

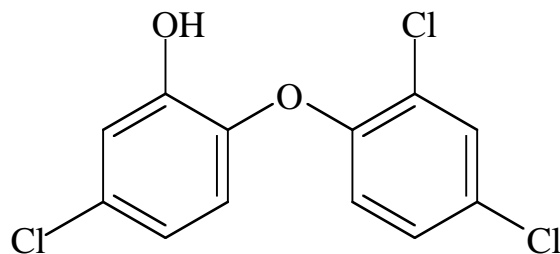
Nomination Profile

Triclosan
[CAS 3380-34-5]

Supporting Information for Toxicological Evaluation
by the
National Toxicology Program

July 2008

Prepared by:
U.S. Food & Drug Administration
Department of Health and Human Services



Summary of Nomination:

Triclosan is used as an antibacterial and bacteriocidal agent in a large number of consumer products in the U.S. and worldwide. It is used commercially (*e.g.* hospitals), in personal hygiene applications such as handwashes, body washes, toothpastes, and mouth rinses, and in fabrics and plastics to inhibit microbial growth. The bacteriocidal mechanism of action of triclosan is reported as integration and interruption of membrane function and inhibition of bacterial enoyl-acyl carrier protein reductase. The FDA is requesting dermal toxicity characterization of triclosan be conducted due to inadequate dermal toxicity data. The studies that are requested are dermal carcinogenesis in an appropriate animal model and phototoxicity studies.

A. CHEMICAL INFORMATION

Molecular Identification

Chemical Name: 2,4,4'-trichloro-2'-hydroxydiphenyl ether

Chemical Abstracts Service Registry Number: 3380-34-5

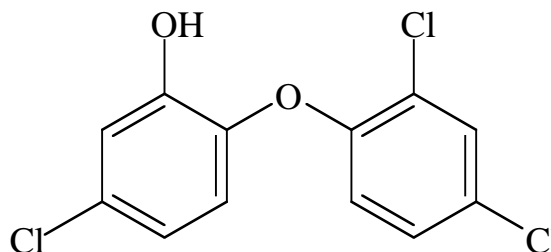
Synonyms:

5-chloro-2-(2,4-dichlorophenoxy)phenol

Trade Names: Irgasan; Irgasan DP300; CH 3565; Irgacare MP; Lexol 300; Cloxifenolum; Aquasept; Gamophen

Formula: C₁₂H₇Cl₃O₂

Molecular Weight: 289.546



Physical Chemical Properties

Physical State of Pure Material: White to off-white crystalline powder

Melting Point: 55-57 °C

Vapor Pressure: 4x10⁻⁶ mm Hg at 20 °C

Solubility: water, 0.01 g/L; 0.1 N NaOH, 23.5 g/L; ethanol, acetone, highly soluble

pKa = 7.9

Lop P = Log K_{ow}: 4.76

B. EXPOSURE POTENTIAL

Use

Triclosan is used as an antibacterial agent in a number of personal hygiene products and as an anti-plaque agent in dentifrices. Triclosan is also used as a preservative, fungicide and biocide in several household cleaning products and other household items.

Triclosan preparations have been used to control the spread of methicillin-resistant *Staphylococcus aureus* (MRSA) in clinical settings (Brady et al. 1990; Coia et al. 2006; Jones et al. 2000; Zafar et al. 1995), pre-operatively to decolonize skin (Brady et al. 1990; Coia et al. 2006) and in sutures to prevent bacterial colonization of surgical wounds (Ming et al. 2007a).

In July 1997 the FDA approved triclosan (0.3%) for use in Colgate Total® Toothpaste as a copolymer to prevent gingivitis and cavities (<http://www.fda.gov/cder/da/da0797.htm>).

Triclosan is listed by the U.S. Environmental Protection Agency (EPA) as a pesticide under active chemical code 054901 (www.epa.gov).

A total of 2385 patents containing the word "triclosan" were issued by the U. S. Patent and Trademark Office between 1976 and April 2008 (www.uspto.gov).

Human Exposure

Consumer Exposure:

Triclosan exposure may occur through ingestion of toothpaste, mouthwash, or dentifrices containing triclosan and through dermal contact with consumer products containing triclosan, or through consumption of contaminated food and drinking water (HSDB, 2004).

Triclosan is found in a wide variety of consumer products including a number of personal hygiene products: “antibacterial” soaps (hand and body washes, facial wash, and dish liquids), toothpaste, mouthwash, cosmetics, deodorant, shaving cream, feminine hygiene products (sponges and wipes), anti-acne products, skin cream and first aid products (Glaser 2004). In addition, triclosan has been incorporated into a number of household items such as kitchen utensils, cutting boards, kitchen wipes, mop heads, computer equipment, clothing, blankets, flooring, paint, air filters, children’s toys, and some small appliances (Glaser 2004). Medical devices (*e.g.* sutures) have also been impregnated with triclosan to inhibit bacterial growth in wounds (Ming et al. 2007a; Storch et al. 2004). In a survey of brand name soaps available in the U. S., 76% of liquid soaps and 29% of bar soaps were “antimicrobial”, containing either triclosan or triclocarban (Perencevich et al. 2001). Among these, 100% of antimicrobial liquid soaps and 16% of antimicrobial bar soaps contained triclosan (Perencevich et al. 2001).

Occupational Exposure:

Triclosan exposure may occur through inhalation and dermal contact at workplaces where it is produced or used (HSDB, 2004).

The National Occupational Exposure Survey (NOES), conducted from 1981 to 1983 by the National Institute for Occupational Safety and Health (NIOSH), estimated that a total of 188,670 employees in 16 different industries were potentially exposed to triclosan (www.cdc.gov/noes). The NOES database does not include information about the extent of exposure to chemicals that were evaluated. Further information on occupational exposure to triclosan is available in the EPA document “Occupational and residential Exposure Assessment” (USEPA 2008).

