A pH Cycling Model as an Alternate to Animal Testing Under the FDA Anticaries Monograph

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ABSTRACT

Objective: Seven pH cycling studies were conducted to establish the fluoride dose response for comparison with dose response data from our animal caries database. **Methods:** Sterilized, caries-free, human crowns of molars and pre-molars were cleaned and polished with a 5µm alumina slurry. Crowns were painted with nail varnish leaving one small window of enamel exposed. The windows were exposed to test slurries at all times during the treatments and subsequent incubations in de- and remineralizing solutions. The treatments and incubations in de/remineralization solutions (referred to as pH cycling) were repeated daily for a total of 14 days. Specimens were treated with dentifrice slurries for one minute, placed into demineralizing solution for 6 hours, treated with dentifrice slurries for one minute again, and then placed in remineralizing solution for 16 hours. Specimens were evaluated using a cross-sectional microhardness technique and reported as Delta Z (lesion severity, micrometers x vol% mineral). **Results:** The treatment groups compared were a) 1100ppm F, b) 250ppm F and c) < 1ppm F (placebo). The mean Delta Z values (± stdev) for the series were a) 945 ± 302, b) 2238 ± 873 and c) 4291 ± 1181, with a > b > c performance (significant @ p = 0.05). These results were compared to results observed in three previous animal caries studies where mean caries scores were a) 21 ± 4, b) 30 ± 3 and c) 36 ± 5. Good agreement was observed indicating the two models have similar capability of separating treatments based on concentration of available fluoride and thus similar capability for discriminating against potentially inferior dentifrice products with lower levels of available fluoride. **Conclusion:** Based on the variances and response to fluoride dose, we conclude that this pH cycling model may provide equivalent accuracy to the animal caries model when evaluating NaF and SnF_2 based formulations under the Anticaries Monograph.

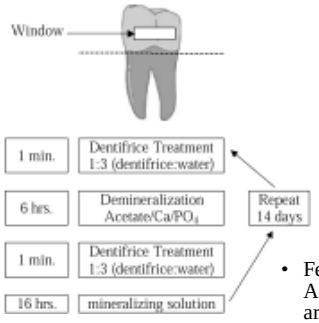
BACKGROUND

All new fluoride dentifrice formulations in the USA are required to meet animal caries testing requirements defined by the Anticaries Final Monograph. P&G is actively pursuing alternate models to the current animal caries monograph requirement. The Anticaries Final Monograph allows for alternative models to be considered providing the alternate model can demonstrate results of 'equivalent accuracy' to the existing model. A demonstration of equivalent accuracy should include, first and foremost, the ability to show a fluoride dose response with separations in fluoride levels previously shown in the animal caries models (i.e., 1100ppm F > 250ppm F > 0ppm F).

MATERIALS AND METHODS

pH Cycling: Caries free human enamel crowns were prepared according to standard procedures (Featherstone et al., 1983). Crowns were cleaned, polished and covered with acid resistant nail varnish leaving one exposed window (approximately 3.0 x 2.0 mm) on the enamel surface. Windows were exposed to the treatment schedule shown graphically below. Following 14 days of treatments crowns were assessed by cross-sectional microhardness and mineral loss (ΔZ) was calculated (White and Featherstone, 1987). An example of this specimen analysis technique is provided below.

Treatment Schedule



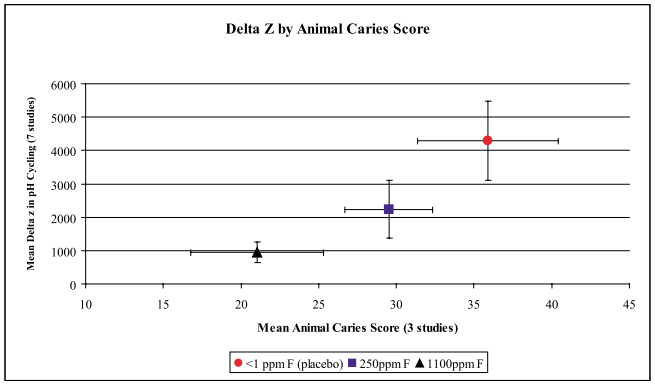
Example of Cross-Sectional Microhardness Analysis

- Featherstone JDB, ten Cate JM, Arends J, Shariati M. Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. Caries Res 1983; 17:385-391.
- White DJ, Featherstone JDB. A longitudinal microhardness analysis of fluoride dentifrice effects on lesion progression *in vitro*. Caries Res 1987; 21:502-512.

Rat Caries: Modified Method #37 is a gross lesion animal caries model in which rats are inoculated with *Streptococcus sobrinus* and placed on high sucrose diet. Fluoride dentifrice treatments were applied as slurries (1:1, paste: water) twice a day for 21 days including once a day on weekends for three weeks. Lesions were disclosed with 2% murexide, teeth are scored for smooth surface lesions and assessed using the Keyes grading method. [These rat caries studies were conducted, under the direction of Dr. George Stookey, at Indiana University OHRI using the accepted FDA modified Method #37. This model is widely used by dentifrice manufacturers to assess products for FDA monograph qualification.]

RESULTS

The results of the 7 recent pH cycling studies and 3 Modified Method #37 studies have been used to demonstrate a historical positive correlation. The correlation is shown in the chart below. The mean values are plotted, with error bars representing standard deviation between studies, for both animal caries results (x-axis) and pH cycling results (y-axis). Only those studies that included placebo, 250ppm, and 1100ppm fluoride legs are included in this figure.



CONCLUSION/NEXT STEPS

- Based on the variances and response to fluoride dose, the proposed pH cycling model appears to be a promising alternative to required animal caries testing under the Anticaries Final Monograph.
- Additional efforts are underway to demonstrate 'equivalent accuracy'. These efforts include comparisons between clinically effective formulations and formulations with attenuated fluoride activity versus appropriate controls in both models.

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