Risk assessment on the use of triclosan in cosmetics
I: Development of antimicrobial resistance in bacteria

Panel on Biological Hazards

Contents

I- Summary

Triclosan is a widely used biocide. As well as being an effective antimicrobial agent against various bacteria, it also acts anti-inflammatory. It is included in many contemporary consumer and professional health-care products, particularly oral and dermal products, but also in household items including plastics and textiles.

At lethal doses, triclosan appears to act upon multiple non-specific targets, causing disruption of bacterial cells. At sub-lethal concentrations, triclosan has been shown to affect specific bacterial targets. Although currently widely accepted, the utility and efficacy of triclosan in some products, particularly plasticware, is debatable. During the 1990s, bacterial isolates with reduced susceptibility to triclosan were produced in laboratory experiments by repeated exposure of bacteria to sub-lethal doses of triclosan. During the last four years, a number of studies have verified the occurrence of triclosan resistance amongst dermal, intestinal and environmental bacterial species, including some of clinical relevance. However studies involving clinical isolates have been relatively limited. A number of different mechanisms for the development of triclosan resistance in bacteria have been demonstrated.

Of major concern is the possibility that triclosan resistance may contribute to reduced susceptibility to clinically important antimicrobial agents, due either to cross-resistance or co-resistance mechanisms. Whilst the number of studies concerning association between triclosan resistance and resistance to other antimicrobial agents in clinical isolates has been limited, recent laboratory studies have confirmed the potential for such a link.

In our opinion, in some situations, particularly in clinical settings, triclosan is a useful, broad-spectrum biocide. However, widespread use of triclosan, including in cosmetic products, selects for development of triclosan resistance. Furthermore, such use represents a public health risk in regard to development of concomitant resistance to clinically important antimicrobial agents. Therefore, to protect public health, use of triclosan ought to be restricted to purposes where there is a well documented desirable effect in order to prevent development and spread of resistance to triclosan and other antimicrobials. There is a need for further information in a number of areas associated with triclosan use and its potential to contribute to the development and spread of antimicrobial resistance. These areas should be addressed by independent research.

II- Background

Triclosan has been widely used since its introduction 40 years ago. However, in more recent years the use of triclosan as a preservative, antiseptic and disinfectant in the USA and Europe has risen dramatically (16).

Based on formulation and application, the United States Food and Drug Administration (FDA) currently recognises triclosan as an over-the-counter (OTC) or prescription drug (20). In the United States, triclosan has been used in underarm deodorants and deodorant soaps since the 1960s and, in the health care industry, in a surgical scrub since 1972. In Europe, triclosan has been incorporated into toothpastes since 1985 (35). In the 1990s the FDA approved a new drug application for the use of triclosan for OTC oral care (toothpaste) at similar concentration levels as found in topical products (21). In June 2000, the American Medical Association (AMA) adopted the recommendation of its own Council on Scientific Affairs. The AMA:
1- Encourages the FDA to expedite its regulation of the use in consumer products of antimicrobials for which acquired resistance has been demonstrated.
2- Will monitor the progress of the current FDA evaluation of the safety and efficacy of antimicrobials in consumer use in OTC hand and body washes; and
3- Encourages continued research on the use of common antimicrobials as ingredients in consumer products and their impact on the major public health problems of antimicrobial resistance (71)

As a part of FDA's ongoing review of OTC drug products, FDA has recently announced a call-for-data on safety and effectiveness of triclosan, at a concentration of 0.3% maximum, as an anti-gingivitis ingredient in dental pastes and oral rinses (22). The deadline for submission of data, information and general comments was October 4, 2004. FDA will evaluate the submitted data and information to determine whether this proposed OTC use of triclosan can be generally recognised as safe and effective.

In Europe also, new concerns have been raised regarding the widespread and increasing use of triclosan, in view of the potential for selection of resistant bacterial strains that may confer cross-resistance to other antimicrobial agents. The former Norwegian Food Control Authority (SNT) therefore asked scientific experts at the Norwegian Institute of Public Health to perform a risk assessment on the use of triclosan in cosmetics (4th September 2000). The conclusion of this assessment (57) was as follows:
"…care must be taken to contribute as little as possible to the selection of resistant bacteria.....with this in mind, particularly in light of recent indications of its association with the development of antibiotic resistance in bacteria, we recommend against the use of triclosan in cosmetics and other products in general use, in which disinfectant action is neither useful nor desirable.".

In 2002, the former Scientific Steering Committee (SSC) (64) of the EU appointed a working group of experts with the mandate to draft a scientific report that could be used as input for the preparation of a scientific opinion of the SSC regarding the safety of triclosan, especially related to the risk for resistance development in certain microorganisms. The SSC concluded that:

"There is no convincing evidence that triclosan poses a risk to humans or to the environment by inducing or transmitting antibacterial resistance under current condition of use."
The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) of EU evaluated the opinion of the SSC and concluded that:
"1: Under current conditions of use of triclosan as a preservative in cosmetic products, it is safe taking into account the risk of resistance by certain micro-organisms.
2: There is no need for setting a new concentration limit for the use of triclosan in cosmetic products."

The experts at the Norwegian Institute of Public Health evaluated the assessment of the SSC and SCCNFP, and concluded that their own recommendations of September 2000 remain valid (8th November 2002). The Norwegian Food Control Authority, in a letter to the European Commission, DG Enterprise (Biotechnology, competitiveness in pharmaceuticals, cosmetics), called for a re-evaluation of the permission to use triclosan in cosmetic products (20th December 2002). The recommendations from the Norwegian experts were also stressed at a meeting in the Working Party on Cosmetics in the European Commission. The call for re-evaluation was answered in March 2003, where the European Commission asked the Norwegian Food Control Authority for an assessment based on new data and after consideration of the SCCNFPs opinion on triclosan (ENTR/F/3/RS/bl D (2003) 735109).

