Appendix 3

LITERATURE REVIEW ON

BACTERIAL RESISTANCE AND TRICLOSAN

BETWEEN 2002 AND 2005

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ANTIBACTERIAL RESISTANCE AND TRICLOSAN

I. INTRODUCTION

In 2002 the EU Scientific Steering Committee, SSC, concluded that

"...there is no convincing evidence that triclosan poses a risk to humans or
the environment by inducing or transmitting antibacterial resistance under
current conditions of use."

This conclusion was based on a review of current literature pertaining to the
safety of triclosan in personal care products

Subsequently, the SCCNFP stated that it is "of the opinion that

1. under current conditions of use of triclosan as a preservative in cos-
metic products, it is safe taking into account the risk of resistance by cer-
tain micro-organism.
2. there is no need for setting a new concentration limit for the use of
triclosan in cosmetic products."

In the intervening years additional studies have investigated the safety of tri-
closan. The purpose of this document is to provide DG Enterprise with an over-
view of those studies. In this submission, we are going to present the evidence
that the recently published scientific information demonstrates the following:

- Further work has been done and progress has been made to eluci-
date the many mechanisms of action of triclosan including membrane de-
stabilization, inhibition of fatty acid synthesis, efflux mechanisms, and for-
formation of biofilms;
- A number of additional studies have shown the safety, efficacy, and
benefit of triclosan in oral care and skin care products;
- Laboratory studies continue to demonstrate that bacterial strains with
increased tolerance to triclosan can be developed in the laboratory by se-
lecting mutants based on growth in the presence of either sub-inhibitory
concentrations of triclosan or supra-inhibitory concentrations of triclosan;
- A number of surveillance studies have been conducted looking for
organisms with decreased susceptibility to triclosan in natural environ-
ments where there has been repeated exposure to triclosan. There was
no evidence of shifts in populations or development of resistance to tri-
closan in any of these studies. There is little evidence of a correlation of
resistance between triclosan-tolerant strains and antibiotic resistant
strains. While a study of human and animal Campylobacter isolates re-
ports a small proportion (4.5%) of strains examined that were less suscep-
tible to triclosan and more resistant to six (6) antibiotics, this is thought to be related to a general mechanism of resistance, such as efflux and unlikely to be related to use of triclosan; and
- A number of expert reviews have concluded that while cross-resistance to biocides and antibiotics can be demonstrated in the laboratory using pure cultures, it does not necessarily equate to the development of such resistance in the natural or clinical environment where complex, multispecies biofilms predominate, that the use of triclosan in cosmetic and over-the-counter drug products was safe and not expected to select for antimicrobial resistant bacteria, and that triclosan has a low potential for acquired bacterial resistance.

It is our opinion that the new evidence overwhelmingly supports the previous findings of the SSC and the SCCNFP.

II. USES OF TRICLOSAN

Triclosan is used in some personal care products including dentifrices, bar soaps, liquid hand and body soaps, and deodorants, where those products provide a consumer benefit. Usage of triclosan in those products has not significantly changed in the past three to five years.

III. MECHANISMS OF ACTION

Further work has been done and progress has been made to elucidate the many mechanisms of action of triclosan including membrane destabilization, inhibition of fatty acid synthesis, efflux mechanisms, and formation of biofilms. The different mechanisms described below are dependent on organism type and level of triclosan, and at higher concentrations, it is likely that the non-specific mechanisms tend to prevail, resulting in lower risk of decreased susceptibility developing. It is clear that different mechanisms/targets exist in different bacteria and their ability to mount a response to the effects of triclosan will be dependent on these various modes of action.

A. Membrane Destabilization

The perturbing effects of triclosan on membrane structures suggest that this molecule would alter membrane functions, affecting not only lipids, but also indirectly the proteins of the membrane, the functions of which are highly dependent on membrane structure (Lygre et al., 2003).

B. Inhibition of Fatty Acid Synthesis

Numerous studies have been conducted on the pathway of fatty acid synthesis resulting in a clearer understanding of this mechanism in the various bacteria. At
sub-lethal concentrations triclosan has been shown to target ENR, the enoyl-acyl carrier protein (ACP) reductase (FabI enzyme) or enzymes of similar function in both Gram negative and Gram positive bacteria.