Environmental Exposure:

Triclosan was among the top 7 organic wastewater contaminants found in samples from a network of 139 streams across 30 states during 1999 and 2000 by the U. S. Geological Survey (Kolpin et al. 2002). Triclosan concentrations up to 74 ng/L were found in lakes and a river in Switzerland (Lindstrom et al. 2002). In 2003 triclosan concentrations in the influent of wastewater treatment plants in the U.S., Sweden, Switzerland and Denmark ranged from 0.1 to 16.6 µg/L, while concentrations in the effluent ranged from 0.1 to 2.7 µg/L and concentrations in the sludge ranged from 0.028 to 15.6 µg/L (Samsøe-Petersen et al. 2003). Further information on environmental risk assessment of triclosan is available in the EPA document “Preliminary Ecological Hazard and Environmental Risk Assessment Science Chapter for the Triclosan Reregistration Eligibility Decision (RED) Document” (USEPA 2008).

Effectiveness/efficacy

Mechanism of action in bacteria:

Two mechanisms of action of triclosan inhibition of bacterial growth have been described. Triclosan has been shown to intercalate into bacterial cell membranes and disrupt membrane activities without causing leakage of intracellular components (Guillén et al. 2004; Villalaín et al. 2001). In addition, triclosan inhibits bacterial type II fatty acid synthase enoyl-reductase (Heath et al. 1998; McMurry et al. 1998b; Ward et al. 1999). Triclosan resembles an enoyl intermediate in fatty acid synthesis with a K_D in the low picomolar range (Ward et al. 1999). It is unclear the degree each mechanism contributes to the bacteriostatic or bacteriocidal properties of triclosan.

Antimicrobial activity:

At low doses triclosan is bacteriostatic and at higher doses it becomes bactericidal (Kampf & Kramer 2004; Yazdankhah et al. 2006). Lower concentrations of triclosan favor specific action against type II fatty acid synthase enoyl-reductase (FabI), while higher concentrations allow action against multiple targets, including less specific targets, such as the cell membrane (Yazdankhah et al. 2006). Triclosan also has some *in vitro* antiviral and antifungal activity (Jones et al. 2000).

While triclosan has *in vitro* activity against a broad spectrum of both gram-negative and gram-positive bacteria, it has greater activity against gram-positive species (Bhargava & Leonard 1996; Jones et al. 2000). Triclosan is particularly effective against *Staphylococcus aureus* (Bamber & Neal 1999; Bhargava & Leonard 1996; Jones et al. 2000). However, some clinical isolates of *S. aureus* are not as susceptible to triclosan (minimum inhibitory concentration [MIC] of 1-2) due to overexpression (3- to 5-fold increase) of FabI (Fan et al. 2002). In addition to overexpression of the triclosan target, the FabI of these isolates carries a single amino acid change, which prevents stable triclosan-NAD⁺-FabI complex formation (Fan et al. 2002).

Gram negative bacteria are generally more resistant to triclosan. In particular, *Pseudomonas aeruginosa* has several multi-drug efflux pumps that remove a number of drugs, including triclosan, from the cells (Chuanchien et al. 2001; Chuanchien et al. 2003).

Soaps:

A review of studies on the efficacy of triclosan in soap revealed that it does not reduce bacterial counts on hands significantly more than plain soap unless used repeatedly and in relatively high concentrations ($\geq 1\%$) compared to the 0.1-0.45% in consumer antibacterial soaps (Aiello et al. 2007). This may drive manufacturers to reformulate with higher concentrations of TCS, which would increase the human exposure and environmental contamination. However, any increase in the concentration of triclosan in consumer products in an attempt to improve efficacy would directly increase consumer exposure. In addition, without the implementation of more aggressive use of such products, efficacy might still be equivocal.

There have been few studies that have been adequately designed to assess the impact of the use of triclosan containing products on infection rates. A study of reasonable design evaluated the household use of multiple antimicrobial products, including a liquid handwash containing 0.2% triclosan, for 48 weeks. The study that measured efficacy by infectious illness symptoms indicated no statistical significance between use of "antimicrobial" household cleaners, detergents and handwash, and the use of similar products lacking antimicrobial ingredients (Larson et al. 2004). This study used several types of antimicrobial household cleaners (liquid handwash was the only one containing triclosan) and focused primarily on symptoms consistent with a viral illness as a measure of efficacy.

Studies that used bacterial counts to determine the efficacy of triclosan-containing soaps produced variable results. An epidemiological study of household use over 11 months found that 0.2% triclosan liquid soap was not significantly better at reducing bacterial levels on hands than liquid soap without triclosan (Larson et al. 2003). When overall bacterial counts were used to determine the efficacy of triclosan-containing soaps, those with less than 1% triclosan were not significantly more effective than plain soap (Aiello et al. 2007), except in 2 studies, one where 0.3% triclosan soap was used 18 times daily for 5 days (Larson et al. 1989), and another where 0.75% triclosan was used in 2 min hand washes 6 times (Lilly & Lowbury 1974). When 1% triclosan soaps were compared to plain soap using bacterial counts, one study found no significant difference when hands were washed using a standard surgical technique (Faoagali et al. 1995), but another study found that 1% triclosan significantly reduced bacterial counts when hands were washed for 30 sec or for 3 min (Leyden et al. 1991).

In studies employing artificial contamination of hands with *Serratia marcescens*, 10 hand washings for 10 sec each using 1.0% triclosan soap [5 subjects; (Sickbert-Bennett et al. 2005)] or as little as 1 hand washing with 1.5% triclosan soap [12 subjects; (Bartzokas et al. 1987)] reduced bacterial counts significantly more than washing with plain soaps, as measured by log reduction starting with heavy initial inocula (overnight culture and 10^9 colony forming units [cfu]/ml, respectively). In a study using artificial contamination of fingertips with *Escherichia coli*, when hands were washed 30 sec with 1.5% or 2.0% triclosan soaps, reduction of bacterial counts was not significantly greater than when plain soap was used (Ayliffe et al. 1988). A soap containing 2.0% triclosan exhibited residual antimicrobial activity on the skin (n=20) for up to at least 2 hr after three applications, as compared to plain soap (Bartzokas et al. 1983).

