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Occurrence, Efficacy, Metabolism, and Toxicity of Triclosan

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Triclosan has broad-spectrum anti-microbial activity against most gram-negative and gram-positive bacteria. It is widely used in personal care products, household items, medical devices, and clinical settings. Due to its extensive use, there is potential for humans in all age groups to receive life-time exposures to triclosan, and, indeed, triclosan has been detected in human tissues and the environment. Data gaps exist regarding the chronic dermal toxicity and carcinogenicity of triclosan, which is needed for the risk assessment of triclosan. The US Food and Drug Administration (FDA) nominated triclosan to the National Toxicology Program (NTP) for toxicological evaluations. Currently, the NTP is conducting several dermal toxicological studies to determine the carcinogenic potential of triclosan, evaluate its endocrine and developmental-reproductive effects, and investigate the potential UV-induced dermal formation of chlorinated phenols and dioxins of triclosan. This paper reviews data on the human exposure, environmental fate, efficacy of anti-microbial activity, absorption, distribution, metabolism and elimination, endocrine disrupting effects, and toxicity of triclosan.

Keywords: triclosan; metabolism; endocrine disruption effect; toxicity

INTRODUCTION

Triclosan is a synthetic, lipid-soluble, broad-spectrum anti-microbial agent that was first introduced in the health care industry in 1972 and in the toothpaste in Europe in 1985 (1). In the United States, triclosan has been used for

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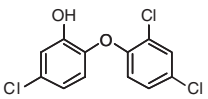
more than 40 years in personal care products, household items, medical devices, and hospitals to control the spread of bacteria (1). In 1997, the US Food and Drug Administration (FDA) approved triclosan (0.3%) for use in Colgate Total® toothpaste to prevent gingivitis and cavities. This paper reviews data on the human exposure, environmental fate, efficacy, absorption, distribution, metabolism and elimination, endocrine disrupting effects, and toxicity of triclosan. We will not discuss whether the benefits of using triclosan-containing products in personal care and household outweigh the potential hazards associated with this use, such as the emergence of antibiotic-resistant bacteria, because these issues are still the subject of ongoing scientific and public debate.

Triclosan is regulated by both the FDA and US Environmental Protection Agency (EPA). Within the FDA, triclosan is considered an over-the-counter drug for use in hand soaps, toothpaste, deodorants, laundry detergent, fabric softeners, facial tissues, antiseptics for wound care, and medical devices. Triclosan is currently registered with the EPA under the Federal Insecticide Fungicide and Rodenticide Act as an anti-microbial agent for the protection of polymers and plastics.

Chemical Identification

Molecular structure:



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; CH 3565, Irgasan CH 3565, Irgasan DP300, Ster-Zac, Tinosan AM110 Antimicrobial, Invasan DP 300R, Invasan DP 300 TEX, Irgaguard®B 1000, VIV-20, Irgacare MP, Lexol 300, Cloxifenolum, Aquasept, Gamophen, Vinyzene DP 7000, Vinyzene SB-30, Sanitized Brand, Microbanish R, Vikol THP, Ultra-Fresh, Microban Additive "B", and AerisGuard.
Formula: $C_{12}H_7Cl_3O_2$
Molecular weight: 289.54
Physical state: colorless to off-white crystalline powder
Ordor: slightly aromatic
Taste: tasteless
Specific gravity: 1.55×10^3 kg/m^3 at 22°C
Stability: stable under normal use conditions
Melting point: 55–57°C
Vapor pressure: 4×10^{-6} mm Hg at 20°C
Thermal decomposition (by DTA): 280–290°C
pKa = 7.9

Octanol-water partition constant (log K_{ow}): 4.76
Solubility: water, 0.01g/L; 0.1 N NaOH, 23.5 g/L; ethanol, acetone, propylene glycol, Tween 20, benzene, methylcellosolve, highly soluble (> 1,000 g/L)