The x-ray crystal structure of ENR from *Escherichia coli* has been determined (Roujeinikova et al., 1999; Stewart et al., 1999). Triclosan sits in the binding pocket of the enzyme and forms hydrogen bonds with the 2-hydroxyl group of the nicotinamide. The 2-hydroxyl group is crucial for antibacterial activity.

The enoyl-acyl carrier protein reductases have been described from *Bacillus subtilis* (Schujman et al., 2001), mycobacteria (Kremer et al., 2003; Rawat et al., 2003), and *Staphylococcus aureus* (Fan et al., 2002). In the study on *S. aureus*, it was concluded that overexpression of the FabI enzyme did not appear to be advantageous, since it appeared in only one of the 31 clinical strains tested. Presence of these reductases in various bacteria indicates that they may well be susceptible to triclosan.

C. **Efflux Mechanisms**

The efflux of potentially harmful molecules is a common defense mechanism in bacteria. The mechanism by which triclosan is effluxed from bacteria has been elucidated in *Pseudomonas aeruginosa* (Chuanchuen et al., 2002; Chuanchuen et al., 2003), *E. coli* (Levy, 2002), *Salmonella enteria* (Braoudaki and Hilton, 2005), and *Stenotrophomonas maltophilia* (Sanchez et al., 2005). In some cases, the up-regulation of the efflux mechanism by sublethal levels of triclosan resulted in some cross-resistance to antibiotics (Levy, 2002; Sanchez et al., 2005) but not in all cases (Chuanchuen et al., 2002; Sanchez et al., 2005).

D. **Biofilms**

Two studies using oral plaque biofilms have shown that, as expected, triclosan was less effective against organisms in biofilms when compared to the same organisms in planktonic suspensions (Fine et al., 2001; McBain et al., 2003a). The mean MIC of triclosan for the biofilm strains did not change significantly after five (5) days of treatment (McBain et al., 2003a).
IV. SAFETY AND EFFICACY OF TRICLOSAN

A. Oral Care

Since the previous review a number of additional studies have shown the benefit of triclosan in oral care products. The use of triclosan-containing products has resulted in a significant reduction in plaque and gingivitis, a significant reduction in periodontitis, and a significant reduction in supragingival calculus. While there are other mechanisms available to reduce these clinical endpoints, industry consumer knowledge shows that a large majority of consumers do not clean teeth with sufficient care. So, in reality, triclosan plays an important role in providing a convenient and efficacious means of achieving enhanced oral health in the wide population. Furthermore, several researchers have investigated the effect of triclosan-containing dentifrices on the development of resistance or even of some reduced susceptibility of oral bacteria to triclosan and found no causal link.

The benefits of a triclosan/copolymer/fluoride dentifrice were reviewed by DeVizio and Davies (2004). Approximately 2000 subjects participated in thirteen (13) independent, double-blind studies. Those subjects had on average 27% less plaque than subjects using a fluoride dentifrice. This improved level of plaque control was accompanied by a 57% reduction in gingival bleeding. Data from clinical studies of a triclosan/copolymer dentifrice of up to one (1) year duration clearly indicate that there is no evidence to support the acquisition of bacterial resistance in the supragingival oral microflora.

Hoang (2000) reviewed the safety and efficacy of triclosan in oral care products with particular attention to the benefits found in the reduction of periodontitis.

Jin and Yip (2002) found that a triclosan-containing dentifrice is effective at significantly reducing the severity and occurrence of supragingival calculus after prophylaxis.

Sreenivasan and Gaffar (2002) reviewed antiplaque biocides and bacterial resistance. The effect of triclosan on oral flora was studied in eight (8) dentifrice clinical trials lasting from 6 to 36 months. In all, over 700 subjects were evaluated. On examination of their dental flora, pathogenic bacteria were not found at the end of the study in any subject, nor were opportunistic pathogens found in most of the populations. Following the studies the subjects were followed for up to twelve (12) months to determine if resistant strains had developed during the trial; no resistant strains were identified. In one of the reviewed studies, that also investigated bacterial resistance of isolates, the MIC of clinical isolates was determined at 3-month intervals for twelve (12) months, including a 6-month period of post-therapy monitoring. At all sample times, there were no differences in the MICs of the isolates from the control and and triclosan groups. Triclosan has been shown to penetrate the oral biofilm and reduce the viability of the bacteria therein. However, no adverse effects of this reduction have been reported.
Cullinan et al. (2003a; 2003b) followed the progression of periodontal disease as well as the acquisition and loss of the *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, and *Prevotella intermedia* in the plaque of adult volunteers over a five (5) year period using triclosan/copolymer dentifrice or a placebo. This study showed that in a normal adult population, unsupervised use of a triclosan/copolymer dentifrice is effective in slowing the progression of periodontal disease. Subjects using triclosan were more likely to have *P. intermedia* than those not using the dentifrice; however this did not translate into these subjects having higher levels of *P. intermedia*, and its presence was uniform showing no signs of increasing over the time of the study.