Based on the opinion from Norwegian experts, as well as SSC and SCCNFP, regarding use of triclosan and the possibility for development of resistance in bacteria, it was concluded that an updated risk assessment was necessary. In March 2004, the newly-established Norwegian Food Safety Authority (Mattilsynet) asked the Norwegian Scientific Committee for Food Safety to address this issue. In response, a working group of experts was appointed, with the mandate to draft a risk assessment regarding the use of triclosan in cosmetic products and its association with development of antimicrobial resistance in bacteria.

III- Exposure assessment

Triclosan (also known as Irgasan) is used as an ingredient in a diverse and extensive range of products, due to its broad-spectrum antibacterial properties. It is found in soaps, detergents, dishwashing liquids, lotions, deodorants, mouth rinses, toothpastes, toothbrush handles, slippers, towels, pillows, mattresses, carpets, fabrics clothing, baby toys, plastics, chopping boards, chopsticks, pizza cutters, food storage containers, garbage bags, kitty litter, finger inserts in bowling balls, and paints.

In Norway, the total quantity of triclosan in cosmetic products in 2001 was approximately 2300 kg. The contribution from different products was as follows; toothpastes (1700 kg), mouth rinse (50 kg), deodorants (60 kg), soaps (270 kg), and other unspecified cosmetics (200 kg). Since autumn 2002, the use of triclosan in toothpastes has been reduced by 50% (55). In contrast to the quantities of triclosan used in cosmetic products, the sales of triclosan for use in the Norwegian food processing industries did not exceed 4.4 kg and 1.3 kg in 2001 and 2002, respectively (www.mattilsynet.no). According to a Norwegian report (56), triclosan

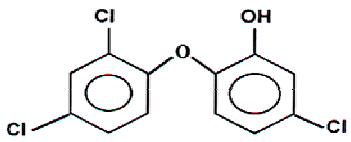
seems to be less widely used in the food processing industry in Norway and North Europe than in other European countries. The maximum allowed concentration of triclosan according to the Norwegian regulations is 0.5% in soaps and products that are removed after use (rinse-off-products), and 0.3 % in leave-on -products. In EU countries, triclosan is mainly used in soaps, creams and solutions (in concentrations of up to 2%) for the disinfection of hands and wounds, and for the disinfection of skin prior to surgery, injections, and venepuncture (64). According to this document, prepared by Scientific Steering Committee (SSC) of EU, of the total amount of tricolsan used in Europe, more than one third appears to be used in products for oral care, and a similar amount is used in products for skin care. Much less than one third is used in other items, including household products. At the request of the SSC, The European Cosmetics, Toiletry and Perfumery Association (COLIPA) tried to trace the distribution of triclosan-containing cosmetics in the EU market. COLIPA focused on the major manufactures of finished oral-care products containing triclosan, since it was impossible to obtain market information from the numerous manufactures of triclosan-containing skin-care products. The results showed that the percentage use of triclosan in oral care products per individual was highest in Germany (19.74%), Italy (17.03%), and France (16.54%), respectively. The percentage was lower in Norway (0.82%) than in the other EU Member States (64).

IV- Triclosan characteristics

IV A- Chemical structure

Triclosan (3380-34-5) is a non-ionic, odourless, and tasteless powder, which was developed by the Ciba Geigy Company in Switzerland in the early 1960s. Triclosan is a diphenyl ether (bis-phenyl) derivative, known as 2, 4, 4'- trichloro-2'-hydroxydiphenyl ether or 5-chloro-2-(2,4-dichlorophenoxy).

Triclosan



Triclosan has both hydrophobic and hydrophilic properties. It has poor aqueous solubility, but is solubilized in the flavour/surfactant phase. In commercial products like toothpastes, one or more solubilizing agents such as detergents (e.g. sodium dodecyl sulphate, sodium lauryl sarcosinate) or propylene glycol and polyethylene glycol are used to solubilize tricolsan (62).

IV B- Antimicrobial activity

Triclosan is active predominantly against Gram-positive bacteria, but also against some Gram-negative bacteria, fungi and viruses (35). It has been regarded as a biocide, acting by non-specific (multi-target) disruption of bacterial cells. Most data on the antimicrobial activity of triclosan are collected from studies using planktonic phase microorganism (unattached micro-organisms living freely in suspension) rather than microorganisms in more natural conditions, such as in a biofilm. Notably, gene expression in microorganisms living in a biofilm differs from that in planktonic cells,

and the concentration of an agent needed to kill microorganisms in biofilms may be 10-500 times higher than in the planktonic phase (62). Use of triclosan in oral care formulation is intended to control accumulation of plaque, which is indeed a biofilm (73). The effect of triclosan incorporation into acrylonitrile-butadiene-styrene (ABS) plastic on biofilm accumulation has recently been published (36). The authors concluded that the results of the study call into question the long-term utility of triclosan incorporation into ABS plastic and highlight the need for proof of efficacy regarding the antimicrobial properties of such materials.
Significant data indicate that use of antimicrobial wash products containing triclosan has an important role in preventing nosocomial infections in clinical settings such as hospitals, nursing homes, and neonatal nursery facilities (7,8,19,24,29,30,54,76,78).

IV C- Anti-inflammatory effect
The anti-inflammatory effect of triclosan within oral formulation for gingivitis control has been reported in a number of studies (73). Barkvoll and Rølla (1994) studied the anti-inflammatory effect of triclosan and confirmed its activity, in combination with sodium lauryl sulphate, in the prevention of an inflammatory skin reaction (6). The anti-inflammatory action of triclosan has been shown to be related to a reduction in prostaglandin production (6,74). Furthermore, triclosan reduced the interferon-δ (INF-γ) production induced by phytohemagglutinin (PHA), which is relevant as the major histocompatibility complex (MHC) expression is stimulated by rIFN-δ (51). These data support the view that triclosan affects the formation of important mediators in the inflammation process (52,53).

