

Genotoxic Assessment of BriteSmile Whitening Procedure Gel in *Salmonella typhimurium*

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Abstract

Objective: To determine the potential for BriteSmile Whitening Procedure Gel (BriteSmile Gel) to induce mutations in tester strains of *Salmonella typhimurium*. 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 mutagenic potential of BriteSmile Gel, five tester strains of *Salmonella typhimurium* (TA97a, TA98, TA100, TA102, and TA1535) were exposed to BriteSmile Gel at levels up to 5 mg/plate in the presence and absence of an external metabolic activation source (S9, an Aroclor-induced rat liver homogenate). Results: No mutagenic activity was seen in any of the five tester strains in the presence of S9, and no mutagenic activity was seen in TA97a, TA98, TA100, and TA1535 in the absence of S9. A weak mutagenic response was seen in TA102, a tester strain that is sensitive to oxidative mutagens. For comparison, aqueous hydrogen peroxide was also included in TA102 without S9. HP was mutagenic (exceeded twice background) at 0.15 and 0.2 mg/plate. BriteSmile Gel was also weakly mutagenic at similar levels of HP without S9. These results are similar to other published results that show a weak mutagenic response for HP in tester strain TA102 in the absence of S9. **Conclusion:** The biological relevance of this positive response is questionable as intracellular and extracellular defense mechanisms are present in the oral cavity that decompose HP. The response of BriteSmile Gel is more appropriately compared to tests performed in the presence of S9 in which no mutagenic activity was detected. Supported by BriteSmile, Inc., Walnut Creek, CA.

Materials & Methods

Determination of Hydrogen Peroxide Levels

Hydrogen peroxide content 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:

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

Bacterial Cultures

Salmonella typhimurium tester strains TA97a, TA98, TA100, TA102, and TA1535 were grown in Oxoid Nutrient Broth #2 for approximately 16 hr at 37±2° C with agitation at 300±25 rpm. For each mutagenicity assay, confirmation of phenotypic markers was also performed. The presence of the rfa wall mutation in TA97a, TA98, TA100, TA102, and TA1535 was confirmed by demonstrating sensitivity to crystal violet. For UV sensitivity, bacteria were irradiated at 7,000 µJoules. TA102 is relatively insensitive to UV; all other tester strains are sensitive to UV. The presence of the pKM101 plasmid in tester strains TA97a, TA98, TA100, and TA102 was confirmed by resistance to ampicillin. Tester strain TA1535 lacks the ampicillin resistance plasmid, and it was sensitive to ampicillin. The presence of the pAQ1 plasmid in TA102 was demonstrated by growth in tetracycline; tester strains TA97a, TA98, TA100, and TA1535 were sensitive to tetracycline. Tester strains grew on plates containing 0.0005% histidine and 3 µM biotin, but not on plates that lacked histidine.

General Information - Plating

Minimal media (MGA) plates containing reduced glucose levels (0.4%) were purchased from Molecular Toxicology, Inc. (Boone, NC) and stored at room temperature. Sterile 1X NADP cofactor mix was diluted with reconstituted lyophilized Aroclor-induced rat liver homogenate (S9, Molecular Toxicology, Inc. Boone, NC) at a ratio of 1 mL S9 to 9 mL 1X NADP cofactor mix immediately prior to use.

Negative Controls

Negative control plates were run concurrently with each assay. Three plates containing only minimal media and tester strains served as medium controls and three minimal media plates with 50 µL of Aroclor-induced rat liver homogenate (S9) and tester strains served as S9 controls. Plates containing 100 µL of water and tester strains were used as solvent controls.

Positive Controls

In the presence of S9, 2-aminoanthracene (2.5 µg/plate) was used in the range-finding with TA98 and TA100 and in the definitive test with TA97a, TA98, TA100, and TA1535. Danthron (100 µg/plate) was used in TA102 in the presence of S9. In the absence of S9, ICR-191 (1.0 µg/plate) was used in TA97a; 2-nitrofluorene (5 µg/plate) was used for the range-finding and definitive study in TA98; NaN₃ (2 µg/plate) was used for the range-finding and definitive test with TA100 and in the definitive test in TA1535; mitomycin-c (0.25 µg/plate) was used in TA102.