Sullivan et al. (2003) conducted a clinical study which showed that no major changes occurred in the MICs of triclosan in the normal oral microflora and streptococcal strains examined over the five (5) day treatment period or in the two weeks following treatment.

B. Skin Care

Some work has continued to be published on the safety and efficacy of triclosan as it relates to skin care products.

Johnson et al. (2002) surveyed transient and resident skin isolates for their susceptibility to triclosan. The variation in susceptibility was typical for the strains surveyed. They found that the concentration of triclosan in products is in excess of that needed to kill bacteria on the skin, indicating a low potential for survivors to lead to the development of resistance.

Wilcox et al. (2003) evaluated a pre-surgical regimen including prophylaxis with nasal mupirocin for five days and a single bath or shower with 2% triclosan in a clinical trial studying the incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) surgical infections. A statistically significant reduction in surgical site infections and MRSA carriage resulted from this treatment. The authors note that while they could not determine the relative contributions of these two elements, it was felt that the triclosan enhanced the eradication of MRSA carriage over that achieved by nasal mupirocin.

V. LABORATORY STUDIES

There are two fundamentally different mechanisms for obtaining a more resistant organism in the laboratory: genotypic studies wherein specific resistance genes are introduced to a new host, and phenotypic studies where mutations are selected based on growth in the presence of either sub-inhibitory concentrations of an antimicrobial agent (serial passage procedures) or supra-inhibitory concentrations of the antimicrobial agent (direct selection procedures). A number of studies
were published where bacterial strains with increased tolerance to triclosan were developed in the laboratory by selecting mutants based on growth in the presence of either sub-inhibitory concentrations of triclosan or supra-inhibitory concentrations of triclosan.

A. Genetic Studies

Levy (2002) inserted an antibiotic resistance plasmid into *S. aureus* which conferred antibiotic resistance and an elevated MIC towards disinfectants. Such plasmids contain the qac genes which code for energy dependent efflux pumps. However, it is documented in microbiological literature that the addition of external genetic material does not necessarily confer a survival advantage to the organism. Fitness to survive may only be expressed in the presence of a specific pressure e.g. triclosan. In the absence of the specific pressure, the organism may be at a disadvantage relative to the wild type (McBain *et al.*, 2002) and may revert to wild type (Russell, 2000).

B. Phenotypic Studies

1. Resistance mechanisms

Since 2002, a number of phenotypic studies have been published that report on the mechanisms of the induced "resistance" to triclosan (Braoudaki and Hilton, 2005; Chuanchuen *et al.*, 2002; Chuanchuen *et al.*, 2003).

Brenwald and Fraise (2003) studied triclosan-resistant mutants and clinical isolates of MRSA. The amino acid sequences of the FabI gene products of these isolates were determined. Only two (2) of the six (6) isolates had mutations in FabI that were known to confer triclosan resistance. This suggests that genetic loci other than FabI may be involved in triclosan resistance.

Fan *et al.* (2002) studied the inherent variation in MIC for triclosan of clinical isolates of *S. aureus*. All of the isolates studied were within the MIC bounds of what would be considered susceptible. These levels are also well within the normal range of susceptibility of *S. aureus* to triclosan. Strains with higher MICs were shown to have elevated levels of ACP reductase (FabI enzyme) as compared to strains with lower MICs. This study shows one mechanism whereby there is natural variation in the MICs of varying strains of *S. aureus*.