IV D – Mode of action
Triclosan, like various other biocides (e.g. alcohols, bleaches, and peroxides), is considered to have multiple mechanisms of action and numerous cellular targets (35). This is considered to provide protection against development of resistance in microorganisms;products containing alcohol act non-specifically and have been used for decades, without any concerns about resistance development. Although, the bactericidal activity of triclosan involves the action on multiple cellular targets, several studies have demonstrated that at sub-lethal concentrations triclosan inhibits a specific bacterial target. This target is known as enoyl-acyl carrier protein (ACP) reductase (Fab1 enzyme) in *Escherichia coli* (47), *Pseudomonas aeruginosa* (27) and *Staphylococcus aureus* (25), or its homologue, the *InhA* gene in *Mycobacterium smegmatis* and *Mycobacterium tuberculosis* (45,66). The information generated from several studies during the last six years confirms that the former claim that the action of triclosan is non-specific is not valid at low triclosan concentrations,

V- Triclosan resistance

The typical in-use concentration of triclosan is substantially higher than the concentration required for inhibiting most bacteria (MIC-value). It has therefore been concluded that microbial resistance is unlikely to occur under conditions of normal usage of cosmetics/health care formulations containing triclosan. However, in contrast to alcohol, bleaches and peroxidase, triclosan-containing consumer products leave residues on kitchen and bathroom surfaces and in the environment, where they might be diluted to less-than-effective concentrations (39). This could contribute to a more prolonged effect on the microflora, both at the application site and in the

environment and raises concerns regarding the use of triclosan, particularly inappropriate use, possibly leading to the development of microbial resistance.

The driving force for development of bacterial resistance to antimicrobials is the use of antimicrobials themselves. Widespread use of antimicrobial agents, including biocides, has created a strong selective pressure, which has lead to the development and spread of bacteria resistant to different antimicrobials. This is a good example of the major tenet of Darwinian evolutionary theory of “survival of the fittest”. The introduction of a new antimicrobial is often soon followed by reports of bacteria with acquired resistance to the antimicrobial in question. The amount of antimicrobial agent used, the duration of its effect, and its mode of use are all determining factors in the development of resistance. The use of antimicrobial agents is known to favour the selection of resistant strains by broadly eliminating the competing susceptible normal flora. Horizontal transfer of resistance between bacteria may also occur by different mechanisms; 1) the acquisition of exogenous DNA containing resistance genes by transformation, 2) the acquisition of resistance genes by transduction mediated by bacteriophages, and 3) the acquisition of resistance genes on mobile genetic elements such as plasmids or transposons by conjugation. In addition, the development of compensatory mutations can also be involved in the stabilization and further development of resistance traits. Furthermore, resistance to one antimicrobial can confer resistance to another antimicrobial through cross-resistance in which the same mechanism of resistance is applicable to both drugs. Also selection of one type of antimicrobial can select for resistance to another antimicrobial through co-selection (resistance genes are linked). Exposure to antimicrobial agents plays an important role in the development of resistance. It has been demonstrated that use of triclosan could contribute to selection of bacteria possessing intrinsic triclosan resistance. Bacteria like *Enterococcus faecalis* and *Streptococcus pneumoniae*, which lack the *fabI* gene and have a related enoyl reductase gene, *fabk* (26), are naturally resistant to triclosan. Therefore triclosan usage has the potential to enhance their growth at the expense of susceptible strains (38). Development of antimicrobial resistance is often a multi-step process involving many different factors. It may take place in different ecological niches and may need a long period of time to develop.

V A- Genetic basis for triclosan resistance mechanisms in bacteria

Bacterial resistance to triclosan can be considered as being either intrinsic or acquired, and it is the latter is of the highest public health concern. The resistance may disturb the balance of normal flora in patients with a tendency to acquire opportunistic infections (e.g. in patients with cystic fibrosis, immunosuppression etc).

Intrinsic resistance

Resistance to triclosan in *Pseudomonas aeruginosa* is intrinsic and could be due to a non-susceptible enoyl-reductase. Outer membrane permeability barrier or efflux may cause resistance to triclosan in this bacterium and the efflux mechanism has been stated to be the major reason for the lack of triclosan-susceptibility in *P. aeruginosa* (11). Intrinsic resistance can also be dependent upon enzymatic degradation: two soil isolates (*Pseudomonas putida* strain TriRY and *Alcaligenes xylosoxidans* spp. *Dentrificans* strain TR1) exhibited high levels of resistance to triclosan due to production of a triclosan-degrading enzyme (48). *A. xylosoxidans* is a multi-drug resistant pathogen of importance in patients with opportunistic infection and the

bacteria were grown on medium containing 1% triclosan, a concentration which is frequently found in many commercially available products.

Acquired resistance

There are a number of possible acquired resistance mechanisms (illustrated in Figure 1), which have been identified as being associated with development of resistance to triclosan in bacteria:

1) Target mutations; mutation in the enoyl acyl carrier protein (ACP) reductase gene (*fabI1)* in *E. coli* (46,47), *S. aureus* (25), and its homologue *inhA* gene in *M. smegmatis* and *M. tuberculosis* (45,66),
2) Increased expression of *fabI* gene in clinical isolates of *S. aureus* (18),
3) Active efflux; expression and overproduction of such efflux pumps may be induced through sublethal exposure to an agent. McMurray *et al*. (45,46) reported that mutation causing overexpression of *marA* and *acrAB* were associated with exposure and reduced susceptibility to triclosan,
4) Unknown resistance mechanisms which have not yet been identified.