Range-finding Assay

A range-finding test was performed prior to the definitive bioassay to determine appropriate dose levels of the test substance to be used in definitive bioassays. The test substance was analyzed in duplicate at eight dose levels for toxicity at various concentrations up to 5 mg/plate. Toxicity was defined as a decrease in the number of revertant colonies per plate and/or by a thinning or disappearance of the bacterial lawn, or by the appearance of pinhead colonies in treated cultures.

Definitive Assay Performance

Assays were performed within a hood in subdued lighting. After overnight incubation, tester strains were refrigerated; S9 homogenate and cofactor solutions were mixed at a ratio at 1 mL reconstituted S9 homogenate to 9 mL cofactor solution; and top agar was melted and maintained at 42-45°C. Dose selection was determined from the range-finding study. Dilutions were arranged so that the test substance was delivered in 100 µL water. The test substance and tester strain were added to sterile tubes containing 2 mL top agar (-S9) or 0.5 mL S9/cofactor mix was added prior to test substance and tester strain (+S9); and the mixture was vortexed and poured onto MGA plates. After solidifying, plates were inverted and incubated 48-72 hr at 37±2°C. An independent repeat bioassay was conducted to confirm results of the initial study. After 48-72 hr, plates were removed from the incubator, and revertant colonies on each plate were counted manually or with an automatic colony counter. The background bacterial lawn was evaluated for toxicity and precipitate formation, and the mean number of revertant colonies for all replicates was determined.