An evaluation of the available data by the American Medical Association in 2002 determined that when properly used in clinical settings, triclosan-containing soaps were efficacious; however, since patterns of use in consumer settings are not usually as rigorous as those in the clinical setting, this efficacy does not necessarily translate to the use of consumer products (Tan et al. 2002).

Deodorants:

When used *ad libitum* for 6 months, deodorant sprays containing 0.15% and antiperspirant deodorant sprays containing 0.25% triclosan reduced bacterial counts per cm² of skin from 5.2×10^5 to 1.4×10^3 and 3.74×10^2 , respectively (Cox 1987). Upon

discontinuing use of the triclosan-containing deodorants, bacterial levels returned to the pre-test levels within 4 to 7 days (Cox 1987).

Dentifrices:

A review of studies on the efficacy of triclosan in dentifrices revealed that when combined with 2.0% Gantrez®, 0.3% triclosan acts as an effective anti-plaque and anti-gingivitis agent in dentifrices (Gunsolley 2006). Dentifrices with combinations of triclosan and soluble pyrophosphate or zinc citrate, however, were not effective against plaque and gingivitis (Gunsolley 2006). Following a dose of 1 g toothpaste containing 0.5% triclosan/ml water, triclosan is orally retained in plaque (0.31 µg/ml) and saliva (20.87 µg/ml at 5 min and 3.91 µg/ml at 2 hr) (Gilbert et al. 1987). Following a dose of 1 g toothpaste containing 0.2% triclosan, triclosan was detected in bacterial plaque for at least 8 hr (0.1 µg/ml of protein) and in the oral mucosa for at least 3 hr (0.21 µg/ml of protein) (Gilbert & Williams 1987).

Plastics:

Triclosan-incorporated plastics do not release sufficient amounts of triclosan to inhibit bacterial growth when used in cutting boards (Junker & Hay 2004) or in toothbrushes (Efstratiou et al. 2007). A low density polyethylene film containing triclosan (1 g/kg) reduced bacterial growth *in vitro*, but did not effectively reduce spoilage bacteria when used on refrigerated vacuum-packaged chicken breasts (Vermeiren et al. 2002). While triclosan in plastic (Microban®) has been registered with the EPA to inhibit bacterial growth in plastic, the EPA has taken action to prevent manufacturers from claiming that the use of such products provides protection from disease (Pugliese & Favero 1998).

Sutures:

Sutures impregnated with triclosan inhibit bacterial growth *in vitro* (Ming et al. 2007b; Rothenburger et al. 2002) and *in vivo* at suture sites in guinea pigs and mice (Ming et al. 2007a; Storch et al. 2004) without affecting wound healing or suture performance (Storch et al. 2002a; Storch et al. 2002b). When monofilamentous suture implants in mice were challenged with 6×10^7 cfu of *E. coli*, triclosan-containing sutures produced more than a 2-log reduction in colonizing bacteria over control sutures (Ming et al. 2007a). In the guinea pig model using monofilamentous sutures, challenge with 4×10^5 cfu of *S. aureus* resulted in colonization near the challenge level in the majority of sites using control sutures, with a mean of 8.6×10^4 cfu, while 90% of sites with triclosan-impregnated sutures were free of *S. aureus* (Ming et al. 2007a). When multifilamentous suture sites in guinea pigs were challenged with 2.1×10^4 cfu of *S. aureus*, the mean recovery of colonizing bacteria for triclosan-impregnated sutures was 5.6×10^2 cfu and that for control sutures was 1.7×10^4 cfu (Storch et al. 2004). Together, these results suggest that triclosan impregnated sutures reduce bacterial colonization; however, whether this translates to reduced colonization in humans was not addressed with these or other studies.

C. ACUTE, SUBCHRONIC, AND CHRONIC TOXICITY

Acute Toxicity:

Triclosan demonstrates a low level of toxicity in acute studies. Studies on the acute oral toxicity of triclosan were first reported by Lyman and Furia (1969), where LD₅₀ values of 4,350 mg/kg were reported for mice, and between 3,700 and >5,000 mg/kg for Sprague-Dawley rats. The LD₅₀ of triclosan administered by intravenous injection in triethylene glycol:water (1:2) was 29 mg/kg in white rats. Subcutaneous administration of triclosan in ethanol led to a LD₅₀ of 14,700 mg/kg in male and female white rats.

Lyman and Furia (1969) reported that the LD₅₀ of triclosan administered to rabbit skin as a slurry in propylene glycol was greater than 9,000 mg/kg.

The acute toxicity of triclosan (0.25% solution in gum tragacanth) was determined following oral doses to adult Wistar rats at a dose of 10 mL/kg (authors state doses ranged from 0.625 to 2.5 g/kg Chow et al. 1977). A mortality of 20% occurred in the highest dose group, and no differences were noted in serum glutamate oxaloacetate transaminase, glutamate pyruvate transaminase or blood urea nitrogen, suggesting no hepatotoxicity.

The acute lethal effects of triclosan were additionally summarized by DeSalva et al., 1989. The LD₅₀ following oral administration was 3,750-5,000 mg/kg in rats, 4,350 mg/kg in mice, 580 mg/kg in neonatal mice, and >5,000 mg/kg in dogs. The LD₅₀ following intravenous administration was 19 mg/kg in mice and 29 mg/kg in rats, while intraperitoneal administration led to a LD₅₀ of 184 mg/kg in mice and a LD₅₀ of >14,700 mg/kg in rats following subcutaneous administration. Although not specifically cited in DeSalva et al., 1989, these data obviously come from the same studies as reported earlier in Lyman and Furia (1969).

