

Genotoxic Assessment of BriteSmile Whitening Procedure Gel in the Mouse Micronucleus Test

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Abstract

Objective: To determine the potential for BriteSmile Whitening Procedure Gel (BriteSmile Gel) to induce chromosome damage in mice following oral administration. BriteSmile Gel contains 15% hydrogen peroxide (HP), and it is used in conjunction with a visible wavelength light source to whiten teeth. BriteSmile Gel is formulated with a light-activated component to reduce the contact time needed for tooth bleaching. Inclusion of a light activated component enables a lower HP concentration to be used to provide tooth whitening.

Methods: To assess the clastogenic potential of BriteSmile Gel, male and female CD-1 mice (5/group) were administered BriteSmile Gel by gavage at levels up to 2,000 mg/kg body weight. Bone marrow was obtained 24, 48, and 72 h after administration, and immature polychromatic erythrocytes (PCEs) were evaluated for the presence of micronuclei as an indicator of chromosome damage.

Results: There was no evidence of chromosome damage in PCEs from mice administered BriteSmile Gel at levels of 500, 1,000, or 2,000 mg/kg body weight, compared to the vehicle control, water. Cyclophosphamide was included as a positive control, and the number of micronuclei induced in PCEs with cyclophosphamide was significantly greater than that of the control.

Conclusion: These results indicate BriteSmile Gel does not have the potential to cause chromosome damage in animals following ingestion. Supported by BriteSmile, Inc., Walnut Creek, CA.

Materials & Methods

Preparation of Test Substance

Analysis of the BriteSmile Whitening Procedure Gel test substance revealed a mean hydrogen peroxide percentage of 16.03%. The vehicle used to deliver the test substance was deionized water. The average amount of hydrogen peroxide present in the test substance used for dosing in the range-finding assay was 320.65 mg/kg body weight. Analysis of dosing solutions used in the definitive assay revealed hydrogen peroxide concentrations of 15.7, 7.82, and 3.92 mg/ml for the high, medium, and low doses, respectively.

Determination of Hydrogen Peroxide Levels

Hydrogen peroxide content of BriteSmile Whitening Procedure Gel and dosing solutions was determined by titration with KMnO₄ according to procedures published in the USP. Briefly, a 20-ml volume of 2.0 N H₂SO₄ was added to a 20-ml sample, and this mixture was titrated with 0.1N KMnO₄ until a slight pink color remained in the solution after addition of KMnO₄. Each ml of 0.1N KMnO₄ is equivalent to 1.7005 mg H₂O₂, and the following formula was used to determine peroxide content:

$$\% \text{ H}_2\text{O}_2 = (\text{ml KMnO}_4 \text{ titrant}) \times 0.1\text{N} \times 1.7005 \text{ mg H}_2\text{O}_2/\text{Sample weight (g)}$$

Animals

CD-1 mice were obtained from Charles River Laboratories, Inc. (Raleigh, NC). The animals were dosed by gavage using a stainless steel ball-tipped dosing needle and syringe at a volume of 20 mL/kg.

Extraction of Bone Marrow

All animals were sacrificed by carbon dioxide asphyxiation at approximately 24, 48, or 72 hours after administration of test or control material. Right femurs were removed from all animals, opened at both ends, and a 1 mL syringe was inserted into the opening in the center of the bone. A syringe containing approximately 0.2 mL fetal bovine serum was forced into the bone opening, and the bone marrow was flushed into a conical centrifuge tube. The suspension was centrifuged at approximately 1,000 rpm for approximately three minutes. The supernatant was removed, leaving a small amount of fetal bovine serum above the remaining cell button. The cell button was resuspended in the residual fetal bovine serum, and two drops of the mixture was placed on two glass microscope slides per animal.

Smears were prepared by drawing a slide held at an angle of approximately 45° to the slide on which the smear was prepared from the frosted end of the slide to the distal end of the slide.

Slide Staining

Slides were coded prior to application of bone marrow. After staining, the slides were air-dried overnight, dipped in absolute methanol, and allowed to air dry to fix the cells. The air-dried slides were stained for approximately 10 minutes with Wright-Giemsa (Harleco, diluted with deionized water with 1 part stain added to 3 parts deionized water), rinsed in deionized water to remove excess stain, air-dried and coverslipped using Acrytol (Surgipath Medical Industries, Inc.) to affix coverslips.