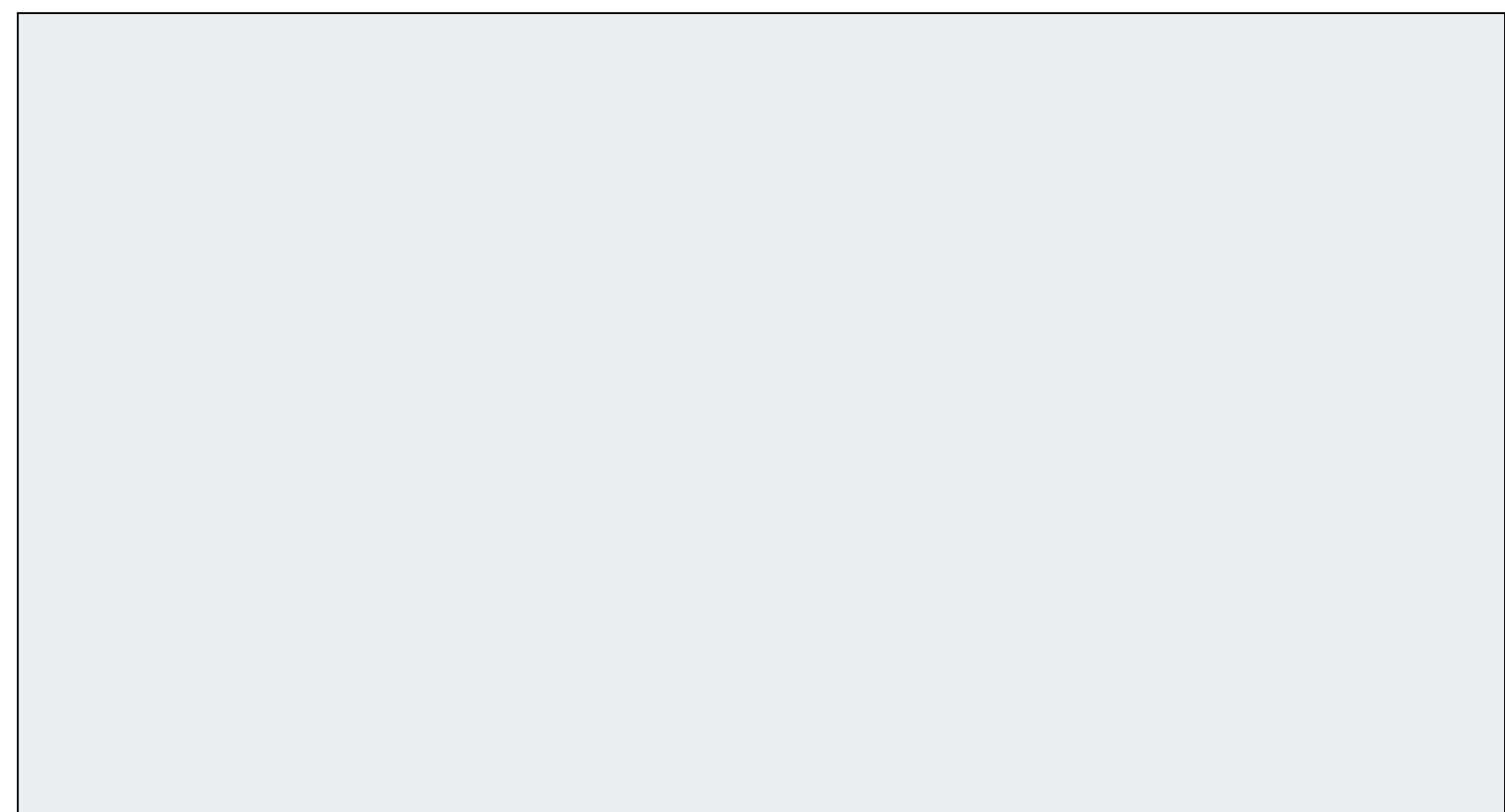


Figure 1: Mutagenic Response of BriteSmile Gel in Tester Strains TA97a, TA98, TA100, and TA1535 Without S9

Results

A range-finding study was conducted in tester strains TA98 and TA100 at levels of 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, and 5 mg BriteSmile gel/plate (Table 1); all additions of test substance and controls were made in 100 µL volumes. The positive and negative controls were within acceptable ranges for tester strains TA98 and TA100. Because toxicity was not observed in tester strain TA98 at 5 mg/plate, this dose level was selected as the highest dose level of BriteSmile Whitening Procedure Gel for definitive testing in the presence and absence of S9.

Initial and Repeat Bioassays

Overview

Initial and repeat plate incorporation bioassays were conducted in tester strains TA97a, TA98, TA100, TA102, and TA1535 at levels of 0.03, 0.08, 0.1, 0.3, 0.8, 1, and 5 mg/plate in the presence and absence of S9. All additions of test substance BriteSmile Whitening Procedure Gel were made in 0.1 mL of water.

TA97a

The spontaneous reversion frequencies as well as positive and negative controls for tester strain TA97a were acceptable in the initial and repeat bioassays. There was consistent evidence of toxicity in the initial and repeat assays in the absence of S9 at 5,000 ?g/plate. The mutagenic response of BriteSmile Whitening Procedure Gel never exceeded twice background at any of the dose levels examined (Figure 1). Therefore, BriteSmile Whitening Procedure Gel was not mutagenic in tester strain TA97a in the presence or absence of S9 in the plate incorporation assay.

TA98

The spontaneous reversion frequencies as well as positive and negative controls for tester strain TA98 were acceptable in the initial and repeat bioassays. Toxicity was observed in the initial and repeat assays at 5,000 ?g/plate in the absence of S9. Because BriteSmile Whitening Procedure Gel never exceeded twice background at any of the dose levels examined (Figure 1), it was not mutagenic in tester strain TA98 in the presence or absence of S9 in the plate incorporation assay.