Kremer *et al.* (2003) presented *in vitro* data to support the InhA gene in *M. smegmatis* and potentially in *M. Tuberculosis*, being the primary target for isoniazid activity. Rawat *et al.* (2003) used triclosan as a tool to explore the molecular basis of fatty acid synthesis and for isoniazid resistance in clinical isolates of *M. tuberculosis* carrying InhA mutations. They found that the mutation which correlates with resistance to triclosan in *M. smegmatis* slows the rate of inactivation of the *M. tuberculosis* by isoniazid, but does not prevent the formation of the drug-
enzyme-cofactor complex. Consequently, the overall potency of inhibition by isoniazid is only slightly reduced in the presence of triclosan, with overall potency decreased only five-fold. The clinical significance of this is unknown.

2. Development of Triclosan-Resistant Strains

McBain et al. (2003b) studied the effect of triclosan exposure on population dynamics and antimicrobial susceptibility of established sink drain microcosms over three months. Viable cell counts were largely unaffected by exposure to a triclosan-containing domestic detergent at use level, but species diversity decreased. Triclosan susceptibilities ranged widely within bacterial groups. Triclosan-tolerant strains (including aeromonads, pseudomonads, stenotrophomonads and Alcaligenes spp.) were isolated before and after triclosan exposure. Triclosan addition did not significantly affect the community profiles of susceptibility to the biocides or antibiotics tested. Additionally, the bacterial consortia were found to degrade triclosan. The authors concluded that the insusceptibility of drain isolates to a range of antimicrobials was related to innate resistance or insusceptibility as opposed to developed, transferable resistance. The sample used to inoculate the microcosm was taken from a household that had not used biocide-containing products since installation of the sink, so biocide insusceptibility could not be attributed to past exposure to triclosan.

McBain et al. (2004) investigated the effect of triclosan exposure on the antimicrobial susceptibilities of important oral bacteria (Neisseria subflava, Porphyromonas gingivalis, Actinomyces naeslundii, Streptococcus mutans, Streptococcus sanguis, Fusobacterium nucleatum, Lactobacillus rhamnosus, Provotella nigrescens, and Veillonella dispar) versus E. coli. These data fail to demonstrate biologically significant drug resistance in triclosan-exposed bacteria and suggest that markedly decreased triclosan susceptibility, although confirmed for E. coli, is not a universal phenomenon. The results suggest that triclosan acts upon targets such as membrane components of oral bacteria or that more sensitive, highly conserved targets exist. Similarly, triclosan-exposure caused no net significant decreases in susceptibility towards chlorhexidine, metronidazole, and tetracycline. This work is similar to earlier work in Russell's laboratory where passage studies failed to product an adapted organism (McDonnell and Russell, 1999; Suller and Russell, 1999) and supports the view that long-term use of triclosan products in the oral cavity does not select for triclosan-resistant populations.
3. Cross-resistance Studies using Laboratory Strains

Research continues into the investigation of potential cross-resistance of triclosan with other biocides and antimicrobials.

Walsh et al. (2003) developed *E. coli* mutants to triclosan that were not cross-resistant with eugenol, thymol, triclocarban, and the quaternary ammonium compounds DDDMAC or ADMAO.

Loughlin et al. (2002) adapted *Pseudomonas aeruginosa* by serial passage to benzalkonium chloride and looked for cross-resistance with other biocides. Cross-resistance with triclosan was not found.

Tattawasart et al. (1999) adapted *Pseudomonas stutzeri* to chlorhexidine diacetate or cetlypyridinium chloride (CPC) by serial passage. The CPC-resistant strains showed a 2-10 fold increase in MIC to triclosan. This reduced susceptibility was lost after subculture in antimicrobial-free medium.

Braoudaki and Hilton (2004a) showed that triclosan resistance in *E. coli* is strain specific rather than a general resistance mechanism. Triclosan-adapted strains were cross resistant to a small number of antibiotics. They found that a strain of *E. coli* O157, developed resistance to triclosan more quickly than two other strains of *E. coli* (K-12 and an O55 strain). They also found triclosan-resistant *E. coli* O157 to be cross resistant to a larger number of antibiotics than the triclosan-resistant strains of O55 or K-12. Strains repeatedly expressing elevated levels of resistance to a wide range of structurally unrelated antibiotics/antimicrobials are thought to be related to increased levels of active efflux. Disease caused by *E. coli* O157 and other verocytotoxigenic *E. coli* is not treated with antibiotics, since this is likely to lead to adverse effects, so there is no clinical significance related to these findings. The response of different strains, as with tolerance to other stresses such as low pH, is likely to vary considerably between strains.