The LD₅₀ of triclosan (>99% pure) has been reported as 1090 ± 20 mg/kg in male ddY mice (22 g body weight) following intraperitoneal injection in an ethanol/olive oil mixture (Kanetoshi et al. 1992). In this study, the mice were injected at doses ranging from 272-1090 mg/kg triclosan and observed for up to one week.

Subchronic Toxicity:

Lyman and Furia (1969) reported the results of subchronic toxicity studies where triclosan was administered by gavage at 0, 0.5, 1; 2, 5, and 10% by weight in gum Arabic to rats, 6 days/week, for 4 weeks to achieve doses of 50, 100, 200, 500, and 1000 mg/kg/day. Mortality (2/10) occurred in the high dose group, and there was no report of histopathological results.

The subchronic toxicity of triclosan has been summarized in DeSalva et al. (1989) primarily from unpublished, industry sponsored studies. Oral administration of triclosan to rats for 2 weeks resulted in decreased weight gain and mortality at 1000 and 2000 mg/kg, respectively. No toxicities were detected at doses as high as 1000 mg/kg following 4 weeks oral administration. After 13 weeks administration, hepatic, thymic, and renal changes were noted at 125 mg/kg but not at 315 mg/kg. Administration of

triclosan for 13 weeks in the diet resulted in hepatic and hematopoietic changes at 150 and 300 mg/kg in male rats. These results were also reported in Bhargava and Leonard (1996).

Oral administration of triclosan to rabbits induced mortality and hematologic changes at 30 and 150 mg/kg in 13 weeks, but no toxicity at 125 mg/kg when included in the diet for 13 weeks (DeSalva et al. 1989). Similarly, administration of triclosan in the diet of dogs for 13 weeks as high as 25 mg/kg did not induce toxicity, while administration in a capsule induced hepatic changes at 25, 50, and 100 mg/kg in one study and at 100 and 200 mg/kg in a second study (DeSalva et al. 1989). Administration of triclosan at up to 300 mg/kg by oral capsule to baboons induced no pathological findings in 52 weeks, although emesis and diarrhea were reported (DeSalva et al. 1989).

In 1998, Colgate-Palmolive submitted and the Agency reviewed a 13-week dermal subchronic study of triclosan in rats: Signs of severe dermal irritation were seen in the treated groups, especially in the high-dose group. These signs were erythema, edema, desquamation, and eschar formation. Microscopically, hyperplasia of sebaceous glands, inflammation, and focal necrosis were seen on the skin of treated animals. The dermal effects were reversible during the recovery period. There were no systemic effects that could be treatment-related, although liver masses were observed in two treated animals.

Skin Sensitization:

Subchronic dermal studies were conducted by applying 0.4 mL of a 2.5% or 5% suspension of triclosan in gum Arabic five times each week for four weeks to the shaved backs of male and female rats (5/sex) (Lyman & Furia 1969). No local dermal irritation or systemic toxicity was reported.

Lyman and Furia (1969) also tested the dermal toxicity of triclosan as a powdered soap (100 mg/kg dose, 15% triclosan) applied to the shaved abdominal skin of three rabbits for 23.5 hrs, with three daily applications, followed by 1 week of observation. No compound related toxicity was reported.

Dermal administration of triclosan at 2.5-5% as a suspension in gum Arabic for 1-4 weeks did not result in toxicity in rats (Bhargava & Leonard 1996; DeSalva et al. 1989). Another study with daily application to rats of 3% triclosan in corn oil (2 mL) for 2 weeks also did not result in toxicity (Bhargava & Leonard 1996). Dermal application of a 3% solution resulted in some skin irritation in rabbits (DeSalva et al. 1989).

Lyman and Furia (1969) conducted skin sensitization studies using several modalities in guinea pigs including: intracutaneous injection of 0.1% triclosan in gum Arabic using 33 male albino guinea pigs; intradermal injection of 0.1 mL of 1% triclosan in 5% polyethylene glycol for 10 injections (3/week) followed by challenge injection 2 weeks later; delayed contact sensitization; dermal application of 50 ppm triclosan in water: isopropanol for 6 days/week for 3 weeks followed by dermal challenge 3 weeks later. In all cases, the author concluded that no consistent triclosan-related sensitization occurred in the guinea pigs.

DeSalva et al. (1989) summarized several non-published studies from industry concluding that application of body powder (0.1% triclosan), body lotion (0.1% triclosan), non-woven wipes (0.0005% triclosan), shower gel (0.25% triclosan), aerosol deodorant (0.0375% triclosan), and soap (0.1-1.0% triclosan) did not induce dermal toxicity in rabbits using the rabbit acute dermal lethality test or primary dermal irritation test when applied at 2 g/kg test article.

Human Skin Irritation and Sensitization:

Studies were conducted on the skin of human volunteers to determine the compatibility of dermal application of triclosan (Lyman & Furia 1969). The subjects were topically treated with 0.5% triclosan in 1% soap solution according to the Draize method (Draize 1965). In the soap control, 0/50 subjects had sensitization or irritation, while 2/50 subjects receiving 0.5% triclosan had a very mild reaction. The conclusion was that triclosan was not a sensitizer or irritant.

In a second study with human volunteers (Lyman & Furia 1969), 0.75 g of a 25% solution of triclosan in petrolatum was applied to the skin of subjects pretreated with 5% sodium lauryl sulfate solution (Maximization protocol). No sensitization occurred in 25 subjects.

The sensitization of volunteers to patch test of 0.5% triclosan in soap solution with and without solar exposure was conducted with 116 subjects (Lyman & Furia 1969). The author concluded that skin irritation of triclosan did not exceed the irritation of the soap solution.

DeSalva et al. (1989) summarized several studies regarding the human toxicity of triclosan. A total of 1,246 volunteers used toothpaste or mouth rinse containing 0.2-0.6% and 0.01-0.06% triclosan, respectively, for varying lengths of time up to 12 weeks. The authors reported that no difference existed between the treated and control groups (blood chemistry and hematology) concluding that oral use of triclosan was safe, although the power of the study in regards to detecting mild irritants was not discussed.

