

4. New Data and Comments on Cetylpyridinium Chloride (CPC)

The Dental Plaque Subcommittee recommended cetylpyridinium chloride as a Category I ingredient based on information submitted during the call-for-data and in presentations at the Subcommittee meetings. These data establish the safety and effectiveness of CPC as an antigingivitis/antiplaque active ingredient. In this submission, Procter and Gamble provides additional data regarding the *in vitro* antimicrobial activity of CPC to support an optional antibacterial indication identical to that recommended for essential oils.

- 4.A. New microbiology studies demonstrate that CPC has antimicrobial activity against representative organisms responsible for, or associated with, gingivitis. Based on these data, we request that FDA grant CPC the same optional indication of “helps (select one) ‘control’, ‘inhibit’, or ‘kill’ plaque bacteria that contribute to the development of (select one or more) ‘gingivitis’; ‘gingivitis, an early form of gum disease’; or ‘bleeding gums’.”**

As noted in Section 3 for stannous fluoride, during the Subcommittee proceedings, Warner-Lambert presented data demonstrating the antimicrobial properties associated with the fixed combination of essential oils. Details of the Subcommittee recommendation for this optional “*helps kills plaque bacteria*” indication for essential oils were provided earlier. Likewise, in this current section we are requesting the Agency allow this optional indication for CPC.

Procter & Gamble has generated the same type of data, as well as additional data, that Warner Lambert provided to the Subcommittee in order to demonstrate a relative comparison of the bacterial properties of a 0.05% CPC mouthrinse and to support an antibacterial indication. This includes a series of five microbial studies to establish the antimicrobial activity of a 0.05% CPC mouthrinse which demonstrate that CPC can

provide significant antimicrobial benefits. Specific details of the study designs, results, and conclusions from these studies are found in the referenced appendices specified below. Results of these studies are briefly summarized below. Details of study objectives and methods are the same as discussed in Section 3 for stannous fluoride.

4.A.1. Time Kill Study (Appendix 8)

The objective of the time kill study was to evaluate the *in vitro* antimicrobial activity of CPC. This testing included the same organisms used by Warner Lambert in support of their antibacterial claim for essential oils. These data demonstrate a substantial antimicrobial kill of plaque organisms that are exposed to a 0.05 % CPC rinse formulation containing at least 360ppm CPC [0.05% CPC rinse x 72% bioavailable = 360ppm CPC] for 30 seconds. Testing indicates that a 0.05% CPC mouthrinse resulted in predominately a 2 to 5 log reduction in the plaque organisms associated with gingivitis. The percent reductions are calculated from the raw data: to give an example, if an inoculum count of 3×10^8 cfu/mL is reduced to 3×10^7 cfu/mL the percent reduction or kill is 90%. This means a 1-log reduction corresponds to a 90% germ kill. A 2-log reduction corresponds to 99%, 3-log reduction corresponds to 99.9%.

**Time Kill Kinetic Study Data after 30 Seconds Exposure of 0.05% CPC to
 Representative Plaque Bacteria**

Representative Organisms	0.5% CPC, Log cfu/ml	Water, Log cfu/ml	% kill relative to water	Log reduction relative to water
<i>A. viscosus</i> ATCC19246	3.6	7.22	99.97	3.62
<i>C. albicans</i> ATCC 10231	4.18	6.47	99.86	2.29
<i>C. rectus</i> ATCC 33238	3.6	4.84	93.08	1.24
<i>F. nucleatum</i> ATCC 10953	3.6	7.65	99.99	4.05
<i>H. actinomycetem- comitans</i> ATCC 29522	5.01	7.90	99.97	2.89
<i>L. casei</i> ATCC 393	7.64	7.94	99.99	0.3
<i>P. intermedia</i> ATCC 25611	3.6	8.76	99.99	5.16
<i>P. aeruginosa</i> ATCC 27853	6.02	8.29	74.6	2.27
<i>P. gingivalis</i> ATCC 33277	3.6	8.25	99.99	4.65
<i>S. sanguinis</i> ATCC 10556	3.6	6.12	99.69	2.52
<i>S. mutans</i> ATCC 35668	5.33	7.77	99.99	2.44
<i>Eikenella corrodens</i> ATCC 23834 (GC agar)	4.95	6.82	99.89	1.87

Time Kill Kinetic Study Data after 30 Seconds Exposure of 0.05% CPC to Representative Plaque Bacteria