Slide Evaluation

Microscopically, 1,000 polychromatic erythrocytes (PCEs) per animal were evaluated for the incidence of micronuclei. In all instances, a single slide was used to evaluate 1,000 PCEs. All slides were evaluated for the presence of micronuclei using an Olympus BH-2 microscope with a 100X oil objective. The ratio of PCEs to normochromatic erythrocytes (NCEs) was determined by counting 200 erythrocytes. Typically, micronuclei are round, with a diameter 1/20 to 1/5 that of PCEs. Micronuclei are uniform, dark staining, typically round bodies in the cytoplasm of PCEs. Inclusions in PCEs that were not in the focal plain of the cell or were reflections, improperly shaped, or stained were considered artifacts and not scored as micronuclei. Cells containing more than one micronucleus were scored as positive for micronuclei, and the number of micronuclei per PCEs was recorded. A positive response was defined as a statistically significant dose-related increase in the number of PCEs over the control.

Results

Range-finding Study

For an initial assessment, one dose level of test article BriteSmile Whitening Procedure Gel (2,000 mg/kg) was administered orally to three male and three female CD-1 mice, and survival was determined 72 hours later. All three males and three females at the 2,000 mg/kg dose level survived for 72 h. Thus, 2,000 mg/kg was selected as the highest dose level to be used in testing, along with dose levels of 1,000 mg/kg and 500 mg/kg as intermediate and low dose levels, respectively.

Definitive Study

Treatment Groups

The following treatment group designations were used for this study (Table 1).

Table 1: Treatment Groups

Treatment	Dose	24 Hours		48 Hours		72 Hours	
		Males	Females	Males	Females	Males	Females
Vehicle Control	20 ml/kg	5	5	5	5	5	5
Positive Control ¹	80 mg/kg	5	5	ND ²	ND	ND	ND
Low Dose	500 mg/kg	5	5	5	5	5	5
Medium Dose	1,000 mg/kg	5	5	5	5	5	5
High Dose	2,000 mg/kg	5	5	5	5	5	5
Total Number of Animals		30	30	25	25	25	25

¹ Cyclophosphamide

² ND, not done

Positive and Negative Controls

A statistically significant difference (p<0.001) in the number of PCEs with micronuclei was observed by one-way analysis of variance (ANOVA) within sexes at the 24-hour time point between vehicle-treated animals and those receiving 80 mg/kg cyclophosphamide, the positive control. The spontaneous incidence of micronucleated PCEs was 5.4/1,000 PCEs in males and 3.0/1,000 PCEs in females. Tukey's honestly significant difference (HSD) test of the number of micronuclei observed at 24 hours showed a significant difference in males and females when cyclophosphamide was administered compared to vehicle-treated animals. The statistical significance was decreased, but still present (p<0.01) using Scheffé's test, which is more conservative than Tukey's HSD test. There was no statistically significant evidence of toxicity, based on weight changes, between vehicle-treated animals and cyclophosphamide-treated animals (Figure 1).

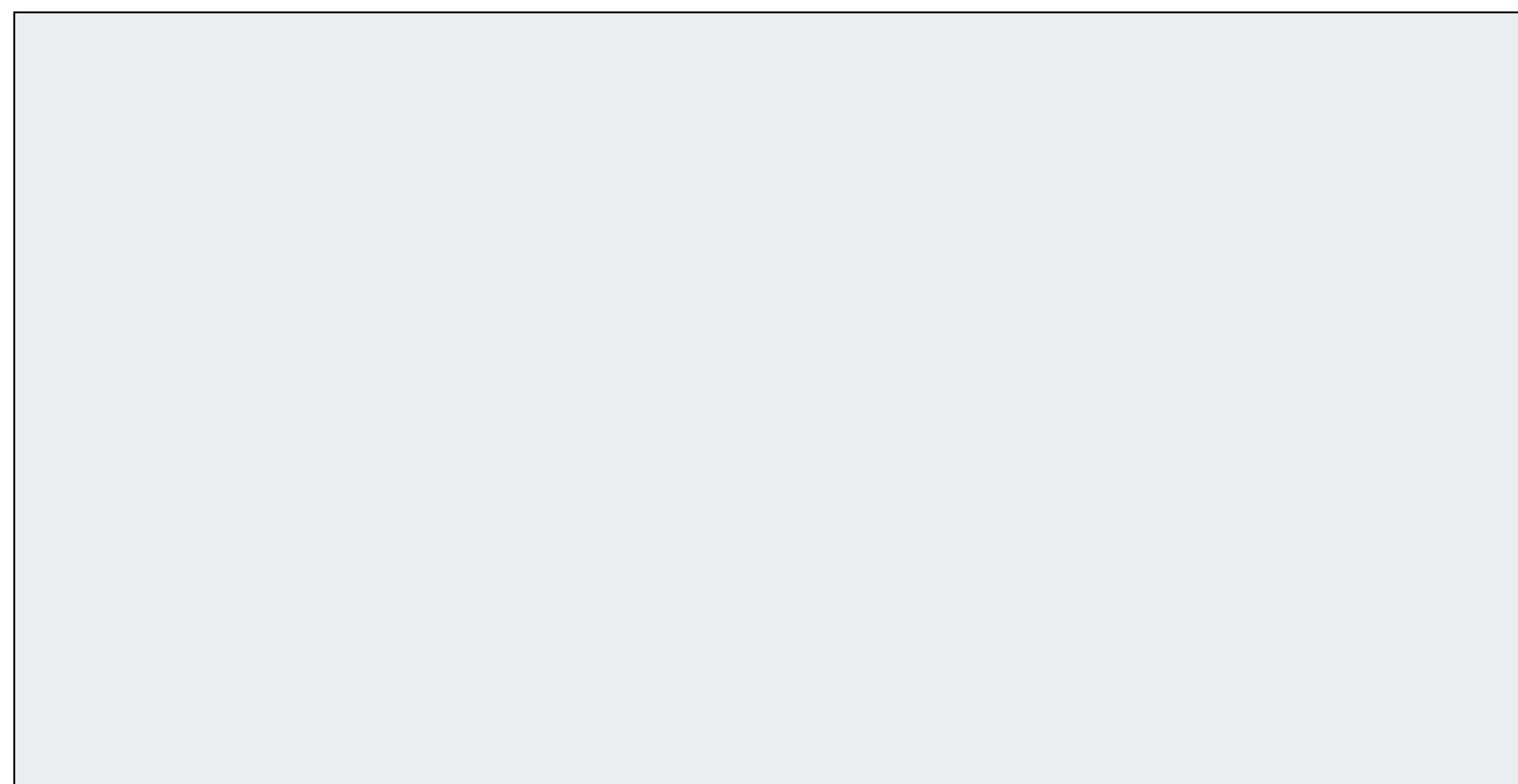


Figure 1: Body Weight Changes in Male and Female Control Animals at 24 Hours

Effects of BriteSmile Whitening Procedure Gel on Micronucleus Formation

BriteSmile Whitening Procedure Gel was administered orally, and the extent of micronucleus formation was examined in femoral bone marrow approximately 24, 48, and 72 hours after administration (Figure 2). There was no evidence of a dose- or time-related increase for micronuclei detected at any of the time points examined, compared to controls. There were also no statistically significant differences by one way ANOVA between the mean values of micronuclei obtained for vehicle-treated-animals of either sex and animals receiving 500 mg/kg, 1,000 mg/kg, or 2,000 mg/kg BriteSmile Whitening Procedure Gel at 24, 48, or 72 hours.