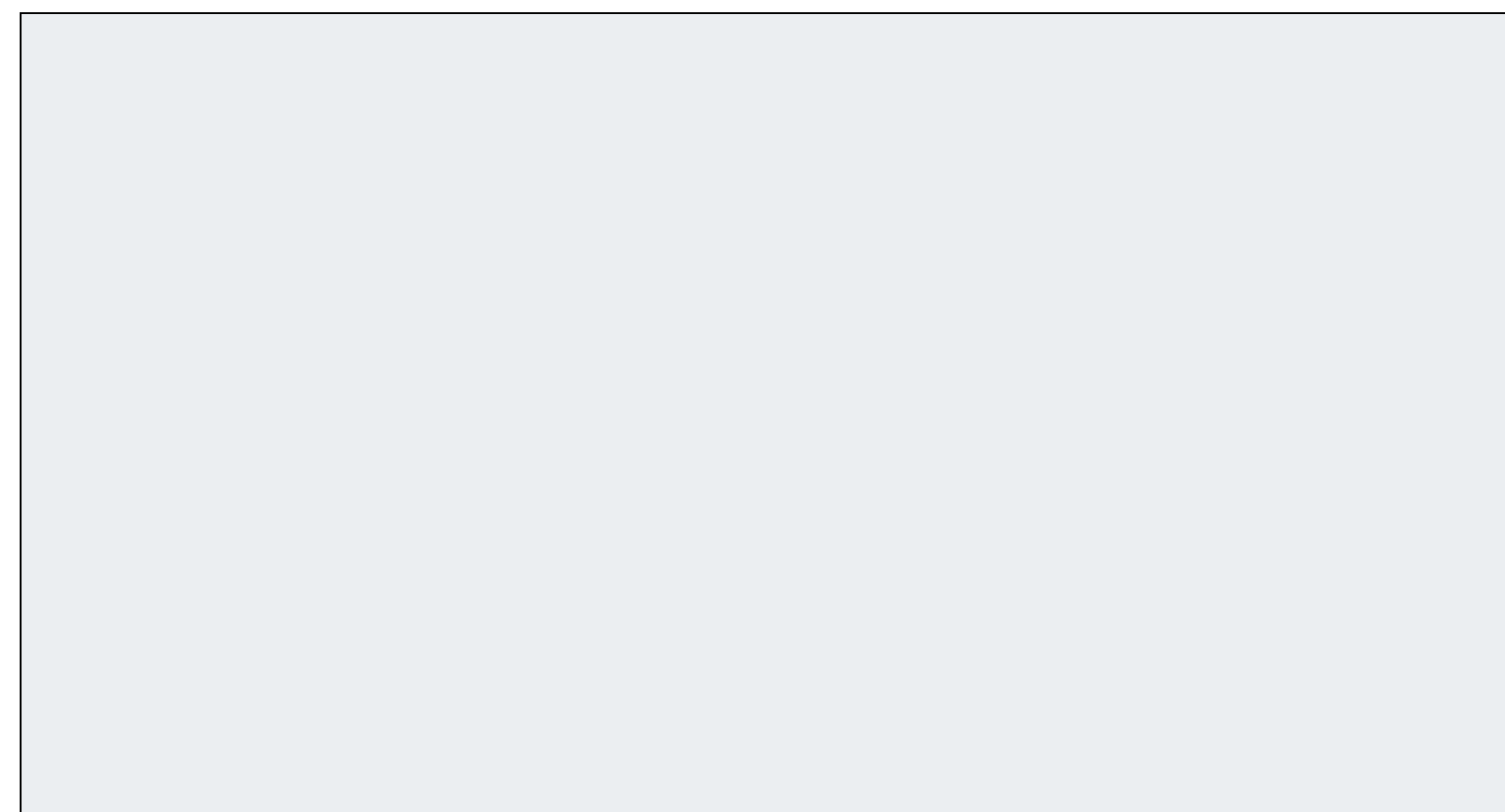


Figure 2: Mutagenic Response of BriteSmile Gel in Tester Strain TA102 Without S9

TA100

The spontaneous reversion frequencies as well as positive and negative controls for tester strain TA100 were acceptable in the initial and repeat bioassays. Toxicity was seen with BriteSmile Whitening Procedure Gel in the absence of S9 at 5,000 ?g/plate. Because BriteSmile Whitening Procedure Gel never exceeded twice background at any of the dose levels examined (Figure 1), it was not mutagenic in tester strain TA100 in the presence or absence of S9 in the plate incorporation assay.

