ and $CH_3SCH_3$ (t = 0.00 hr. time-point control) induced by the Ardox-X and Corsodyl oral rinse products and the water placebo treatment. Each value represents the mean of n = 30 trial participants.

**APPENDIX: ANOVA Tables and Associated Figures**

**Type I Sum of Squares analysis (Variable $H_2S$):**

| Source | DF | Sum of squares | Mean squares | F | Pr > F |
|---|---|---|---|---|---|
| Time | 5 | 467.1888 | 93.4378 | 11.0958 | < 0.000000001 |
| Participant | 29 | 665.2124 | 22.9384 | 2.7239 | 0.0000 |
| Product | 2 | 105.3132 | 52.6566 | 6.2530 | 0.0022 |
| Time*Participant | 145 | 1221.4555 | 8.4238 | 1.0003 | 0.4927 |
| Time*Product | 10 | 111.8476 | 11.1848 | 1.3282 | 0.2146 |
| Participant*Product | 58 | 872.5991 | 15.0448 | 1.7866 | 0.0011 |

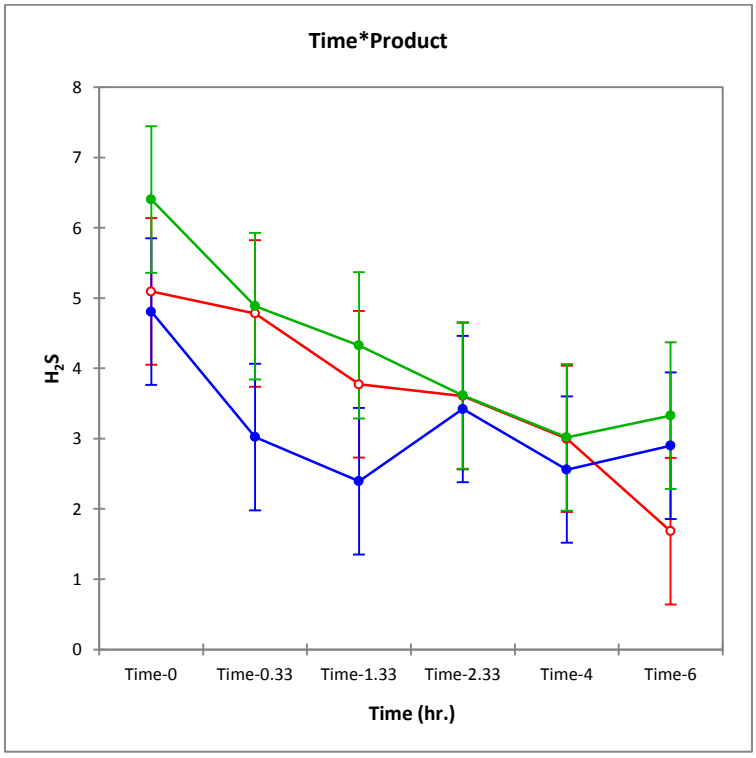


**Figure. Individual Product Response: $H_2S$**
**Key: Red - Ardox-X; Blue - Corsodyl; Green - Water Control**

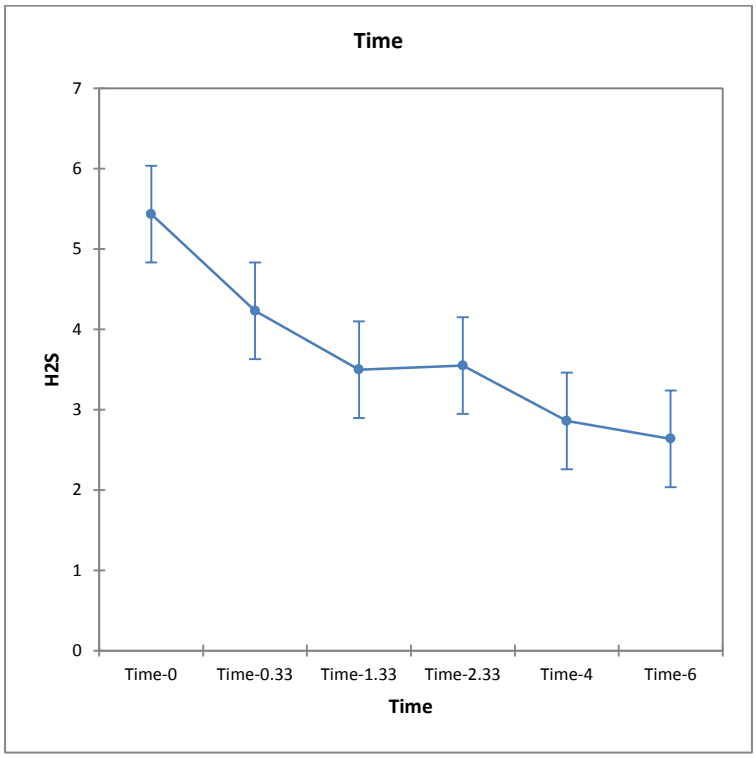


**Figure. Overall Response: $H_2S$ (all 3 products)**

**Product / Fisher (LSD) / Analysis of the differences between the categories with a confidence interval of 95%: $H_2S$**

| Contrast | Difference | Standardized difference | Critical value | Pr > Diff | Significant |
|---|---|---|---|---|---|
| CORSODYL vs $H_2O$ | -1.0789 | -3.5271 | 1.9682 | 0.0005 | Yes |
| CORSODYL vs ARDOX-X | -0.4716 | -1.5417 | 1.9682 | 0.1242 | No |
| ARDOX-X vs $H_2O$ | -0.6073 | -1.9854 | 1.9682 | 0.0480 | Yes |
| LSD-value: | | | 0.602 | | |