Triclosan-containing dermatological products have been used by volunteers in case studies. DeSalva et al. (1989) summarized several non-published studies from industry concluding that application of body powder (0.1% triclosan), body lotion (0.1% triclosan), and soap (0.1-0.25% triclosan) did not induce sensitization in human subjects using the repeated insult patch test or prophetic patch test for sensitization.

Reproductive/Developmental Toxicity:

Reproductive studies were reported by DeSalva et al. (1989) where a NOEL of 50 mg/kg/day was reported for the dams based on effects on the pups. A NOEL based on developmental outcomes was listed as 150-300 mg/kg/day (DeSalva et al. 1989); however, no reproductive tract or fertility abnormalities were reported.

Further Information:

Further information regarding toxicity studies involving triclosan is available in the EPA Docket for the Reregistration of Triclosan (USEPA 2008) and in supplements within the

FDA Docket 1975N-0183H (FDA 1975N-0813H)

(www.fda.gov/ohrms/dockets/dailys/01/Sep01/091701/cp00009.pdf ;

www.fda.gov/ohrms/dockets/dailys/03/Sept03/090303/75n-0183h-c000085-01-vol170.pdf)

D. ABSORPTION, DISTRIBUTION, METABOLISM, ELIMINATION (ADME)

Absorption:

Administration of ¹⁴C-triclosan to rats at 400 mg/kg on an occluded dermal patch, resulted in approximately 15% of the dose absorbed and excreted in the feces (DeSalva et al. 1989).

Kanetoshi et al (1992) studied the absorption of triclosan when applied to the skin of ddY mice. They applied 1.6 mg of ³H-triclosan (in ethanol: olive oil) to the shaved backs of ddY mice and the absorption was quantified 6, 12 and 18 hours later. Maximum levels of ³H-triclosan appeared to occur between 12 and 18 hours, with greatest concentration in the gall, liver, body fat, lungs and kidneys. The levels in the tissues were approximately 14-67% the levels achieved in a comparable study where ³H-triclosan was given orally.

Triclosan is readily absorbed in humans by the skin (Chedgzoy et al. 2002; Moss et al. 2000), through the oral mucous membranes (Lin 2000), through the gastrointestinal tract (Bagley & Lin 2000; Sandborgh-Englund et al. 2006), and through mucosal tissues following intra-vaginal administration (Siddiqui & Buttar 1979).

When absorbed through the oral mucous membrane during tooth brushing (n=9), plasma levels of triclosan reach only 9-14% of those attained when the triclosan-containing dentifrice is swallowed (n=9) (Bagley & Lin 2000). When subjects (n=21) brushed with and then swallowed a single dose of dentifrice containing 3.75 mg triclosan, the maximum plasma triclosan concentration (243 ng/ml) was reached by 4 hr, and when this regimen was repeated 3 times daily for 12 days, the mean plasma triclosan concentration was 352ng/ml by days 12-13 (Bagley & Lin 2000). Following a single dose (swallowed) of 4 mg triclosan, plasma levels of triclosan increased from a median baseline of 0.4ng/ml to maximum concentration (218 ng/ml, n=10) within 1 to 3 hr (Sandborgh-Englund et al. 2006). Prior to this gastrointestinal exposure to triclosan, urinary excretion of triclosan ranged from 0.1 to 91 µg/d, increasing after exposure with removal of 24–83% of the original 4 mg dose within 4 days (Sandborgh-Englund et al. 2006). During use of a mouth rinse containing 4.5 mg of triclosan for 30 sec twice daily for 21 days, mean plasma triclosan concentrations were 74.5-94.2 ng/ml (Lin 2000). When absorbed through the oral mucous membrane in mouthwash or the gastrointestinal tract in dentifrice, triclosan levels in human blood plasma return to baseline approximately 8 days following final exposure to triclosan (Lin 2000; Sandborgh-Englund et al. 2006).

Triclosan absorption through skin is less efficient, with only 6.3% penetration in 24 hr when directly applied (Moss et al. 2000), and up to only 0.7% penetration in 24 hr when applied in a transdermal adhesive formulation patch model (Chedgzoy et al. 2002) to human skin *in vitro*.

Enzyme Induction and Inhibition:

Exposure to triclosan has an effect on drug and chemical metabolism in rodents. Kanetoshi et al. (1992) showed that intraperitoneal administration of triclosan (50 or 100 mg/kg) for three consecutive days to male Wistar rats, which were then sacrificed 1 day later, resulted in increases in hepatic cytochromes P450. The authors used enzyme assays for the cytochromes P450 (as opposed to Western blots), and demonstrated that triclosan induced P450 activity differently than phenobarbital or 3-methylcholanthrene inducing aminopyrine *N*-demethylase, biphenyl hydroxylase (2- and 4-) and 7-ethoxycoumarin, *p*-nitroanisole, and *p*-nitrophenetole *O*-deethylase activities in male Wistar rats. Only aminopyrine *N*-demethylase activity was induced in male ddY mice. Extending this work, Hanioka et al. (1997) determined that intraperitoneal injection of triclosan into rats resulted in induction of cytochromes P450 2B1, 2B2, 3A2/1 and 4A1. A subsequent study by Jinno et al. (1997) using rat hepatocytes cultured on Matrigel demonstrated that triclosan induced cytochromes P450 2B1/2 with some induction of cytochrome P450 3A. Jinno et al. (1997) additionally showed that triclosan inhibited uroporphyrinogen III synthetase leading to an accumulation of uroporphyrin I in the tissues.

Triclosan has also been shown to inhibit cell growth in MCF-7 and SK Br-3 human breast cancer cell lines resulting in cellular apoptosis (Liu et al. 2002). The authors demonstrated that triclosan reversibly inhibited mammalian fatty acid synthesis (enzyme from SK Br-3 cells and goose uropygial gland). Triclosan was shown also to induce apoptosis in Smulow-Glickman human gingival epithelial cells *in vitro* (Zuckerbraun et al. 1998).

