Introduction:
LoxaSperse is a powder excipient base used for nebulization and irrigation. LoxaSperse is a blend of specially micronized xylitol with an optimized ratio of micronized poloxamers, designed to improve the dispersability and solubility of APIs (PCCA, 2013). The use of xylitol and poloxamers in nebulization and irrigation is thoroughly referenced in the literature and there is ample evidence of their safety and efficacy (Durairaj et al., 2000; Jagannath et al., 1995; Pliatki et al., 2011; Zabner et al., 2000). Gentamicin is an aminoglycoside antibiotic and has bactericidal action against many gram-negative aerobes and against strains of staphylococci (Martinade 35, 2007). PCCA tested the performance of Formula #10337 which is gentamicin (80 mg) in a LoxaSperse mixture and measured efficacy against microbial activity when mixed with sterile water.

Methodology:
The efficacy of gentamicin LoxaSperse dilutions were evaluated by serially diluting the formula in sterile water and plating for colony counts with minimum inhibitory concentrations of 101 to 104 CFU/mL product challenge was performed according to universal AET procedures (Moser and Meyer, 2011). 1 mL aliquots of the test articles were prepared in 15 mL polycarbonate test tubes. 10 µL of cell culture diluted in Phosphate Buffered Saline (PBS, Sigma-Aldrich®) was added to each 1 mL aliquot to initiate the AET assay. 10 µL of cell culture was also added to 1 mL PBS for initial colony counts at the start of the AET assay.

During the AET assay, 100 µL of the mixture was removed at intervals of 0.5h, 6h, 28h, and 168h, serially diluted, and plated for colony counts. Final colony counts, reported in CFU/mL and Log10 reductions in viable cell numbers are discussed in this report.

Results and Discussion:
Initial colony counts of E. coli, P. aeruginosa, S. aureus, and C. albicans indicated that a 102 to 104 CFU/mL product challenge was performed for these organisms (Table 1). A. niger colonies were not obtained from these initial plates (≤10 CFU/mL, Table 1), but counts from subsequent plates indicated that 101 to 102 spores were present at the start of the AET (Table 2).

Over the course of the AET, viable cell/spore counts varied depending upon the test article, where it was prepared, and the test organism.

No viable cells of E. coli, S. aureus or P. aeruginosa were recovered after 0.5h exposure.

C. albicans: a 1-Log reduction observed after 0.5h and no viable cells were observed after 24h.

A. niger: colony forming spores were recovered up to 128h in solutions.
Conclusions:
The Test Article containing Gentamicin Sulfate USP and LoxaSperse reduced the number of viable bacteria (E. coli, S. aureus and P. aeruginosa) within 0.5h of exposure and no bacterial growth was observed up to 168 h after exposure. A 3-Log to 4-Log reduction in viable bacteria was observed within 0.5h (Tables 1-2). A 1-Log reduction in the number of viable C. albicans cells was observed within 6h and no C. albicans cells were recovered after 24h (a 2-Log reduction). The gentamicin and LoxaSperse formulation continued to reduce the number of viable A. niger spores throughout testing. Additionally, the low number of spores introduced at the initiation of the AET and the subsequent low limit of detection prevented the observation of a significant 1-Log reduction of viable spores. The chosen formula when intentionally contaminated with microorganisms specified in USP 51 resisted microbial growth. Further, this study demonstrated this formulation after reconstituted was not at risk or did not support microbial growth.

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References: