Safety assessment: benzalkonium chloride as sanitising agent

Benzalkonium Chloride

Benzalkonium chloride or BAC is an antibacterial agent belonging to the quaternary ammonium compound group. It is widely used in the health care industry and cosmetic industry as an antimicrobial and cleansing agent. According to the Food and Drug Administration (FDA), 0. 1-0. 13% w/w BAC is considered safe and effective as an " antiseptic drug product". [55] BAC has also been recognized as safe and effective when incorporated into oral mouth rinses. [56]

BAC is also popularly used as a rinse-free hand sanitizer and for hand washing. [57] Short-term and long-term clinical studies have shown that BAC incorporated in intranasal products is safe and well-tolerated. [58]

Furthemore, BAC meets the performance criteria under the Tentative Final Monograph for Health Care Antiseptic Drug Products as an active component in rinse-free hand sanitizers. Sufficient evidence exists to support the usage of BAC as a Category I active ingredient in 1994. It was analyzed as a leave-on sanitizer at the Final Monograph for Healthcare Personnel Antiseptic Drug Products. [59]

A study by Dyer et al concluded that a hand sanitizer with BAC as the main component was a safe and effective way of decreasing illness absenteeism in grade schools. [60]

The safety of BAC was reviewed by an expert panel and they concluded that at concentrations of up to 0. 1%, free active ingredient is “ safe for human use”. [61]

It was acknowledged that BAC can be used beyond being an active ingredient for leave-on products because of its safety and antimicrobial effectiveness profiles.

The FDA has also agreed upon the usefulness of biocidal quaternary amino actives, particularly BAC, in their previous rulemaking. As an example, the FDA stated that “ it was not seriously concerned with the safety of ‘ quats’ for first aid uses like wound cleansers, skin wound protectants, and skin antiseptics” in the 1991 Proposed Rule for Topical Antiseptic Drug Products for Over-the-Counter Human Use; Tentative Final Monograph for First Aid Antiseptic Drug Products. BAC was found to meet the requirements for first-aid antiseptic applications as reviewed by the expert panel at that time.

Furthermore, the FDA has permitted the combination of BAC and Benzethonium Chloride homologues at concentrations of up to 0. 004% as a food preservative or for long-term ingestible applications. [62]

At the 2002 16 th Report of the Cosmetic Ingredients Review Expert Panel, BAC was announced to be safe at concentrations of up to 0. 1% w/w. The cosmetic applications studied here were those that encompassed a daily-use regimen. A publication was done on a cross-over study of 420 grade-school children (5-12 years old) for a period of 10 weeks. They were told to use a leave-on sanitizer with BAC as soon as they entered the classroom, before

eating their snacks and lunch, after sneezing or coughing inside the classroom, and after using the restroom. They observed that even with frequent use, there was no adverse reaction such as edema, rash or erythema, among the students during or after the study. [40]

Another study published in 1998 investigated the performance of an alcohol-free hand sanitizer with BAC. They concluded that the product exceeded the minimum performance standards indicated by the FDA for reducing bacteria. [63]

BAC has been reported to be effective against a wide variety of microorganisms, such as mixed-type viruses and non-enveloped viruses. BAC even demonstrated on some instances, a significantly higher virucidal activity as compared to alcohol applications. One important aspect of BAC that should not be over-looked is its non-flammable nature. In fact, during the SARS epidemic in 2003, BAC-containing sanitizers were very effective against human coronavirus especially in use in airlines and airports as alcohol-containing products were banned because of issues in flammability.

In a study on BAC at reducing illness in public and private schools, they observed a 44. 2% decrease in incidence of gastrointestinal illnesses and a 50. 2% reduction in respiratory-related illnesses. In a different double-blinded study with 769 students, there was a 33% reduction in illness-related absenteeism. It was recommended that alcohol-free leave-on sanitizers, like those with BAC, are the only appropriate composition for usage in settings as school. This is due to the possible misuse of alcohol-containing sanitizers as a fire accelerant or by intentional ingestion.

An excellent review on the issue of bacterial resistance to antibacterial sanitizers was also recently submitted by SDA to FDA in response to the reopening of the docket for comments.

However, the FDA advised that further studies should be done regarding antimicrobial resistance and the potential development of resistance to these agents, including BAC. This was stated at the joint meeting in 1997 of the FDA Advisory Committees.

In the field of Orthodontics, Othman et al [64] confirmed that adding BAC to an adhesive produces antibacterial properties. In the study, BAC was incorporated into a light-cured composite resin, and an evaluation of its antimicrobial and physical properties was done. BAC was incorporated into Reliance Phase II adhesive to produce BAC concentration from 0. 25% to 2. 50% wt/wt. The modified samples were cut into disks and incubated in *Strep. mutans* for two days. Amount of bacterial inhibition was measured with agar disk diffusion assay. Other disks were placed in brain-heart infusion medium with *Strep. sobrinus* to measure its adherence. Cells that adhered were measured. Tensile bond strength was analyzed using a universal testing machine by attaching traction hooks to bovine teeth with the modified adhesives. Results showed an increase in antimicrobial activity in composites with higher BAC. Antimicrobial activity was negative for the composites without BAC. The composite with BAC and without BAC did not show any difference with regard to their diametral tensile stress and tensile bond strength. Therefore, they concluded that when BAC was combined with an

orthodontic adhesive, it added antimicrobial properties without changing its mechanical properties.

Another study that aimed to create an antibacterial adhesive without compromising its bond strength was done by Saito et al. [65] Their aim was to find the dose of BAC that exhibited antibacterial effect and did not compromise bond strength.

In Saito's study, Superbond C&B resin cement was used. A mixture of the polymer, monomer, catalyst and BAC was produced according to the instructions of the manufacturer. Composite disks with the following BAC concentration were produced: 0. 25%, 0. 75%, 1. 25%, 1. 75%, 2. 5%, and 5% (wt/wt). Composite disks of the same size (0. 8 mm x 2 . 0 mm thickness) were made. The test bacteria used were *Strep. mutans* 10449 and PS14, and *Strep. sobrinus* 6715 and B13. A brain-heart infusion medium was used to grow the bacteria.

To evaluate the antibacterial activity, disk diffusion assay was utilized. To analyze the release of BAC as shown by an inhibition of growth of the bacteria, the semidiameter of the growth inhibition zone was measured.

The bacteria were grown overnight in a broth and diluted. An optical density of 0. 5 (550nm) was used. An inoculation of 80 µL (60-fold dilution) was performed on the brain-heart infusion agar plates. A glass rod was used to spread the inoculums evenly providing uniform bacterial growth. The BAC-infused composite disks were placed on the agar and were incubated at 37ºC. Two days after incubation, the inhibition zones were measured.

Saito also tested the shear bond strength using 70 extracted human premolars. The teeth were randomly distributed into seven groups and embedded in acrylic except for the buccal areas which were used for bonding. The teeth were polished using rubber prophylactic cups with pumic for 10 seconds.

The teeth were etched with 65% phosphoric acid for 30 seconds and washed for 20 seconds then orthodontic metal brackets were bonded. Composite with the following BAC concentrations were used to bond the brackets: 0%, 0. 25%, 0. 75%, 1. 25%, 2. 5%, and 5%. A 300-gram force was applied on each bracket (based on the study of Bishara et al [66]).

The results were presented in standard deviation and mean and analyzed with ANOVA to determine if there were significant differences among the groups. For multiple comparisons, Fisher test was used. Statistical differences were detected by a Chi-square test. Significance was predetermined at $P < 0.5$. [38]

Results showed that there were significant differences among the composites with different BAC concentrations. No significant differences were found among the bacterial strains.

When placed on the agar plate with *Strep. mutans or Strep. sobrinus* , no inhibition zone was observed on the composite with 0% BAC. The composite disks with BAC demonstrated a significant antibacterial property when compared with the composite without BAC. The antibacterial effect was found to increase as BAC concentration increased. This was indicated by an

increase in the bacterial inhibition zones that were measured. The disks with 5% BAC had the highest amount of antibacterial activity.

A significant difference in bond strength was noted among the different composites with BAC. However, as BAC concentration was raised, they observed a decline in shear bond strength.

The study of Saito et al concluded that adding BAC into composite resin attributes antimicrobial properties strong enough to inhibit *Streptococcus mutans* and *Streptococcus sobrinus.*

A clinically acceptable range of shear bond strength is yet to be established scientifically. Saito noted that incorporation of BAC does not lessen the strength to less than 10 MPa when an etchant (phosphoric acid) is used. The range of shear bond strength in study was 10. 12 MPa – 20. 94 MPa. Saito et al further concluded that a composite incorporated with BAC “ has a possibility for clinical application as a bonding adhesive”. It was recommended in the study that it is essential to acquire a long-term release behaviour of an antibacterial agent. Therefore, a study investigating the release behaviour of BAC should be done.

It was mentioned in the study by Saito that BAC has been a popular choice of contact lens antiseptic. However, the quantity of BAC used in the study was less than the amount used in contact lenses. A typical bonding procedure in an orthodontic patient requires around 150 mg of composite adhesive. Therefore, 0. 7 mg of BAC is required to achieve 0. 5% BAC concentration.

The same author in 2009 evaluated the antibacterial activity and cytotoxicity of an orthodontic adhesive containing BAC. To achieve the desired concentration of BAC, it was initially diluted to 50% by mixing it with the orthodontic polymer (wt/wt). It was diluted further with polymer to achieve the required concentration. [65]

They achieved the following BAC concentrations: 0. 25%, 0. 75%. 1. 25%, 1. 75%, 2. 5%, and 5. 0% (wt/wt). Custom-made molds were used to produce BAC-composite disks of the same size (8. 0 mm x 2. 0 mm). The disks were evaluated using antibacterial and cytotoxicity assays with three independent runs.

To perform the disk diffusion assay, the BAC composite disks were soaked in distilled water at 37ºC for 0, 30, 90, and 180 days prior to the assessment of antibacterial activity.

The test bacteria used were *Strep. mutans* 10449 and PS14 and *Strep. sobrinus* 6751 and B13. They were routinely grown in a brain-heart infusion medium for 24 hours. The growth inhibition of *Strep. mutans and /* or *Strep. sobrinus* showed the release of BAC into the agar medium. Electric digital callipers were used to take measurements of the zone of growth inhibition around each BAC composite disk.

To test the cytotoxicity, human gingival fibroblast cultures were grown from the cells of healthy gingival tissues from patients who were undergoing extraction prior to orthodontic treatment. This was copied from the method of Somerman, et al.

After the patients underwent extraction, a part of the gingival tissue attached to the interdental papilla was taken. The samples were washed twice in phosphate-buffered saline solution (PBS). The acquired tissues were dissected into 1-mm cubes and were transferred to 35-mm tissue culture dishes with α-minimal essential medium supplemented with 100 µg/mL of penicillin G, 500 µg/mL gentamicin sulphate, 0. 3 µg/mL amphotericin B, and 10% fetal bovine serum. Incubation of the cultures was done in a 37ºC humidified incubator with 95% air and 5% carbon dioxide. When confluence was reached, the cells were detached using 0. 05% trypsin in PBS for 10 minutes. They were subcultured in flasks and seeded. [38]

The controls used in the study were the resin disks without BAC. The harvested gingival cells were laid under resin disks. In between the disks and the gingival cells was an 8 µm PET membrane that allowed the passage of the components of the resins to make contact with the human gingival cells. This was based on the study of Tang et al. Incubation with the gingival cells were done at 37ºC for 1, 3, and 6 days. [38]

A reduction assay kit containing tetrazolium bromide was used to assess the cytotoxicity. This was based on the reduction that occurs to MTT when exposed to living cell mitochondria. It becomes purple fromazan. After 1, 3, and 6 days, the cells were cleaned with PBS after removal of the resins and the medium. Addition of 0. 5 mL MTT medium solution was made to the wells. The solution was incubated at 37ºC for 3 hours. A microplate reader was used to measure the purplish lysate.

They observed significant differences among the different lengths of time the samples were soaked in distilled water and among the BAC concentration. With the different bacteria, they did not detect any significant differences. No difference that was significant enough was also observed between the bacterial strain and BAC.

There were significant differences noted regarding the antibacterial activity at 0 day between samples with 5% BAC compared to other BAC concentrations. There were also significant differences observed regarding antibacterial activity among 0. 25%, 0. 75%, and 1. 25% BAC, and 1. 75%, 2. 5%, and 5% BAC concentration. However, there was not any significant difference detected among 1. 75%. 2. 5%, and 5% BAC concentrations. There was a significant difference at 180 days between 0. 25%, 0. 75%, and between 1. 25% and 5% concentrations. They observed that the higher the concentration of BAC in the composite, the greater the decrease that occurred in antibacterial property based on the assay time prolongation.

They concluded that when the samples were not soaked in water, they exhibited higher antibacterial activity. The resins with 5% BAC had the highest antibacterial property.

There was a significant decrease in antibacterial activity after soaking the samples in water for 180 days at all BAC concentrations. The samples incorporated with 5% BAC had the highest antibacterial activity compared to the resin with 0. 25% at all periods of the experiment. In fact, the antibacterial activity exhibited by the resin with 5% BAC soaked for 180 days

was the same as the antibacterial activity shown by the resin containing 0. 25% and 1. 75% BAC prior to immersion in water.

There were significant differences in MTT activity percentages among the BAC composites with regard to cytotoxicity to the human gingival cells. Saito's study concluded that resins with 1. 25% or 1. 75% BAC exhibited constant antibacterial properties when immersed in water for 180 days due to the continuous BAC release.

However, an increase in cytotoxicity was noted as the antibacterial activity increased. Therefore, composites with a higher concentration of BAC were shown to be more cytotoxic. A concentration of BAC that was 0. 25% or 0. 75% exhibited cytotoxicity similar to the resins without BAC or the controls at all test periods. Saito et al recommended that a similar study be made using saliva instead of water as the immersing medium. This is due to the presumption that the oral environment is different that it may influence the antibacterial activity and cytotoxicity of the resins with BAC.

The study confirmed that adding BAC to a composite resin exhibits an antibacterial effect against *Strep. mutans* and *Strep. sobrinus.* The author suggested that BAC be tested in vivo for safety before it is tested clinically.