Braoudaki and Hilton (2004b) developed adaptive increased tolerance of triclosan in laboratory strains of *Salmonella enterica* and *E. coli* O157. The resistance developed in *E. coli* O157 was stable and conferred some cross resistance with eight (8) antibiotics. The resistance developed in *S. enterica* serovar Virchow showed cross resistance with erythromycin, benzalkonium chloride, and chlorhexidine. The triclosan-adapted *S. enterica* serovar enteritidis showed resistance to chloramphenicol and for serovar Typhimurium, cross-resistance to chlorhexidine. The rate of developing resistance and the level of cross-resistance was less than that seen in *E. coli* O157.

Randall et al. (2004) using a novel in vitro method determined the frequency of mutation of strains of five (5) wild-type strains of *S. enterica* when exposed to antibiotics and sub-inhibitory levels of triclosan simultaneously. The mean frequency of mutation conferring resistance to ampicillin and cyclohexane was
found to increase with such simultaneous exposure, by 10 to 100-fold. In some cases, the level of resistance seen would be clinically significant. The findings suggested that the use of biocides alone or with antibiotic treatment may exert increased selective pressure on bacteria to acquire antibiotic and biocide resistance. Further testing is needed to examine the relevance of this new in vitro method to real clinical situations.

4. Cross-resistance Studies using Clinical Isolates

Fraise (2002) studied the susceptibility of antibiotic-resistant Gram positive cocci to biocides including triclosan, attempting to create biocide-resistant strains in vitro. Parent strains were grown in non-inhibitory concentrations of the biocide and then exposed to a range of inhibitory levels. Less susceptible mutants were created with relative ease and one MRSA isolate was found to have decreased sensitivity to triclosan, with the MIC increasing from 0.002 to 3.12 mg/L.

VI. SURVEILLANCE STUDIES

A number of surveillance studies have been conducted looking for organisms with decreased susceptibility to triclosan in natural environments where there has been repeated exposure to triclosan. There was no evidence of shifts in populations or development of resistance to triclosan in any of these studies. Nor was there any evidence of a correlation of resistance between triclosan-tolerant strains and antibiotic resistant strains.

A. Factory Survey

Lear et al. (2002) examined over 100 isolates from triclosan and PCMX manufacturing sites. The MICs of these isolates were compared with equivalent culture collection strains. They concluded that there was no evidence that the residual levels of biocides in the factory environment had led to changes in susceptibility. This study looked at sites where there was the greatest expectation of finding resistant strains, and none was found. Based on this finding, it would be unexpected to find resistant strains were exposure to these biocides is more casual, i.e. in homes. In reviewing this study Gilbert and McBain (2002) concluded that any changes seen in the flora was due to clonal expansion of pre-existing resistant but less competitive species.

B. Home Surveys

Marshall et al. (2003) compared the incidence of bacteria, including antibiotic resistant bacteria, in the homes of users and non-users of antibacterial agents. The authors concluded that high frequencies of antibiotic-resistant bacteria occurred in the home environment of both groups. However, there were no significant differences in the overall titers of bacteria, potential pathogens or frequencies of an-
tibiotic resistance in a single-time analysis of homes whether using or not using antibacterial-containing products.

Cole et al. (2003) sampled 60 homes split evenly between users and non-users of biocides including triclosan, for the presence of target microorganisms recognized as potential human pathogens. Both environmental (e.g. from the kitchens and bathrooms) and clinical (e.g. from hands and mouths) samples were taken. There was no significant difference found in the level of antibiotic resistance between the users and non-users. The results also showed no evidence of cross-resistance between antibiotics and biocides in either the users or non-users. The non-user group did, however, have a significantly greater number of potential pathogenic organisms present. Also, isolates considered having high MIC values against one or more active ingredients were fully susceptible to all of the preferred/alternative treatment drugs and were also found mostly in non-user homes.

Aiello et al. (2004) examined the triclosan and antibiotic susceptibility of staphylococci and Gram-negative bacteria isolated from the hands of individuals in a community setting. The trial was a one-year double-blinded, randomised home hygiene intervention study. Samples were taken after participants washed, rinsed and dried both hands in their usual manner, with the assigned liquid handwashing product. Triclosan-containing or non-antimicrobial soaps were provided to the study population for one year. There was no statistically significant association between triclosan MICs and susceptibility to the antibiotics tested. The authors state that the results could indicate that such a correlation does not exist.