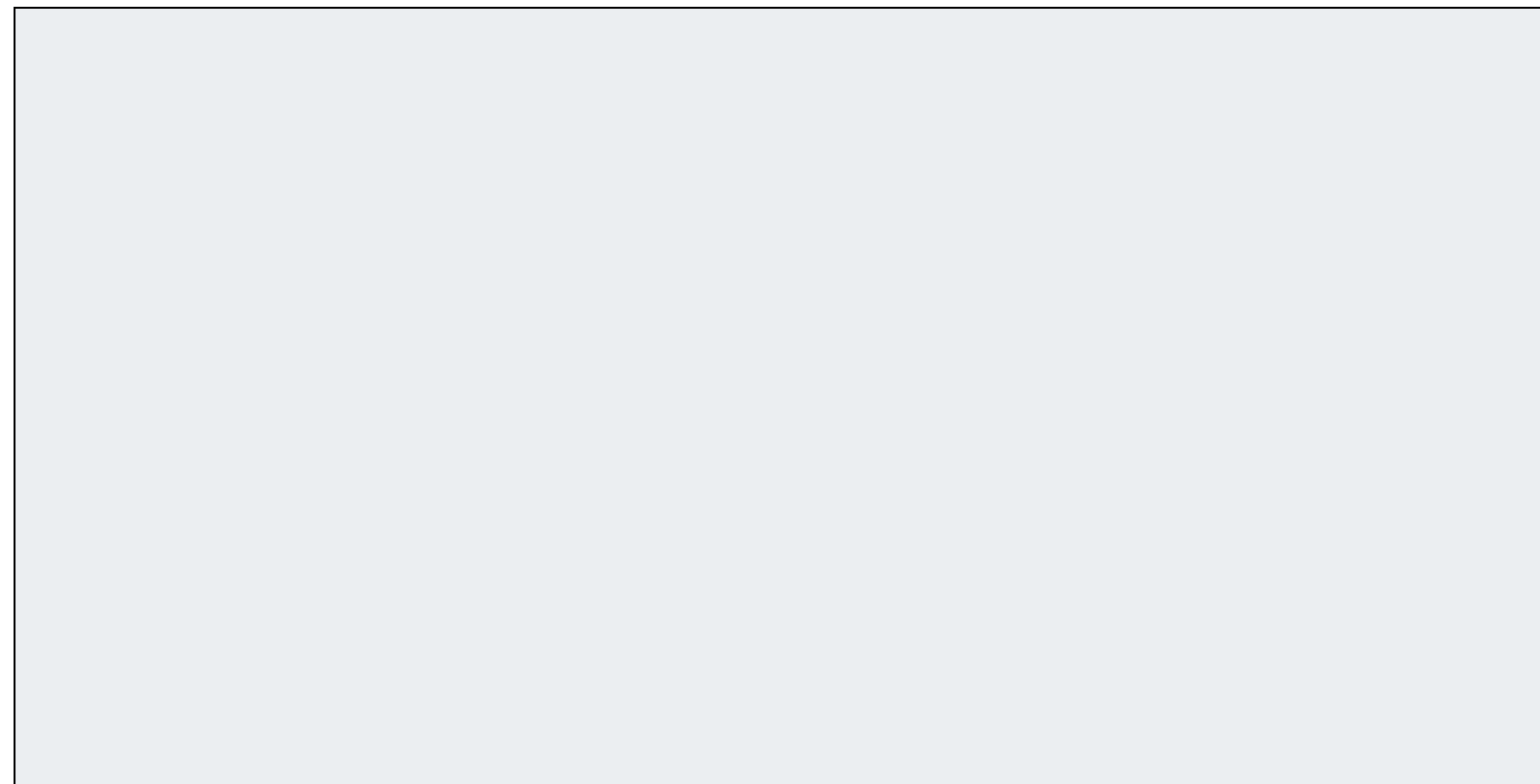


Figure 2: Micronucleus Induction after Oral Administration of BriteSmile Whitening Procedure Gel

Toxicity of BriteSmile Whitening Procedure Gel

Toxicity was assessed by monitoring body weight and by determining the ratio of PCEs to NCEs. In males, body weights decreased for all groups except the 2,000 mg/kg BriteSmile Whitening Procedure Gel group at 24 hours; by 48 hours, body weights increased in all groups except the 1,000 mg/kg and 2,000 mg/kg groups which remained the same; at 72 hours, body weights increased in all dose groups (Figure 3). In females, body weights increased in the 1,000 mg/kg and 2,000 mg/kg groups and decreased in the control and 500 mg/kg groups at 24 h; at 48 h, body weights increased in all groups, and the smallest increase was seen in the 2,000 mg/kg group; at 72 h, body weights increased in all groups. No statistically significant differences in body weights were observed in males or females analyzed by treatment group and sex using one-way ANOVA.