Metabolism and Kinetics:

Two early studies on the metabolism of triclosan summarized that triclosan was excreted predominantly unchanged (Black et al. 1975; Tulp et al. 1979). Triclosan (500 mg/kg) was administered to male Wistar rats (200 g) and feces and urine collected for 7 days followed by sacrifice and determination of the levels of triclosan in tissues and excreta (Tulp et al. 1979). The authors concluded that triclosan was excreted into the urine and feces essentially unchanged with some evidence of conjugation. Triclosan was detected in the liver and fat at the end of the study. The fate of triclosan following administration to rats was summarized in DeSalva et al. (1989). At oral doses of 5 and 50 mg/kg, triclosan is predominantly excreted in the feces of rats (~80% in studies with ¹⁴C-triclosan).

Triclosan is conjugated at the 2'-hydroxyl group to glucuronide and sulfate conjugates by Phase II metabolism. The concentrations of free triclosan and the glucuronide and sulfate conjugates in blood were reported for rats following administration in the diet for up to 24 months (DeSalva et al. 1989). Total triclosan levels increased in a dose-dependent manner in the rats reaching 54 or 86ug/mL (ppm) blood at a dose of 3000 mg/kg in female and male rats, respectively. The levels of triclosan in male rat blood and kidney tissue showed a dose-dependent increase at 3, 6, 12, 18 and 24 months of dosing, with a general trend of time-dependent decrease in blood and liver levels, but time-dependent increase in kidney levels (DeSalva et al. 1989).

The half-life of triclosan has been determined in several studies. Siddiqui and Buttar (1979) showed that following intravenous administration of ¹⁴C-triclosan, it distributed to 42% of bodyweight and disappearance from the blood has a two-compartment half-life with the second phase being 8.8 hrs. In another study triclosan half-life was determined in rats following oral administration as 0.66% in sodium lauryl sulfate solution or 0.2% in toothpaste at 5 mg/kg triclosan (DeSalva et al. 1989). Oral administration of triclosan in the solution led to triclosan and metabolites with half-lives of 7-14 hours. Administration of triclosan as a toothpaste gave glucuronide and sulfate half-lives of 11-14 hours.

In summary, plasma triclosan determinations revealed that triclosan was absorbed through the skin, although insufficient data was available to calculate the percentage absorbed. Triclosan plasma levels were higher using acetone vehicle, than they were using the propylene glycol vehicle. The effects reported in this review occurred in rodents, under a unique set of experimental conditions. It is not known if the same effects would occur in humans using triclosan containing commercial products. However, although there is limited skin absorption of triclosan after topical use, a large exposure skin area together with non-intact skin or abrading during over-the-counter (OTC) use may change the absorption profile.

Human Distribution:

The human blood levels of triclosan following use in either mouth rinses or dentifrices were reported by DeSalva et al. (1989). When used as an aqueous solution for 21 days (2 mg consumed), the mean blood levels 4 hours later were 150-174 ppb (ng/mL) total triclosan. In other studies, brushing twice daily with 2 mg triclosan the blood levels were 15-21 ppb, and use of a dentifrice with 0.2-0.6% triclosan resulted in blood levels of 16-25 ppb.

Triclosan has also been detected in human breast milk, and is probably associated with the fat due to its high lipophilicity (Dayan 2007). Milk samples (n=62) from San Jose, CA, and Austin, TX, had triclosan levels ranging from none (n=2), and at limit of detection (n=9) to a range of 100 to 2,100 ng/g lipid (n=51) (Dayan 2007). The 5 samples with the highest levels averaged 1,742 ng/g lipid or 35.8 ng/g whole breast milk (Dayan 2007). Using average daily consumption values of breast milk by babies, and the highest average concentration of triclosan in breast milk, Dayan (2007) calculates an average maximum daily consumption of 74 µg/kg body weight/day by breast-feeding infants. If the NOAEL for neonatal rats is 50 mg/kg/day (DeSalva et al. 1989), then the margin of safety for human infants is approximately 6,700 (DeSalva et al. 1989); however, this risk estimation is dependent on accurate estimation of the NOAEL for infant mammals and true understanding of the maximum dose of triclosan in human breast milk.

In 2002 triclosan was found in the blood plasma of a random selection of men in Sweden (Hovander et al. 2002). Subsequently, triclosan was found in the plasma and breast milk of nursing mothers in Sweden, regardless of whether the mothers used triclosan-containing soap, deodorant or toothpaste (Allmyr et al. 2006). Triclosan levels

were higher in the mothers who used triclosan-containing products (0.4-38.0ng/g fresh weight in plasma and 0.022-0.95ng/g in milk) than in those who did not (0.01-19ng/g in plasma and <0.018-0.35ng/g in milk) (Allmyr et al. 2006). Among those who used triclosan-containing products, triclosan levels were lowest in those who did not use toothpaste containing triclosan (Allmyr et al. 2006). Triclosan concentrations in the blood serum of Australian women (11ng/g) were higher by a factor of 2 when compared to the levels in plasma of Swedish women (5.8ng/g), most likely due to efforts by the Swedish authorities using national consumer advisories to discourage the use of triclosan (Allmyr et al. 2008). In 2008, triclosan concentrations of 2.4–3,790ng/ml were found in the urine of a random sample of 2,517 people in the general population of the U. S. (Calafat et al. 2008).

Systemic exposure to triclosan occurs following topical application (dermal absorption) and is also possible through indirect ingestion (from hands or use of dentrifices or mouthwashes). Human pharmacokinetic studies conducted in 1970's with oral and topical administration showed that the dermal absorption of triclosan was 2-9% of the administered dose based on the total urine excretion. More than 90% of the absorbed triclosan was metabolized. The bio-distribution, plasma elimination half-life and plasma protein binding were not available. An *in vitro* study with human blood showed that 99% of triclosan bound to plasma proteins.