TA102

The spontaneous reversion frequencies as well as positive and negative controls for tester strain TA102 were acceptable in the initial and repeat bioassays. In tester strain TA102, no toxicity was seen with BriteSmile Whitening Procedure Gel in the presence or absence of S9 at dose levels up to 5,000 ?g/plate. The number of revertant colonies exceeded twice background with the test substance in the absence of S9, but not in the presence of S9. The threshold value for a positive response was exceeded when concentrations of hydrogen peroxide in BriteSmile Gel exceeded 86 ?g/plate. Because BriteSmile Whitening Procedure Gel exceeded twice background in tester strain TA102 in the absence of S9 in the plate incorporation assay, it can be considered weakly mutagenic (Figure 2). The slopes of the linear portions of the dose-response curves were 347.0 revertants/?g HP and 423.4 revertants/?g HP, respectively, for the initial and repeat assays of BriteSmile Gel.

For comparison, aqueous hydrogen peroxide was analyzed for mutagenic activity in tester strain TA102 in the absence of S9 at levels up to 200 ?g/plate. A weak mutagenic response was observed, which is consistent with literature reports on the mutagenic potential of aqueous hydrogen peroxide (Figure 3). The threshold value for a positive response was exceeded with hydrogen peroxide concentrations above 100 ?g/plate. The slopes of the linear portions of the dose-response curves were 430.4 revertants/?g HP and 400.5 revertants/?g HP for the initial and repeat assays, respectively.

TA1535

The spontaneous reversion frequencies as well as positive and negative controls for tester strain TA1535 were acceptable in the initial and repeat bioassays. Toxicity was observed at 5,000 ?g/plate in the absence of S9. Because test substance BriteSmile Whitening Procedure Gel never exceeded three times background at any of the dose levels examined (Figure 1), it was not mutagenic in tester strain TA1535 in the presence or absence of S9 in the plate incorporation assay.