Research performed during the last six years show that bacteria use several mechanisms to develop resistance against triclosan.

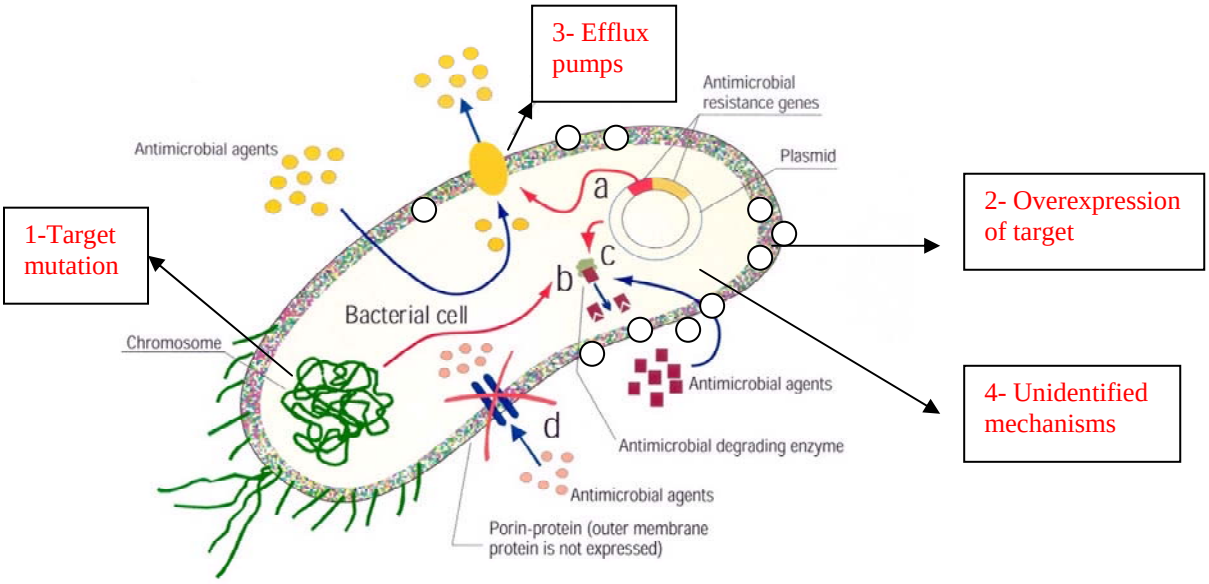


Figure 1- Illustration of different bacterial resistance mechanisms, including mechanisms involved in triclosan resistance.

V B - Susceptibility testing

Testing for triclosan susceptibility in various bacteria is performed using either a micro-dilution technique or an agar-dilution technique (9-11,18,33,43,44,46,59,68,69). However, a standardized method to determine bactericidal and bacteriostatic (Minimum Inhibitory Concentration, MIC) values for triclosan does not exist. Since triclosan is poorly soluble in water, a number of compounds such as ethanol (43,44), dimethyl sulphoxide (DMSO) (18), sodium lauryl sarcosinate, propylene glycol or polyethylene glycol (62) have been used as

solvents. Unfortunately, possible additive or synergistic effects with the diluents during the susceptibility test have often been disregarded. There is a need for standardised susceptibility testing methods enabling improved inter-laboratory comparison of susceptibility data.

V C- Development of resistance to triclosan

Since triclosan is incorporated mainly into personal hygiene products, and thus is of minor clinical importance and has few clinical applications, microbial bacterial resistance testing against this agent is not done on a routine basis. This could also be the reason for lack of research interest in this group of compounds. However, a number of studies have been performed in an attempt to elucidate the development and mechanisms of triclosan resistance in different categories of bacteria.

Oral microflora

The oral cavity is colonized by a diverse range of microorganisms in a complex protective ecosystem that changes constantly throughout life. The oral microflora comprises of over 700 species of bacteria, fungi, and protozoa. Potentially pathogenic bacteria which may be found in the oral cavity include *S. aureus*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Neisseria meningitidis*, members of the family *Enterobacteriaceae*, *Haemophilus influenzae*, Actinomycetes and oral streptococci (70).

The control of dental plaque, a complex microbial biofilm that accumulates naturally on tooth surfaces, is central to regimens for maintaining oral health (40). Triclosan-containing toothpaste is widely used for control of dental plaque. Clinical studies on the long-term daily use of triclosan have shown reductions in plaque and gingivitis (34). A number of studies have reported the effect of triclosan-containing toothpaste on the microflora of the mouth in relation to reduced susceptibility or resistance to triclosan, whereas only a few studies have examined the link between triclosan-containing toothpaste and resistance against other antimicrobial agents. A study performed by Walker *et al*. evaluated the efficacy and safety of a triclosan–containing dental formulation in a double-blind randomised study, which included 144 participants. They reported no significant changes in the antimicrobial susceptibility of the microflora at the end of the trial (75). Similar conclusions were drawn by Jones *et al*., in a 7-month study, evaluating the effect of a triclosan-containing toothpaste on the oral ecology of 13 adult volunteers (34). Sullivan *et al.,* (69) examined the *in vitro* susceptibility of triclosan against oral streptococci and the impact of triclosan on the normal oral microflora after two weeks exposure to a triclosan-containing toothpaste formulation. No major changes in the normal oral microflora were detected during the study period, and no differences in susceptibility of the streptococci prior to, and after, the triclosan exposure were observed. However, a study of two weeks period is insufficient to draw any firm conclusions. Furthermore, a review (67) of nine different (long-term clinical) studies examined triclosan MICs of the microflora from individuals who used triclosan-containing toothpaste. None of these studies reported development of triclosan resistance in oral bacteria. However, in view of the possible time span required for resistance to develop (confer vancomycin resistance development by enterococci and *S. aureus*), the duration of the published studies may be too short to draw firm conclusions on potential resistance development.