E. GENOTOXICITY AND MUTAGENICITY

In the report of DeSalva et al. (1989), the results of 18 mutagenicity tests were summarized, of which 13 were conducted by industry and not reported in the literature. Only one test indicated that triclosan was a mutagen (mammalian spot test), and a repeat of that study was negative. A recent study by Ciniglia et al. (2005) reported that triclosan inhibited the vegetative growth of *Closterium ehrenberghii* at 0.5 mg/L and induced DNA damage (Comet assay) at 0.25 mg/L. More recently, Rodrigues et al. (2007) demonstrated that triclosan was not mutagenic in the *Drosophila* wing spot test. Therefore, the preponderance of data suggests triclosan is not a genotoxic or mutagenic compound.

F. CARCINOGENICITY

Lyman and Furia (1969) reported application of triclosan as a 0.5% and 1% solution in acetone to the shaved intrascapular region of Swiss white mice for 18-months (0.1 mL applied/day). The authors reported no toxicities (body weight, food consumption, behavior, skin reaction, mortality, gross or microscopic pathology) or carcinogenesis as a result of triclosan treatment.

In 1981, a "Two year chronic oral toxicity study with FAT 80'023/A in albino rats (IBT 622 06047, December 1, 1977)" was submitted to the FDA. The agency concluded that the 2-year chronic oral toxicity study and the 18-month carcinogenicity studies were not valid due primarily to the presence of test material in control animals. Other problems were also identified in these studies. In addition, a 3-phase reproduction study was found to be invalid due mainly to the absence of raw data at the time of inspection. The Agency subsequently expressed the need for additional data on 2-year rate feeding study.

In 1986, a 2-year oral rat carcinogenicity study was conducted and submitted to the FDA by Ciba ("*FAT 80'023: new 2-year oral administration to rats*"; and "*Determination of FAT 80'023 in blood and tissue samples taken during a 2-year chronic oral toxicity oncogenicity study in albino rats*"). Several pharmacology/toxicology reviewers came to the same conclusion that the study was inadequate based on the unacceptable high rate of mortality, absence of significant differences in body weights between treated & controls and the presence of hepatocellular lesions that were not consistent with the morbidity/mortality seen in the study. The reviewer (and others who reviewed the data subsequently) recommended that the study be repeated in a dermal route of exposure using Good Laboratory Practice (GLP) guidelines. The reviewer also concluded that triclosan was oncogenic in both male and female rats at 3,000 ppm after 104 weeks of treatment. The sponsor convened an expert panel and a pathology working group who together agreed that the objectives of the study had been reached and that the 2-year carcinogenicity study was valid.

A two-year carcinogenicity study of triclosan (0, 300, 1000, 3000 ppm in diet) was conducted using Sprague Dawley rats (DeSalva et al. 1989). There were no significant dose-related effects on mortality in the study. Rats consuming the 3,000 ppm diet had greater incidences of bradypnea, cachexia, chromatouria, pollakiuria, ptosis and skin lesions. Livers from rats at 1,000 and 3,000 ppm had compound related increases in centrilobular hepatocytes hypertrophy and hepatocytic inclusions. No summary of tumor incidence in the study was reported in DeSalva et al. (1989), and as a result, no conclusions can be made regarding carcinogenicity of triclosan following either oral or dermal application.

In 1991, the Agency recommended that a 2-year mouse carcinogenicity study be conducted according to the current standards, and where the appropriate route of administration (i.e., dermal) and dose selection be used, and that proper endpoints regarding survival and histopathology be obtained.

In 1999, a chronic carcinogenicity study was submitted to the FDA on the potential tumorigenic and chronic toxicity effects of triclosan following prolonged dietary administration to hamsters (up to 95 weeks in males and 90 weeks in females): The adequacy of the reporting and completeness of the histopathology evaluation of the study was questionable by the pharmacology/toxicology reviewer who suggested that sponsor provide the histopathology slides of kidneys, liver, lungs, adrenals and all tumors from all animals on study for review of said tissues by a panel of outside pathologists with the results forwarded to the Agency. No further response was received from the sponsor.

In 2001, at an internal FDA meeting it was concluded that the previous carcinogenicity studies were not adequate and thus a dermal carcinogenicity study was requested to clearly establish the safety of triclosan in skin cleansing preparations.

No further action has been taken since 2001 on the dermal carcinogenicity assessment of triclosan.

G. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

DeSalva et al. (1989) report that 5 reproductive toxicity studies have been conducted by industry on rats, mice and rabbits, without any further publication of the data. Oral administration of triclosan to pregnant mice (gestation days 1-16) resulted in maternal and fetal toxicity at 50 and 100 mg/kg. The authors report no indications of teratogenesis in the mice, or in rats (50 and 100 mg/kg) or in rabbits (10, 25, 50, 100 mg/kg) following administration during gestation.

DeSalva et al. (1989) report a two-generational dose study was conducted in rats at doses of 0, 300, 1000, and 3000 ppm in the diet (equivalent to 0, 15, 50, 150 mg/kg). Toxicity was noted in the neonates from dams consuming the highest dose, and reductions in survival were seen in f_1 and f_2 populations with increased kidney dilations. These studies have not been reported in the literature.

In 1989, a "2-generation reproduction rat study for triclosan" was submitted to the FDA by Ciba. The pharmacology/toxicology reviewer concluded that a NOAEL for fertility (not teratology or development since these were not examined) of 1000 ppm was obtained. However, since no pharmacokinetic data were submitted in support of the study, the reviewer could not establish the level of chronic exposure to triclosan. A number of deficiencies in the study were identified in the review. Subsequently (1990), an internal review recommended that the Agency requests from the sponsor to conduct a proper segment-2 reproductive study following contemporary guidelines, and to submit pharmacokinetic data on the parent drug and metabolites for the reproductive study. At this time, sufficient proprietary data exist on the effects of triclosan exposure on the reproductive and developmental health which appear to be adequate for addressing this aspect of triclosan's safety.