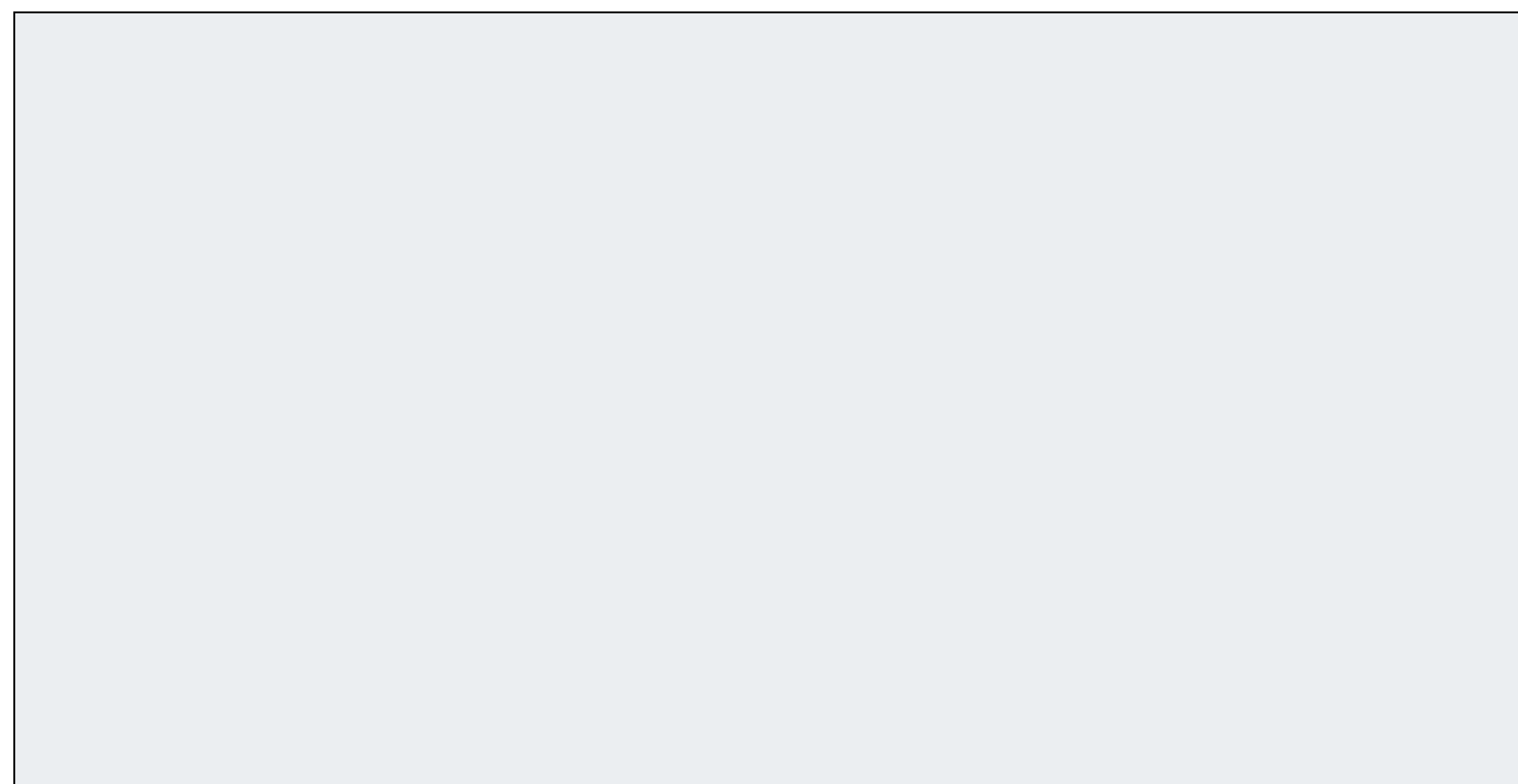


Figure 3: Body Weight Changes in CD-1 Mice Administered BriteSmile Whitening Gel

Toxicity of BriteSmile Whitening Procedure Gel

The ratio of PCEs to NCEs provides an indication of toxicity at the target site. An increase in the ratio of PCE/NCE would indicate greater production of erythrocytes, possibly due to loss of mature erythrocytes. A decrease in the PCE/NCE ratio would indicate destruction of immature erythrocytes, a direct effect on production of erythrocytes in bone marrow. The ratio of PCEs to NCEs is illustrated in Figure 4. In males, an increase in PCE/NCE ratio was observed in Vehicle- and 500 mg/kg BriteSmile Whitening Procedure Gel-treated animals 48 hours after administration, compared to 24 hours. This initial increase was followed by a decrease in PCE/NCE ratio at 72 hours post administration for all males except those in the 1,000 mg/kg group. In females, the PCE/NCE ratio decreased at 48 hours for all groups, except animals receiving 1,000 mg/kg BriteSmile Whitening Procedure Gel. Statistical analyses of the PCE/NCE ratios by time and dose performed by ANOVA revealed these results in males and females were not statistically significant.

Toxicity was observed in animals in this study, based on mortality. Extra animals were dosed in case of mortality. There was a tendency toward increased mortality in animals receiving the highest dose of BriteSmile Gel, 2,000 mg/kg (2 males, 3 females), compared to the lower dose groups: 1 male in the 500 mg/kg dose group and 1 female in the 1,000 mg/kg dose group. Because extra animals received the test substance, replacement animals were used to provide a complete set of animals for analysis.

The hydrogen peroxide contents of the low, mid, and high dosing solutions were 3.92, 7.82, and 15.7 mg H₂O₂/ml, respectively. At 20 ml/kg, animals in these dose groups received 78.4, 156.4, and 324 mg H₂O₂/kg body weight, respectively.