The oral streptococci, previously termed oral viridans streptococci, comprise at least 18 species. They are normal commensals of the human mouth and play an important

role in resistance to colonization by potential pathogens. The colonization may be inhibited by peroxidase synthesis by some members of the streptococcal flora. Oral streptococci are known to constitute a pool of genetic material which can undergo gene shuffling with other bacteria, including pathogenic bacteria (e.g. pneumococci), and cause the emergence of resistant bacteria (61).

Jenkins et al. found that the *in vivo* retention of the triclosan in the oral cavity is limited (31,32). To be efficacious, triclosan must be retained in the oral cavity for an extended period and manufacturers have employed several methods to increase the oral retention of triclosan. One way to increase the *in vivo* efficacy of triclosan is addition of a copolymer; another is addition of zinc citrate. According to our knowledge, no long-term data are available regarding use of either copolymer or zinc citrate in toothpastes.

There are different opinions among dental professionals regarding the use of biocides like triclosan in the oral prophylaxis and treatment of dental diseases. One group is of the opinion that any reduction of dental plaque is beneficial if accomplished safely; thus chemical agents like triclosan may offer an adjunct. The opposing group argues against the use of agents such as triclosan, claiming biocides disturb the ecological balance within the oral cavity and may lead to the development of resistant microorganisms (62).

In contrast to most specific infectious diseases that are caused by infectious pathogenic microorganisms, dental disease is mainly caused by the resident oral microflora, which also has beneficial functions. Therefore, the aim is not the elimination of the microflora, but its control to those levels compatible with oral health and with minimal adverse effects (62). The rationale for unsupervised use of triclosan-containing toothpastes for prophylactic purposes in the general population may thus be questionable. Despite the demonstration, in several short- and long-term studies, that triclosan prevents gingivitis, its effect on dental plaque formation is modest. As to the effect on periodontitis development, the effect on the general population is not significant. Triclosan may, however, possibly slow down progression in predisposed individuals, i.e. individuals with dental pocket depth of more than 3.5 mm at the previous examination (14). Notably, this effect appears to be of moderate magnitude (between 10 and 20 % compared to placebo) and independent of triclosan's antimicrobial properties (13).

Intestinal microflora

Chlorhexidine and related compounds in dental formulations may be swallowed. It has been estimated that from a 10 ml mouthful of 0.2% chlorhexidine mouthwash, 3% is swallowed, 67% spat out and 30% is retained by the oral surfaces, bound through weak chemical bonds (23) The retained chlorhexidine will subsequently be released and swallowed. To our knowledge, similar studies have not been performed with triclosan. It is known that triclosan is absorbed in the gastrointestinal tract, and it has been recovered from most human body fluids (28), including human breast milk (1). The source of triclosan in humans is likely to be toothpastes and mouthwashes containing triclosan. Continuous use of triclosan containing products leads to a steady-state plasma concentration of triclosan (including conjugates) within a few days. Studies with volunteers showed that it is rapidly conjugated in the body into

glucuronides and sulphates, and excreted via the urine (64). Ingestion and excretion of triclosan may influence the microorganisms in the digestive and urinal tract. To our knowledge, there have been no studies which have examined the pharmaco-kinetics of triclosan in detail.

Most studies examining the potential of intestinal bacteria for development of bacterial resistance to triclosan are based on laboratory mutant production. Further, the possible reductions in the susceptibility of the mutant to clinically relevant antimicrobials and other biocides have been investigated. The first observation of the presence of specific targets for triclosan was in a laboratory mutant of *E. coli* (47). Other studies have shown that over-expression of *marA*, *soxS* or *acrAB* produces reduced susceptibility to triclosan in *E. coli* (46).
Adaptive resistance to triclosan was readily achieved for both *Salmonella enterica* and *E. coli* O157, and resistance to a panel of antimicrobial agents was then screened using the adapted strains (9). The adapted *S. enterica* serovar Enteritidis strains showed resistance to chloramphenicol, whereas serovar Typhimurium became resistant to chlorhexidine. In contrast, *E. coli* O157 demonstrated decreased susceptibility to a wide panel of antimicrobial agents, including chloramphenicol, tetracycline, amoxicillin, amoxicillin/clavulanic acid and trimethoprim, as well as to biocides such as benzalkonium chloride and chlorhexidine.

A recent study investigated the potential of *E. coli* K-12, *E. coli* O55 and *E. coli* O157 to adapt to triclosan and the possibility of development of resistance and cross-resistance to a panel of antimicrobial agents (10). The data suggested that whilst there was a degree of cross-resistance in *E. coli* K-12 and *E. coli* O55:H7, it was to a lesser extent than that which was observed in *E. coli* O157. *E. coli* K-12 exhibited reduced susceptibility to chloramphenicol**,** whilst *E. coli* O55:H7 demonstrated resistance to trimethoprim, which is a clinically important antimicrobial agent against *E. coli*. Further, triclosan resistant *E. coli* O157 strains repeatedly showed decreased susceptibility to a range of antimicrobial agents, including ciprofloxacin, tetracycline and trimethoprim, as well as to the biocides benzalkonium chloride and chlorhexidine. The results indicate that differences in resistance profiles between *E. coli* O157, *E. coli* K-12 and *E. coli* O55:H7 are due to strain-specific, rather than general, processes underlying some of the resistance mechanisms observed (10). The authors concluded that *E. coli* O157 not only possesses enhanced virulence compared to closely related *E. coli* strains, but also an increased capacity to become resistant to the activity of triclosan and other antimicrobial agents.