McBain et al. (2003b) examined the triclosan and antibiotic-susceptibility profiles of bacteria in drains where there was constant exposure to dilute triclosan-containing products over a three month period. No shifts in the floral composition were seen, nor were there any significant changes in triclosan or antibiotic susceptibility.

C. Clinical Isolates

Al-Doori et al. (2003) surveyed the MRSA isolates in the Scottish Reference Library. The MIC$_{50}$ was 0.03 mg/L and the MIC$_{90}$ was 0.06 mg/L within a range of <0.015 to 4 mg/L. The two dominant UK epidemic strains were both susceptible to triclosan. The authors concluded that susceptibility of MRSA strains to triclosan has changed little over a 10-year period.

Randall et al. (2003) surveyed 443 Campylobacter species from humans and animals for multiple drug resistance to antibiotics and biocides. The rate of isolation of multidrug resistant campylobacters (3.3% for C. jejuni and 3.8% for C. coli) was lower than expected, compared to recent studies investigating the incidence of multi-drug-resistant strains. The cross-resistance of these strains was also lower than expected. However, some strains (20/443) that were less sus-
ceptible to triclosan were significantly more resistant to six antibiotics suggesting a different, more general, mechanism other than multi-drug-resistance may be playing a role, such as efflux. Evidence for such an efflux pump system in Campylobacter spp. that is effective against antibiotics and a broad range of structurally unrelated antimicrobial agents has been provided by Lin et al. (2002). There is no indication that triclosan was involved in development of this general resistance property and this would be extremely unlikely, given that the mechanism is not specific.

Lambert (2004) found no statistically significant differences in triclosan MIC between MRSA and MSSA strains among 256 clinical isolates. Between 1989 and 2000, a subpopulation of MRSA had acquired a higher resistance to biocides, but this had not altered the antibiotic susceptibility of that group. These strains were surveyed for their susceptibility to a wider group of biocides and antibiotics. Numerous correlations were shown between the MICs of antibiotics and biocides. However, many of these correlations were negative, i.e. an increase in MIC of a particular biocide was correlated with a decrease in the mean MIC of a particular antibiotic. Advanced statistical investigation using the method of principal component analysis grouped, in general, the antibiotics and the biocides separately. The groupings appeared to reflect the mode of action of the antimicrobials. In many cases the groupings showed little interaction, suggesting that little cross-resistance exists between antibiotics and biocides.

Schmid and Kaplan (2004) surveyed clinical isolates of S. aureus and S. epidermidis reconfirming the existing literature that S. aureus are usually highly susceptible to triclosan and S. epidermidis is more heterogeneous as a species and is generally less sensitive with a MIC$_{50}$ of 0.12 μg/mL and a MIC$_{90}$ of 8 μg/mL. Decreased susceptibility to triclosan was more prevalent among methicillin-resistant S. epidermidis in comparison to methicillin-sensitive S. epidermidis isolates. The S. aureus and S. epidermidis strains surveyed were, nevertheless, susceptible to triclosan.

Fan et al. (2002) studied the inherent variation in MIC of clinical isolates of S. aureus. All of the isolates studied were within the MIC bounds of what would be considered susceptible. These levels are also well within the normal range of susceptibility of S. aureus to triclosan. Strains with higher MICs were shown to have elevated levels of ACP reductase (FabI enzyme) as compared to strains with lower MICs. This study shows one mechanism whereby there is natural variation in the MICs of varying strains of S. aureus.

VII. EXPERT REVIEWS ON TRICLOSAN

A number of expert reviews by Russell (2003; 2004) and Gilbert (Gilbert et al, 2002; Gilbert and McBain, 2002) have concluded that while cross-resistance to biocides and antibiotics can be demonstrated in the laboratory using pure cul-
tures, it does not necessarily equate to the development of such resistance in the natural or clinical environment where complex, multispecies biofilms predominate. No convincing evidence has been found to support the contention that triclosan usage has resulted in the clinical development of antibiotic-resistant Gram-negative bacteria, antibiotic-resistant cocci or isoniazid-resistant *M. tuberculosis*.