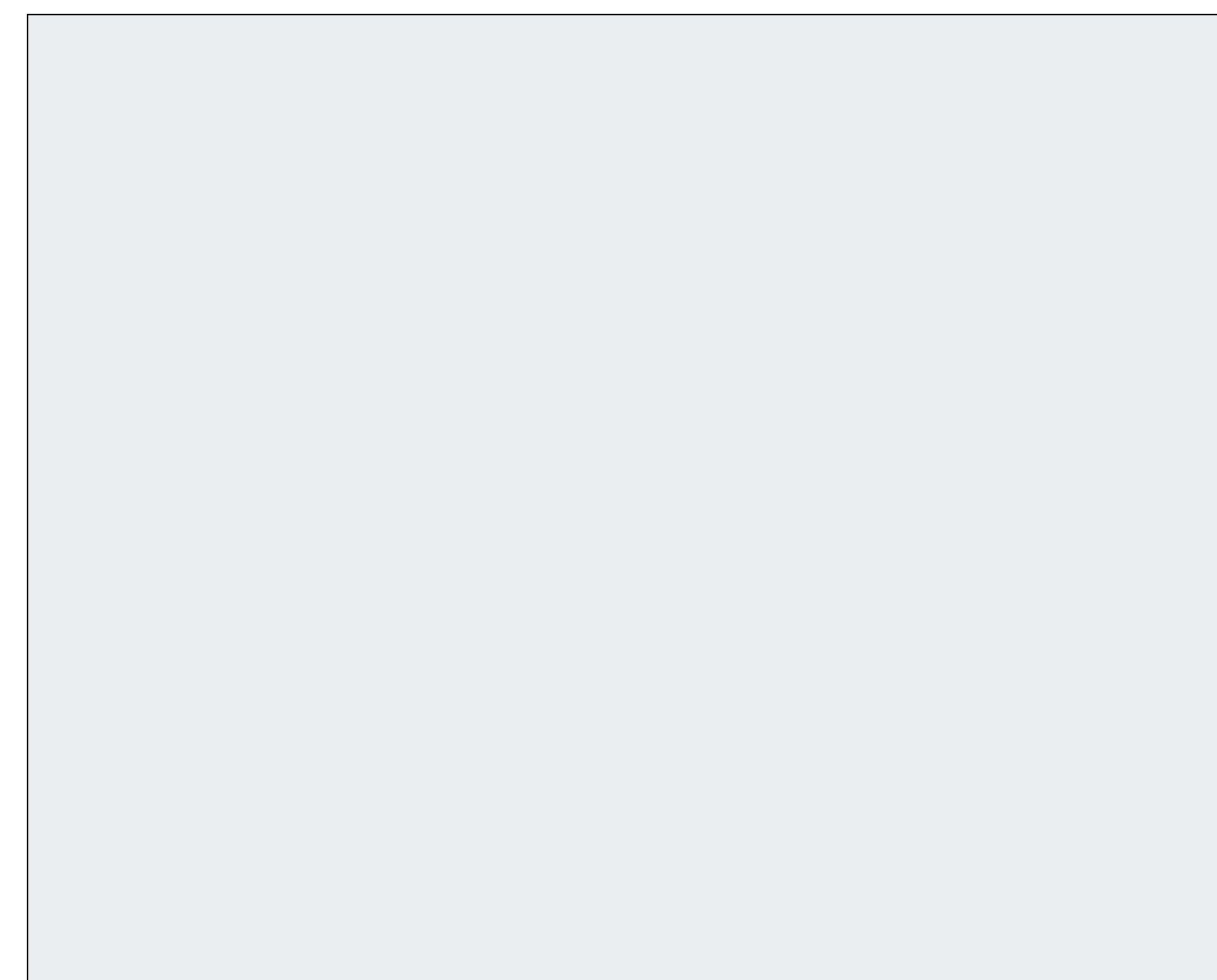


Figure 4: PCE/NCE Ratio in Mice Administered BriteSmile Whitening Procedure Gel Photomicrograph of Micronucleated PCE

Conclusions

- BriteSmile Whitening Procedure Gel was not clastogenic following oral administration at dose levels up to 2,000 mg/kg.
- The positive control, cyclophosphamide, was clastogenic.
- There was no evidence of bone marrow toxicity in mice after ingestion of 500, 1,000, or 2,000 mg/kg BriteSmile Whitening Procedure Gel.