Continued

Representative Organisms	0.5% CPC, Log cfu/ml	Water, Log cfu/ml	% kill relative to water	Log reduction relative to water
<i>Eikenella corrodens</i> ATCC 23834 (BBA agar)	5.08	6.83	99.90	1.75
<i>Salmonella typhimurium</i> ATCC13311	3.6	7.65	99.99	4.05
Stimulated Whole Saliva Total Aerobes	3.65	5.74	99.27	2.09
Stimulated Whole Saliva Total Facultative Anaerobes	3.6	5.16	96.80	1.56
Stimulated Whole Saliva Total GNAs	3.69	6.63	99.90	2.94

These data demonstrate the substantial antimicrobial activity toward representative microorganisms associated with gingivitis within 30 seconds of exposure to the test product. *P.aeruginosa* and *L. casei* seemed least sensitive to the activity of 0.05% CPC. Neither *P.aeruginosa* or *L. casei* are directly implicated as causative agents in gingivitis. These *in vitro* data provide compelling evidence that 0.05% CPC has antimicrobial activity against organisms causing or associated with gingivitis.

4.A.2. Minimum Inhibitory Dilution (MID)/Minimum Bactericidal Dilution (MBD) Analysis (Appendix 9)

The objective of the MID/MBD studies was to evaluate the *in vitro* antimicrobial activity of a CPC rinse formulation against a battery of organisms responsible for or associated with gingivitis. Results of the MID/MBD tests are presented in the table below for an oral rinse containing 0.05% CPC, an analytically prepared standardized

solution containing 360ppm CPC in deionized water and the fixed combination of essential oils.

**Minimum Inhibitory Dilution/Minimum Bactericidal Dilution Values for 0.05%
 CPC Rinse Against Representative Plaque Organisms**

(triplicate data)

Representative Organisms	0.05% CPC mouthrinse		0.036% CPC solution		Listerine	
	MID	MBD	MID	MBD	MID	MBD
<i>A. viscosus</i> ATCC19246	1:450	1:100	1:300	1:100	<1:10	<1:10
<i>C. albicans</i> ATCC 10231	1:100	1:50	1:50	1:30	<1:10	<1:10
<i>C. rectus</i> ATCC 33238	1:800	1:300	1:500	1:150	1:20	<1:10
<i>E. corrodens</i> ATCC 23834	<1:10	1:20	<1:10	1:10	<1:10	1:10
<i>F. nucleatum</i> ATCC 10953	1:550	1:200	>1:100	1:20	<1:10	<1:10
<i>H. actinomycetem-comitans</i> ATCC 29522	1:800	1:100	1:500	1:100	<1:10	<1:10
<i>L. casei</i> ATCC 393	1:150	<1:10	1:100	<1:10	<1:10	<1:10
<i>P. intermedia</i> ATCC 25611	1:150	NR	1:100	NR	<1:10	NR
<i>P. aeruginosa</i> ATCC 27853	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10
<i>P. gingivalis</i> ATCC 33277	1:450	1:450	1:300	1:350	1:10	1:10
<i>S. sanguinis</i> ATCC 10556	1:450	1:200	1:300	1:150	<1:10	<1:10

**Minimum Inhibitory Dilution/Minimum Bactericidal Dilution Values for 0.05%
 CPC Rinse Against Representative Plaque Organisms**

Continued

Representative Organisms	0.05% CPC mouthrinse		0.036% CPC solution		Listerine	
	MID	MBD	MID	MBD	MID	MBD
<i>S. mutans</i> ATCC 35668	1:300	1:100	1:200	1:100	<1:10	<1:10
<i>Salmonella typhimurium</i>	1:100	1:20	1:50	1:20	1:10	<1:10
Stimulated Whole Saliva	1:300	1:200	1:150	1:100	<1:10	<1:10

<1:10 means the product did not inhibit the growth of the microorganism even at 1:10 dilution. Lower dilutions have not been performed here.

>1:1000 means the product inhibited the growth of the test organism at the highest dilution tested in this experiment.

NR – Not Reported *P. intermedia* did grow on BBA plates

The water control did not show any inhibition of the test organisms.

The table above shows the antimicrobial efficacy of three rinse preparations (0.05% CPC rinse, 0.036% CPC in deionized water and Listerine® Oral Rinse (fixed combination of essential oils)) tested against standard representative organisms implicated in gingivitis and wild type organisms present in saliva. The data provide compelling evidence that the CPC containing rinses show good *in vitro* antimicrobial activity against organisms associated with gingivitis.

4.A.3. Oral Biofilm Inhibitory Properties (Appendix 10)

This oral biofilm data represents new data beyond that reported to the Subcommittee for essential oils and also addresses the commentary made by Dr. Bowen on behalf of the Subcommittee with regards to oral biofilms. The current study assessed the ability

of a CPC rinse containing 360ppm available CPC [0.05% CPC rinse x 72% bioavailable = 360ppm CPC] to inhibit the growth of an oral Biofilm. Results demonstrated a significant reduction in both total facultative anaerobes and total facultative gram-negative anaerobes from the CPC rinse compared to water.