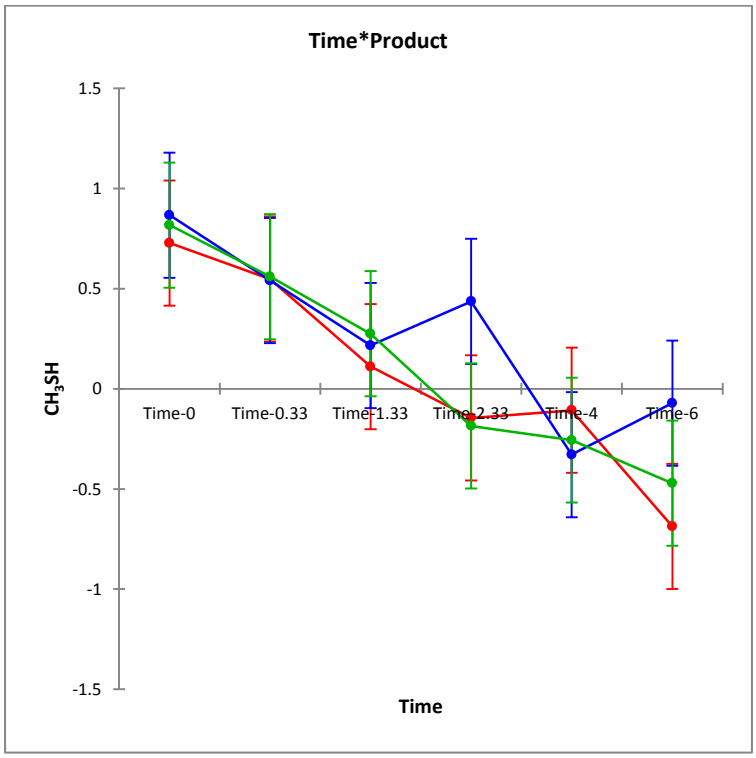


**Figure. Individual Product Response: $CH_3SH$**
**Key: Red - Ardox-X; Blue - Corsodyl; Green - Water Control**

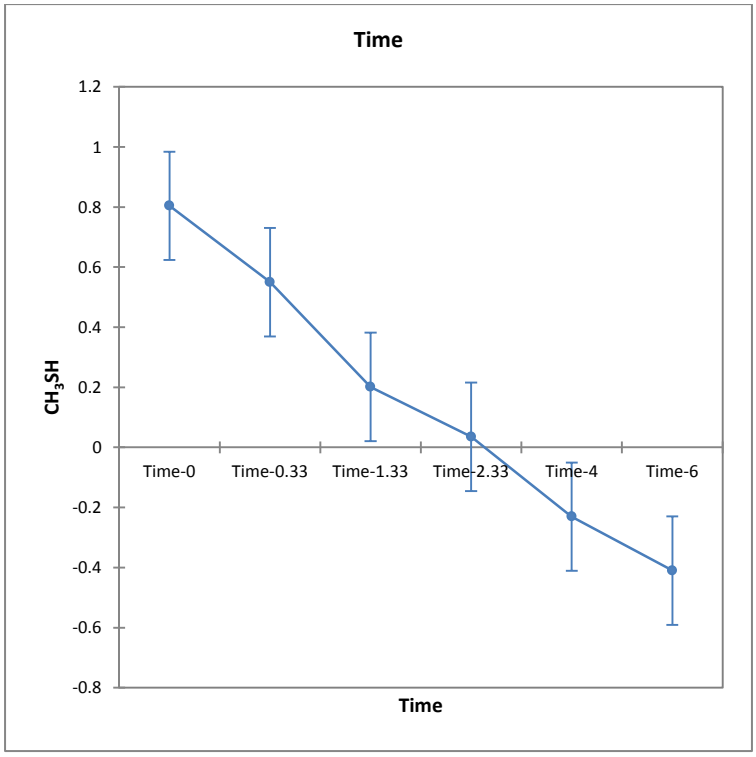


**Figure. Overall Response: $CH_3SH$ (all 3 products)**

**Product / Fisher (LSD) / Analysis of the differences between the categories with a confidence interval of 95%:**

**$CH_3SH$**

| Contrast | Difference | Standardized difference | Critical value | Pr > Diff | Significant |
|---|---|---|---|---|---|
| ARDOX-X vs CORSODYL | -0.2023 | -2.2080 | 1.9682 | 0.0280 | Yes |
| ARDOX-X vs $H_2O$ | -0.0487 | -0.5311 | 1.9682 | 0.5958 | No |
| $H_2O$ vs CORSODYL | -0.1537 | -1.6769 | 1.9682 | 0.0946 | No |
| LSD-value: | | | 0.1804 | | |

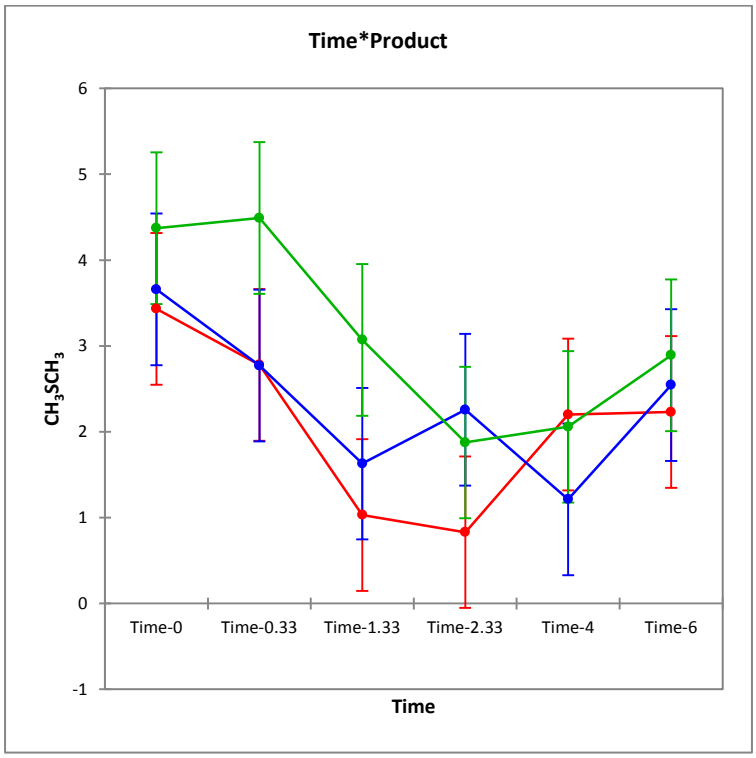


**Figure: Individual Product Response: $CH_3SCH_3$**
**Key: Red - Ardox-X; Blue - Corsodyl; Green - Water Control**

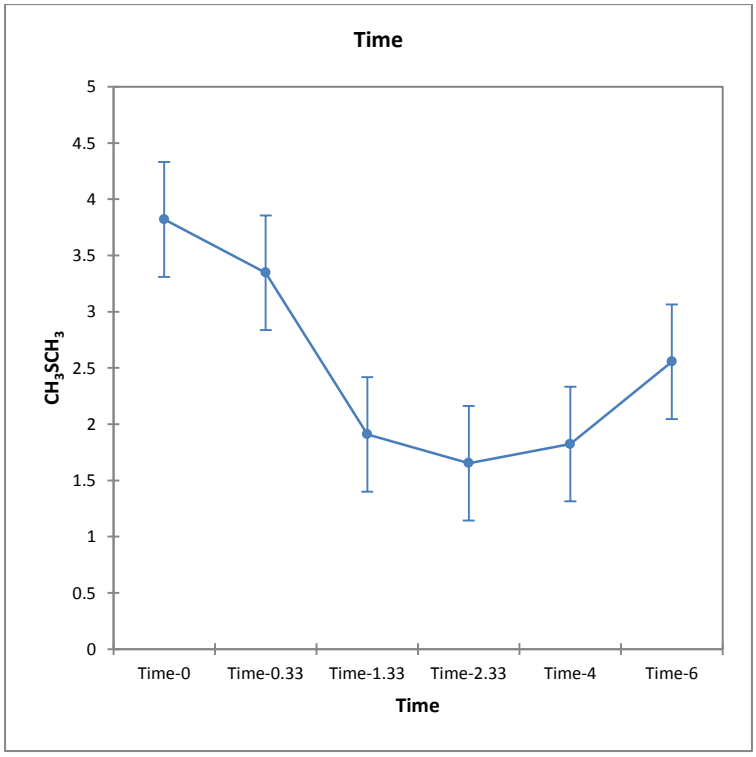


**Figure: Overall Response: $CH_3SCH_3$ (all 3 products)**

**Product / Fisher (LSD) / Analysis of the differences between the categories with a confidence interval of 95%:**

| Contrast | Difference | Standardized difference | Critical value | Pr > Diff | Significant |
|---|---|---|---|---|---|
| ARDOX-X vs $H_2O$ | -1.0422 | -4.0228 | 1.9682 | 0.0001 | Yes |
| ARDOX-X vs CORSODYL | -0.2616 | -1.0096 | 1.9682 | 0.3135 | No |
| CORSODYL vs $H_2O$ | -0.7806 | -3.0131 | 1.9682 | 0.0028 | Yes |
| LSD-value: | | | 0.5099 | | |