Furthermore, Goodfellow *et al.* (2003) reviewed the safety and efficacy of triclosan and concluded that the use of triclosan in cosmetic and over-the-counter drug products was safe and not expected to select for antimicrobial resistant bacteria. Finally, Kampf and Kramer (2004) reviewed the epidemiologic background of hand hygiene and evaluated the most important agents for scrubs and rubs. Their activity includes the mechanical removal of pathogens during hand washing; no removal occurs when hand rubs are used. They concluded that triclosan has a low potential for acquired bacterial resistance.

**VIII. DISCUSSION**

Considering that triclosan has a history of use of over thirty (30) years, it is highly likely that if bacteria were going to develop triclosan resistance, such bacteria would already be evident in the environments where triclosan-containing products have been repeatedly used. Surveillance studies searching for triclosan-resistant bacteria in the triclosan factory, in clinical settings, in the home, on the skin or in the oral cavity of users of triclosan have not found any such organisms. While the surveillance studies can be criticized for being limited in scope, it is affirming that in all of the environmental surveys published to date, no evidence of increasing resistance to triclosan has been shown. While these findings do not preclude the possibility that triclosan resistance can develop outside the laboratory, they indicate that such a development does not commonly or readily occur. They also indicate that intrinsically resistant species do not out-compete susceptible strains in biocide-treated environments.

Laboratory studies have shown some cross-resistance to antibiotics and other biocides in some laboratory-derived mutants of *E. coli*, pseudomonads, and staphylococci. However, there is growing evidence that the generation of such mutants is strain specific and that these mutations do not appear to occur in all species. In fact there have been a number of reported failures by researchers trying to develop triclosan-resistant strains (McBain *et al.*, 2004). Additionally, such mutations are frequently lost when triclosan is removed from the media, suggesting that there is no competitive advantage to the organism to conserve that mutation.
IX. CONCLUSION

Thus in all of the environmental surveys published to date, no evidence of increasing resistance to triclosan has been shown. The natural environment provides many challenges to bacteria including a search for nutrients, appropriate conditions for growth, and competition with other organisms that are not duplicated in laboratory experiments. While these findings do not preclude the possibility that triclosan resistance can develop outside the laboratory, they indicate that such a development does not commonly or readily occur and has not been scientifically demonstrated so far. The scientific data published since 2002 and discussed in this submission has demonstrated that

- Further work has been done and progress has been made to elucidate the many mechanisms of action of triclosan including membrane destabilization, inhibition of fatty acid synthesis, efflux mechanisms, and formation of biofilms;
- A number of additional studies have shown the safety, efficacy, and benefit of triclosan in oral care and skin care products;
- Laboratory studies continue to demonstrate that bacterial strains with increased tolerance to triclosan can be developed in the laboratory by selecting mutants based on growth in the presence of either sub-inhibitory concentrations of triclosan or supra-inhibitory concentrations of triclosan;
- A number of surveillance studies have been conducted looking for organisms with decreased susceptibility to triclosan in natural environments where there has been repeated exposure to triclosan. There was no evidence of shifts in populations or development of resistance to triclosan in any of these studies. Nor was there any evidence of a correlation of resistance between triclosan-tolerant strains and antibiotic resistant strains; and
- A number of expert reviews have concluded that while cross-resistance to biocides and antibiotics can be demonstrated in the laboratory using pure cultures, it does not necessarily equate to the development of such resistance in the natural or clinical environment where complex, multispecies biofilms predominate, that the use of triclosan in cosmetic and over-the-counter drug products was safe and not expected to select for antimicrobial resistant bacteria, and that triclosan has a low potential for acquired bacterial resistance.

Despite the absence of evidence showing the development of decreased sensitivity to triclosan in bacteria found in environments where there has been exposure to this antimicrobial, researchers and reviewers of this subject area remain cautious about the use of biocides and advise on continued monitoring of this area. Industry acknowledges this and fully supports continued study in the area of biocide use. The consumer benefits strongly support the continued availability of triclosan to our consumers in the hygiene products where it is currently used.
Thus, the evidence presented to date supports the 2002 conclusion of the SSC and the SCCNFP that triclosan does not pose a risk to humans or to the environment by inducing or transmitting antibacterial resistance under current conditions of use.
X. REFERENCES