**0.05% CPC Rinse Effects on Bacterial Populations contained within an
In Vitro Oral Biofilm**

Mean Log₁₀ CFU/ml Difference vs. Water

Oral Flora Subgroup	Assay #1*	Assay #2*	Average
Total Facultative Anaerobes	0.84 ^b	0.66 ^b	0.75
Total Facultative Gram-Negative Anaerobes	0.68 ^b	0.88 ^b	0.78

* All data shown here were calculated by subtracting the mean log CFU/ml for the CPC group from that of the water group.

^b For all treatments, the mean differences from water were significant with p<0.05.

Since total facultative anaerobes and total facultative Gram Negative anaerobes are implicated in the etiology of gingivitis, these study data provide strong *in vitro* microbiological evidence that CPC kills the bacteria associated with gingivitis.

4.A.4. Vital Staining of *In Vitro* CPC-Treated Saliva Samples (Appendix 11)

This study assessed the microcidal activity of a 0.05% CPC rinse utilizing a live/dead staining technique to provide a visual representation of the effect.

As is evidenced from the digital color photographs from this study [Appendix 9], the saliva samples treated with CPC resulted in a >90% red color (denoting kill) compared to a water treated sample which was >90% green (denoting live cells). This provides a visual representation of the bactericidal effect of 0.05% CPC rinse.

4.A.5. *In Vivo* Salivary Germ Kill Test (Appendix 12)

The fifth antimicrobial study of CPC's antimicrobial effects was a randomized, two-period, two-treatment (0.05% CPC and water) crossover design trial. During the first period, one group rinsed 13 times over a 5-day period with 0.05% CPC, and the other group rinsed with water 13 times. Saliva samples were collected prior to the first rinse, immediately prior to the 13th rinse, and at several time points from 2 minutes to 2 hours after the 13th rinse. Each saliva sample was assayed to determine the concentration of total aerobic bacteria and total facultative anaerobic bacteria. After a 2-week washout, the treatment and sampling procedure was repeated with the other treatment for each group.

Results showed a significant reduction, relative to water, of mean total aerobes and mean total facultative anaerobes in saliva samples of panelists treated with CPC. It is noteworthy that approximately 9 hours had passed when the sample collected prior to the 13th rinse was taken. This indicates that the 0.05% CPC rinse has the ability to suppress the bacterial concentration in saliva for several hours post use.

**Log Reduction of 0.05% CPC Treatment vs. Water at Three Sampling Times
 Analysis of Covariance for Repeated Measures
 Log Colony Forming Units/mL**

Sampling Time	Total Facultative Anaerobes	Total Aerobes
Immediately prior to the 13 th rinse	- 0.42*	- 0.45*
2 minutes after the 13 th rinse	- 2.74*	- 3.18*
2 hours after the 13 th rinse	- 0.70*	- 0.82*

*p-values < 0.05

4.A.6. Summary of CPC Antibacterial Results and Recommendations

The data submitted herein from five *in vitro* (30-sec Time Kill, MID, MBD, Oral Biofilm, Vital Stain) and one *in vivo* (Germ Kill) microbiological study provide substantial evidence of the ability of a 0.05% CPC rinse to kill plaque organisms that are associated with or responsible for gingivitis. These data include:

- A time kill study demonstrating predominately a 2 to 5 log reduction of plaque organisms exposed to 0.05% CPC rinse for 30 seconds,
- A study showing that dilutions of CPC in ratios of 1:100 to 1:800 are inhibitory against representative plaque organisms
- A study showing that dilutions of 1:20 to 1:450 are bactericidal against those same plaque organisms.
- Total Facultative Anaerobic and Total Facultative Gram Negative Anaerobic plaque organisms within oral biofilms derived from human saliva are significantly reduced following exposure to CPC rinse.
- Fluorescent staining techniques which discriminate live vs dead cells demonstrate a >90% kill following exposure to CPC rinse.
- Significant reductions in total facultative anaerobes and total aerobes are seen at various times up to 9 hours following treatment with CPC rinse when compared to a water control.

Based on these data, Procter & Gamble requests that the Agency provide for the optional indication (i.e. "uses") in CPC labeling that is currently recommended for essential oils, viz, "helps (select one of the following: 'control,' 'inhibit' or 'kill') plaque bacteria that contribute to the development of (select one or more of the following: 'gingivitis,' 'gingivitis, and early form of gum disease,' or ' bleeding gums'."