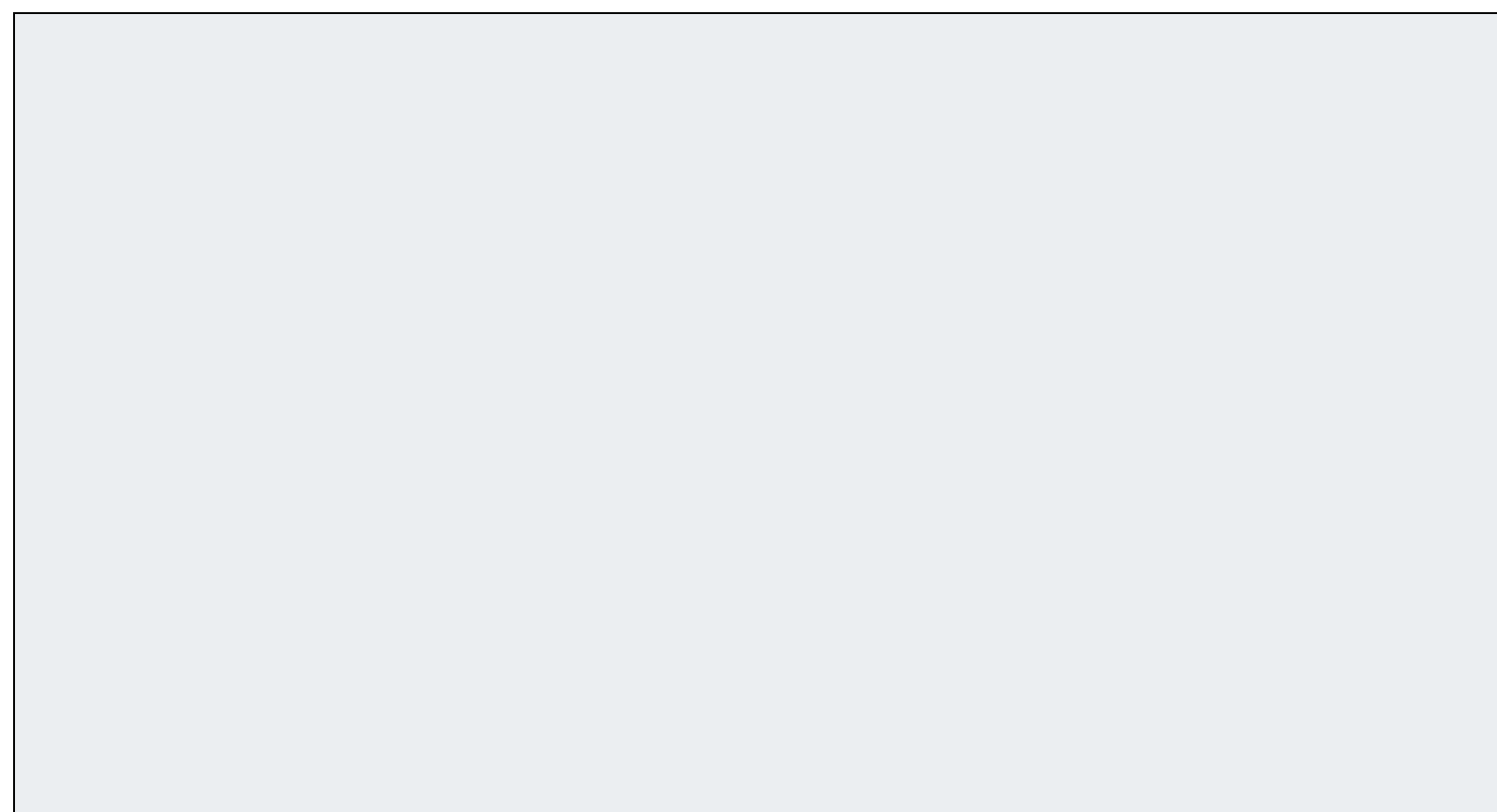


Figure 3: Mutagenic Response of Aqueous Hydrogen Peroxide in Tester Strain TA102 Without S9

Hydrogen Peroxide Concentrations in Test Substance Dilutions			
BriteSmile Dosing Solutions	?g H2O2/plate Initial	?g H2O2/plate Repeat	Theoretical ?g H2O2/plate
30 ?g plate	1.87	4.52	4.5
80 ?g/plate	6.41	12.64	12
100 ?g/plate	10.59	15.40	15
300 ?g/plate	11.15	44.97	45
800 ?g/plate	86.17	122.49	120
1000 ?g/plate	108.32	150.56	150
5000 ?g/plate	548.29	762.21	750
	347.0 Revertants/?g	423.4 Revertants/?g	
BriteSmile Gel %H2O2	15.53	15.65	15%
Aqueous Hydrogen Peroxide	32.4%	31.73%	31.9%
Hydrogen Peroxide Titration			
?g H2O2/plate Initial	?g H2O2/plate Repeat		
1.78	1.33		
11.97	11.07		
26.73	27.12		
54.66	55.81		
108.14	110.16		
168.66	165.08		
18.95	221.68		
430.4 Revertants/?g	400.5 Revertants/?g		

Conclusions

• There was no evidence of an increase in the number of revertant colonies that exceeded twice background in tester strains TA97a, TA98, TA100, and TA1535 at dose levels up to 5 mg/plate in the presence or absence of a metabolic activation source (S9).

• In tester strain TA102, the number of revertant colonies did exceed twice background at dose levels of 0.8, 1, and 5 mg/plate in the absence of metabolic activation, but not in the presence of metabolic activation.

• The lack of a mutagenic response indicates BriteSmile Whitening Procedure Gel is nongenotoxic (non-mutagenic) in tester strains TA97a, TA98, TA100, and TA1535 in a plate incorporation assay.

• BriteSmile Whitening Procedure Gel is weakly genotoxic (mutagenic) in tester strain TA102 only in the absence of external metabolic activation.

• Based on these results, BriteSmile Whitening Procedure Gel is not likely to be carcinogenic in animals or in humans.