Randall *et al*., (59) demonstrated that growth of *S. enterica* at sub-inhibitory concentrations of biocides like triclosan, followed by plating on media containing clinically relevant antimicrobial agents like ampicillin, ciprofloxacin and tetracycline, lead to an increased selection of strains with reduced susceptibility to all antimicrobials included. The study demonstrated that mutants inhibited by >1 mg/ml ciprofloxacin arose only from strains that were multiple antimicrobial resistant (MAR). Reduced susceptibility to ciprofloxacin (at 4×MIC for parent strains) alone was associated with mutations in *gyrA*, a gene that encodes for one of the bacterial topoisomerases that control DNA topology in living cells. MAR mutants did not contain mutations in the *acrR* and *marR* regions. Chromosomal regulatory loci like *mar* and *acr* control efflux pump expression. Mutations within *marR* gene, the negative regulator of the *mar* operon, cause over-expression of *marA* in the

Enterobacteriaceae, e.g., *E. coli*, resulting in an antimicrobial resistance phenotype (41,77). Studies performed with *E. coli, S. enterica* subsp. *enterica* serovar Typhimurium implicate *mar* in fluoroquinolone-resistant phenotypes through the over-expression of the multi-drug efflux pump AcrAB (41,58). Mutations within the repressor (AcrR) have also been shown to lead to *acrA* and *acrB* over-expression. The authors suggested that the use of biocides, including triclosan, either alone or in combination with antimicrobial treatment, may exert increased selective pressure on bacteria to acquire antimicrobial and biocide resistance.

A study on the prevalence of multiple antimicrobial resistance in *Campylobacter* spp. isolated from humans and animals, showed that strains that were less susceptible to triclosan, ethidium bromide and acridine orange, were significantly more resistant to a range of antimicrobial agents (60). The data indicate that multiple antimicrobial resistant *Campylobacter* spp. strains do occur and it may be assumed that they over-express certain efflux mechanisms.

Other food-related microflora

Because of growing public awareness and fear of communicable infections, commercial interests have identified an opportunity for sales of household goods incorporating biocides like triclosan. In a study (15), the antimicrobial effect of triclosan-containing plastic against some food-related bacteria like *Brochothrix thermophacta*, *S. Typhimurium*, *S. aureus*, *Shigella flexneri*, *E. coli*, *Bacillus cereus* and several strains of *E. coli* O157:H7, was examined. The study demonstrated that while antimicrobial activity is seen against bacterial cultures in antimicrobial plate assays, plastic-containing 1500 ppm of triclosan does not effectively reduce bacterial populations on refrigerated, vacuum-packaged meat surfaces. Another study shows that a film containing triclosan at concentrations of 1000 mg/kg^{-1} did not effectively reduce spoilage bacteria or growth of the psychrotropic pathogenic bacteria *L. monocytogenes* on refrigerated vacuum-packaged chicken breast, stored at 7°C (72). None of these studies have examined the effect of triclosan-containing plastic on the development of bacterial resistance to triclosan or other antimicrobials.

Dermal microflora

Triclosan is used in a variety of dermatological products intended for use in a health care setting. The skin is populated by numerous low virulence microorganisms, most of which do not, under normal conditions, cause infection (17). Coagulase-positive staphylococci (CPS) (mainly *S. aureus*) and, to a larger extent, coagulase-negative staphylococci (CNS) constitute a major proportion of the dermal bacteria in humans. Since CNS are inhabitants of the normal skin flora, they are highly exposed to dermatologic products containing triclosan. *S. aureus* is an important cause of bacterial infections in humans, particularly in skin infections and the rapid development and spread of antimicrobial resistance within populations of *S. aureus* is a serious clinical problem. Additionally, resistance to disinfectants seems to develop readily in *S. aureu*s. Furthermore, coagulase-negative staphylococci are considered to be an important source for the maintenance and spread of resistance genes to *S. aureus* (65). A number of studies have reported reduced susceptibility to triclosan among clinical isolates of *S. aureus* (3,4,12). Fan *et al*., (18) studied the mechanism of triclosan resistance among clinical isolates of *S. aureus*. The authors found that strains for which triclosan MIC was above 0.016 μg/ml showed three- to fivefold

increases in their levels of enoyl-acyl carrier proteins (ACP) reductase (Fab1). These data demonstrated that in these isolates not only mutation in the *fabI* gene was required for resistance to triclosan to occur, but also that these altered genes were over-expressed as compared to susceptible strains. In a recently published article (2), the authors examined community-based samples of bacterial isolates obtained from the hands of individuals (randomized to using or not using biocide cleaning and hygiene products) in order to assess the relationship between triclosan MICs and susceptibility to other clinically relevant antimicrobial agents. Both staphylococci (CPS and CNS) and Gram-negative bacteria isolated from hands were included in this study. Triclosan MICs were higher in some of the species compared to earlier reports on household, clinical and industrial isolates, and some of these isolates had triclosan MICs in the range of concentrations commonly used in consumer products. The authors found no correlation between triclosan susceptibility and resistance against clinically relevant bacteria in community isolates compared to laboratory findings. The apparent lack of association may indicate that such a correlation really does not occur, or that it was too low to be detected among the isolates that were studied.

Less attention has been paid to coagulase-negative staphylococci and their susceptibility and mechanisms of reduced triclosan susceptibility. A recent study (63) examined reduced triclosan susceptibility among methicillin-resistant *S. aureus* and methicillin-resistant *S. epidermidis* (CNS). Decreased susceptibility to triclosan was found to be more prevalent among methicillin-resistant *S. epidermidis* than methicillin-resistant *S. aureus* isolates. The reason for the higher proportion of *S. epidermidis* isolates with reduced susceptibility may be greater exposure to triclosan (because of frequent skin contact with triclosan-containing antimicrobial products) of *S. epidermidis* isolates than *S. aureus* isolates, resulting in greater selective pressure. *S. epidermidis* is considered a predominant resident skin bacterium and is also a major nosocomial pathogen associated with implanted medical devices. The authors speculated that the mechanisms and frequencies of resistance might differ between *S. epidermidis* and *S. aureus*.