There have been several reports on endocrine disruptor activity of triclosan. Foran et al. (2000) reported that triclosan was weakly androgenic as evidenced by altered fin length and sex ratio in Japanese Medaka fish starting at age 2 days. Additional studies indicated that triclosan was toxic and had weak estrogenic activity in Medaka (Ishibashi et al. 2004). Estrogen antagonism was induced in frogs following intraperitoneal administration of high doses of triclosan, while lower doses reduced testosterone in male frogs (Matsumura et al. 2005). Additional studies with frogs showed that triclosan bound to thyroid hormone receptor (Veldhoen et al. 2006). Gee et al. (2008) demonstrated that triclosan has estrogenic activity where they demonstrated competitive binding with estradiol at the estrogen receptor and supported growth of the estrogen-dependent MCF-7 cell line. They also demonstrated triclosan bound to the rat androgen receptor, demonstrating androgenic activity. As a result, triclosan endocrine activity may be dependent on the experimental test conditions.

H. ENVIRONMENTAL FATE AND AQUATIC TOXICITY

Heating triclosan to 600 °C to simulate combustion led to the formation of di- and trichlorodibenzo-p-dioxin (Kanetoshi et al. 1988). The addition of sodium hypochlorite

to the combustion led to the formation of di-, tri-, and tetrachlorodibenzo-p-dioxin (Kanetoshi et al. 1988). The levels of these chlorinated dioxins in the environment as a result of combustion of materials containing triclosan have not been established.

Exposure of aqueous triclosan to sunlight results in the formation of 2,7- and 2,8-dichlorodibenzo-p-dioxin (Lores et al. 2005, and references therein). The level of dichlorodibenzo-p-dioxins in the environment following photodecomposition of triclosan, and the levels of dichlorodibenzo-p-dioxins on skin following photodecomposition of topically applied triclosan have not been established.

I. DEVELOPMENT OF RESISTANCE

Resistance to the antimicrobial effects of triclosan is mediated by multiple mechanisms. These mechanisms include target mutation (Fan et al. 2002; Heath et al. 2000; Heath et al. 1998), increased target expression (Slayden et al. 2000), enzymatic degradation (Meade et al. 2001), cellular exclusion (Bayston et al. 2007; Tabak et al. 2007), and active efflux from the cell (Braoudaki & Hilton 2005; Chuanchuen et al. 2003; McMurry et al. 1998a). Of these mechanisms, one of the most significant adaptations to triclosan exposure is the over expression of efflux pumps (Braoudaki & Hilton 2005; Levy 2002).

Efflux pumps are membrane proteins that actively transport a wide range of toxic substances out of the cell thereby preventing accumulation of these substances to toxic levels. They are an important nonspecific defense mechanism that can confer resistance to a number of substances toxic to the cell, including antibiotics. The wide spectrum of efflux pump substrates has prompted the concern that exposure to triclosan could also confer resistance to clinically important antibiotics (Fraise 2002; Gilbert & McBain 2001; Levy 2002; Russell 2002).

Laboratory studies in *Salmonella enterica* (Braoudaki & Hilton 2004; Randall et al. 2004), *Pseudomonas aeruginosa* (Chuanchuen et al. 2002; Karatzas et al. 2007), *Escherichia coli* O157 (Braoudaki & Hilton 2004), and *Staphylococcus aureus* (Brenwald & Fraise 2003) have produced variants with reduced susceptibility to both triclosan and to antibiotics. The significance of these laboratory findings is unclear because little evidence of cross resistance exists outside of the laboratory. In 1991 Cookson described transferable cross resistance to triclosan and mupirocin in a methicillin resistant *Staphylococcus aureus* (MRSA) (Cookson et al. 1991). In 2007 Beier et al. evaluated the antibiotic and antiseptic susceptibilities of vancomycin resistant *Enterococcus faecium* (VRE). Although the authors found no correlation between antibiotic resistance and antiseptic susceptibility, 92% of the 50 VRE isolates examined had a substantially elevated tolerance to triclosan and almost all were resistant to 14 antibiotics including 8 fluoroquinolones. A survey of triclosan sensitivities over a 10 year period found no relationship between triclosan and antibiotic resistance in MRSA and *Pseudomonas aeruginosa* (Lambert et al. 2002). Similarly, surveys of susceptibilities in domestic settings have not documented an association between triclosan and antibiotic resistance (Aiello et al. 2004; Cole et al. 2003). None of these studies, however, address the level of exposure to triclosan.

J. REGULATORY POSITION AND RECOMMENDED STUDIES

The U.S. Food & Drug Administration (FDA) first issued a notice on the need for toxicological data on triclosan in 1972 (FR 1972). The FDA considered the data that was available and in 1978 ruled that triclosan is a Category III product (insufficient information on the safety and effectiveness). The FDA has concluded (FR 1994) that triclosan is still a Category III product due to insufficient data on the dermal carcinogenicity potential of triclosan as a result of dermal application. The only dermal data that exist to date (90 day dermal rat study) showed dose-dependent dermal abnormalities which need subsequent study with a 2-year dermal carcinogenicity bioassay.

The FDA recommends that a properly designed dermal carcinogenicity study be conducted with triclosan to provide reliable data on the effects of long-term triclosan exposure. The primary reasons for these recommendations include: (1) high volume of dermal exposure to triclosan worldwide; (2) a significant level of exposure from various triclosan-containing products in all age groups for life-time duration; and (3) lack of published data on the effects of long term use of triclosan on carcinogenicity by the dermal route.

In addition, the FDA recommends that studies be conducted to address the phototoxicity of triclosan in light of (1) photoactivation to dioxin derivatives and (2) use on solar exposed skin.

Suggest deleting these tables unless really necessary-expect problem with making document section 508 compliant: I don't have any strong feelings about taking them out

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