Environmental microflora

Little information exists regarding the incidence of triclosan resistance amongst environmental bacteria. Two soil isolates, *Pseudomonas putida* and *Alcaligenes xylosoxidans* spp. *dentrificans,* expressed high levels of resistance to triclosan due to production of an enzyme which degraded triclosan (48). The extensive use of triclosan in household, industrial and clinical settings results in widespread disposal, commonly into the wastewater system, which ultimately leads to environmental deposition; triclosan has been found in wastewater, environmental sediments and aquatic biota (42,49,50). The presence of triclosan in sub-lethal concentrations in the environment may lead to the emergence of resistance amongst environmental bacteria.

Much of the research regarding development of antimicrobial resistance involves pathogenic bacteria and is restricted to cultivable, facultative anaerobe bacteria. Knowledge about the impact of triclosan use on the commensally non-cultivable and obligate anaerobes, which are the predominant bacteria in the oral cavity, gut, and skin flora, is limited. These bacteria may constitute pools of resistance determinants potentially transferable to human pathogens (5).

VI- Resistance link between triclosan and other antimicrobial agents

The progressive popularity of domestic cleaning products containing biocides increases exposure of bacteria to sub-lethal biocide doses and thereby represents a risk of development of resistance to them, and the promotion of cross-resistance to a range of antimicrobial agents. Since triclosan inhibits a specific bacterial target (the fatty acid biosynthetic pathway in bacteria) in a similar manner to clinically relevant antimicrobial agents, it is likely that resistance to triclosan can confer cross-resistance to other antimicrobial agents. Observation of mutation in the *fabI* gene has led to the study of its homologue, *inhA*, the gene for one of the proposed targets of isoniazid, an anti-tuberculosis agent. Mutants of *M. smegmatis* selected for either isoniazid or triclosan resistance, demonstrated co-resistance to both drugs via a mutation in the *inhA* gene (38). However, isoniazid-selected *M. tuberculosis* mutants retain their susceptibility against triclosan, suggesting that interactive sites on the FabI enzyme are at least partially separate (66).

The efflux mechanism, which also underlies some mechanisms of resistance to triclosan, can contribute to more widespread antimicrobial resistance. Many efflux pumps are relatively non-specific in their substrate recognition, and these may enable bacteria to pump out a range of substances which are chemically unrelated (37). Hence, broad-spectrum efflux pumps can mediate resistance to a range of antimicrobial agents, including biocides. In a study by Chuanchuen *et al*. (11), triclosan was demonstrated to be a substrate for three distinct efflux pumps in *Pseudomonas aeruginosa*. The study showed that exposure to triclosan could select for multi-drug-resistant mutants via up-regulation of these efflux pumps, and mutants of *P. aeruginosa* with defective efflux pumps became susceptible to triclosan. The authors concluded that exposure to triclosan and antimicrobials can select for multi-drug-resistant bacterial pathogens via over-expression of identical efflux pumps. Further, the research demonstrated that genes encoding efflux pumps might be transferred to other susceptible species or foster proliferation of *P. aeruginosa* and related bacteria in the presence of triclosan. It has been speculated that low-level concentration of triclosan associated with extensive use, might select for bacteria with multi-drug efflux pumps and resistance to multiple antimicrobial agents (46).

Methicillin-resistant *S. aureus* (MRSA) with low level resistance to triclosan, isolated from patients who received daily triclosan baths, were also resistant to the antibiotic mupirocin via plasmid mediated transfer (12). However, the link between muporicin and triclosan resistance could not be confirmed by others (68). The latter study found several clinical isolates of *S. aureus* expressing low-level resistance to triclosan, thus having a high MIC value. These isolates were also resistant to several other antimicrobial agents. This finding was, however, inconsistent as a few strains had low MIC values for triclosan, thus demonstrating triclosan susceptibility, but were resistant to several other antimicrobial agents. Bamber and Neal (4) assessed the MIC value of triclosan for 186 isolates of MRSA and methicillin-susceptible *S. aureus* (MSSA) and concluded that there was no significant difference between the proportion of triclosan resistant strains among MSSA and MRSA. A study performed by Al Doori *et al*. (3), in which 232 clinical *S. aureus* isolates from UK were studied, did not support the association between use of triclosan and selection for resistance to methicillin in MRSA.

Despite the observation of cross resistance between triclosan and antimicrobials in *E. coli* and *Salmonella* in laboratory studies (9,10), the resistance link between triclosan and clinically relevant antimicrobial agents has not been confirmed. However, the apparent lack of evidence for such a definitive link between resistance to triclosan and antimicrobial agents in clinical isolates, does not exclude the possible existence of isolates with such a link. The limited number of studies for resistance against triclosan in clinical isolates may explain the apparent absence of such a link.

VII- Data gaps

Objective consideration of the combined information described above, reveals particular areas where further studies could provide essential data which would enable the performance of a more in-depth risk assessment on the potential contribution of triclosan use to the development of antimicrobial resistance. Areas which should be studied further include:

- Resistance determination and resistance mechanisms among skin flora, especially coagulase-negative staphylococci
- Determination of triclosan resistance amongst anaerobe bacteria
- Resistance determination and resistance mechanisms among non-pathogenic bacteria
- Determination of triclosan resistance among food-related bacteria such as *Listeria*, *Lactobacillus*, and *Enterococcus*
- Determination of triclosan resistance among streptococci isolated from the oral cavity.

VIII- Conclusions

Triclosan acts as a broad-spectrum biocide, targeting multiple targets, including lipid biosynthesis, and thus inhibiting cell growth. Bacteria are infinitely adaptable, and mechanisms of resistance to triclosan have already been demonstrated. It is of significant public health concern that these mechanisms may contribute to, or confer cross-resistance to, clinically important antimicrobials. In some situations, particularly in clinical settings, triclosan is a useful, broad-spectrum biocide.

Based on the published data presented in this report, the Panel on Biological Hazards concludes:

- Widespread use of triclosan, including in cosmetic products, select for development of triclosan resistance,
- Furthermore, such use represents a public health risk in regard to development of concomitant resistance to clinically important antimicrobial agents,
- The assessment regarding use of triclosan in consumer products from 2000 (57) seems strengthened by new evidence.

IX- Recommendations

1. As the risk associated with triclosan's potential contribution to selection of bacteria resistant to clinically important antimicrobial agents is not negligible, use of triclosan ought to be restricted to purposes where there is a well documented desirable effect in order to prevent development and spread of resistance to triclosan and other antimicrobials.

2. There is a need for independently-funded scientific research concerning:
- The effect of triclosan against bacteria under natural conditions (e.g. biofilms),
- Standardisation of methods for susceptibility testing of triclosan,
- Guidelines (regulations) defining what usage is acceptable, in terms of overall, long-term benefits to society,
- Kinetics of triclosan resistance mechanisms and their possible transferability,
- Cross-resistance between triclosan and antimicrobial agents,
- Resistance to triclosan in both pathogenic and non-pathogenic bacteria,
- Consequences and impact on the ecology of microflora during long-term exposure of humans, households and the environment to triclosan,
- Benefits, if any, of the various different triclosan-containing products to consumers.

X- Appendix

Terminology

Nomenclature used in this survey:
Acquired resistance: Describes decreased susceptibility or insusceptibility that is the result of genetic changes in a microorganism due to mutation or the acquisition of extra-chromosomal genetic material.

Antibiotics: Are natural organic compounds synthesised, traditionally, by microorganisms. Antibiotics are usually effective in low concentrations against a limited number of bacteria and are applied on or within living tissues.

Antimicrobial agents: A general term for the drugs (antibiotics), chemicals, or other substances that either kill or stop the growth of microbes. The concept of antimicrobials applies to disinfectants, preservatives, sanitizating agents and biocidal products in general.

Antimicrobial resistance: Refers to a strain of microorganism that is not killed or inhibited by a defined antimicrobial concentration. The resistant microbes haved altered in ways that reduce or eliminate the effectiveness of drugs, chemicals, or other agents used to cure or prevent infections. While the terminology regarding antimicrobial action and resistance is well understood, that relating to biocide resistance is still the subject of debate. A culture is considered resistant to a biocide when it is not inactivated by an in-use concentration of a biocide or a biocide concentration that inactivates other strains of that organism.
Susceptibility describes the degree to which a target microorganism is affected by an agent. Susceptibility can be measured through a number of standard methods, including broth dilution, agar dilution, or diffusion techniques. Insusceptibility to biocides is often a relative term and certainly there are no “cut-off” points that are widely accepted to denote sensitivity or resistance of bacteria to antibiotics.

Antiseptic: A substance applied topically to living tissue that prevents or inhibits the growth of microorganisms.

Biocide/ Biocidal products: According to the Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market, biocidal products are defined as “Active substances and preparations containing one or more substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert to controlling effect on any harmful organism by chemical or biological means”.
Biocide is in common usage and means a “biocidal product”.

Biofilm: Microbial biofilms are populations of microorganisms that are concentrated at an interface (usually solid/liquid) and typically surrounded by an extracellular polymeric slime matrix. Flocs are suspended aggregates of microorganisms surrounded by an extracellular polymeric slime matrix that is formed in liquid suspension.

Co-resistance: Selection of one type of antimicrobial can select for resistance to another antimicrobial (resistance genes are linked).

Cross-resistance: Resistance to one antimicrobial can confer resistance to another. Thus, the same mechanism of resistance applies to both drugs.

Dental plaque: Organized coherent gel-like or mucoid masses consisting of microorganisms in an organic matrix derived from saliva and microbially produced extracellular bacterial products, such as glucans, fructans, enzymes, toxins and acids.

Disinfectant: A substance that is used in the inanimate environment to destroy or eliminate a specific species of microorganism that is infectious or of public health significance. , Disinfectants are not necessarily effective against bacterial spores.

Gingivitis: An inflammatory lesion of the gingiva that is most frequently caused by dental plaque.

Intrinsic resistance: A natural property of an organism resulting in decreased susceptibility to a particular antimicrobial.

Minimum Inhibitory Concentration (MIC): The lowest concentration of a given agent that inhibits growth of a microorganism under standard laboratory conditions. MIC data can provide information about the activity of biocides; it is important to remember that in-use concentrations and MICs refer to distinct and separate values

Normal flora: Indigenous microbial flora of human external, and some internal, surfaces like the skin, mouth and gastrointestinal tract and the upper respiratory tract. The normal flora contains numerous bacterial species, and numerous strains within each species. Although it may contain pathogens, the vast majority are commensals that contribute to general health as well as to colonization resistance.
However, some of these low-virulence bacteria of the normal flora may, under certain circumstances, become opportunistic pathogens.

Selection: a process by which some bacterial species or strains of bacteria, in a population are selected for by having a specific advantage over other microorganisms. Antibacterial substances may provide a more resistant sub-population with such an advantage, enabling them to increase their relative prevalence.

Strain: a subset of a bacterial species differing from other bacteria of the same species by some minor, but identifiable, difference.

XI- References

Data source

MEDLINE database 1966-2004 and World Wide Web using the search strategy such as triclosan OR biocide AND antimicrobial resistance.

Data extraction

English, Norwegian, Danish and Swedish language articles were read and assessed for their provision of information, both direct and indirect, regarding the development of resistance against triclosan in microorganisms.

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