HUMAN EXPOSURE TO TRICLOSAN

Because of its high anti-microbial effectiveness and the ease with which it is processed into solutions and solids, the popularity of triclosan has increased continuously over the past 40 years (2). The annual production of triclosan has risen dramatically from 0.01 to 0.5 million pounds in 1990 to > 1 to 10 million pounds in 1998 (Table 1). A total of 2,385 patents containing the word "triclosan" has been issued by the US Patent and Trademark Office between 1976 and April 2008 (www.uspto.gov). Triclosan is formulated as an anti-microbial active component in consumer care products, such as soaps, deodorants, toothpastes, and mouthwashes, in household cleaners, and even in textiles, such as sportswear, bed clothes, shoes, and carpets (3). Triclosan preparations are also used to control the spread of methicillin-resistant *Staphylococcus aureus* in clinical settings (1, 4, 5) and in surgical scrubs, pre-operative skin preparations, and sutures to prevent bacterial colonization of surgical wounds (4, 6). Due to its widespread use in consumer care products, household items, clinical settings, and medical devices, there is the potential for the general population to be exposed to triclosan through the ingestion or dermal contact with consumer products containing triclosan or through the consumption of food and drinking water contaminated with triclosan.

During the manufacture of triclosan, workers may be exposed by dermal contact and inhalation. Based on a National Occupational Exposure Survey conducted from 1981 to 1983, the US National Institute for Occupational Safety and Health estimated that 188,670 workers in 16 different industries are potentially exposed to triclosan in the United States (www.cdc.gov/noes).

Table 1: Production Volume History for Triclosan

Year	Million pounds per year*
2002	No reports
1998	>1–10
1994	>0.5–1
1990	0.01–0.5
1986	0.01–0.5
1977	>0.5–1

*Data obtained from the *Toxic Substances Control Act Chemical Substances Inventory* EPA: Washington DC; 2003.

The production and the widespread use of triclosan may result in it being disposed in sewage systems, which ultimately leads to environmental deposition. As a result, triclosan is found in finished drinking water, surface water, wastewater, and environmental sediments, as well as in the bile of wild fish, indicating extensive contamination of aquatic ecosystems (7–10). Triclosan has been detected in both raw and finished drinking water in Southern California, at levels of 56 and 49 ng per liter, respectively (7). Based on a US Geological Survey conducted from 1999 through 2000, triclosan was detected in 85 of 139 water samples from a network of streams across 30 US states (10). Triclosan was also found in lakes and in a river in Switzerland at concentrations of up to 74 ng per liter (9), and has been detected in bile samples of wild fish, caught at least 1 km downstream of three different wastewater treatment plants in Sweden, at concentrations ranging from 0.44 to 4.4 mg per kg fresh weight (11). In addition, methylated triclosan was present in fish and shellfish from the Tama River and Tokyo Bay in Japan (12) and in 45 samples corresponding to the influents and effluents from eight domestic wastewater treatment plants located along the Llobregat and Ebro rivers in Spain (13).

Triclosan has been identified in human breast milk. It was found in three of five randomly selected Swedish human milk samples, at concentrations ranging from <20 to 300 μg per kg lipid (11). The concentration of triclosan was 81 and 345 μg per kg lipid in two of four milk samples collected in 2007 from four anonymous lactating American women with no known occupational exposure to triclosan (14). Additionally, single samples of breast milk obtained from 62 women, who had donated milk in 2001 to the Mothers Milk Bank in San Jose, CA, and Austin, TX, have been analyzed for triclosan. Triclosan levels ranged from undetectable (two samples) and barely detectable (nine samples) to readily detectable at the levels between 100 to 2,100 μg per kg lipid (51 samples) (15).

Triclosan has been detected in a pooled plasma sample from 10 randomly selected male blood donors in Sweden (16). Sandborgh-Englund et al. (17) found triclosan in plasma at 0.1–8.1 ng per ml in 10 individuals, of which five were exposed and five were not exposed to triclosan via personal care products. Regardless of whether the nursing mothers used triclosan-containing soap, deodorant, or toothpaste, triclosan was detected in their plasma and breast milk (18). The levels, however, were higher in the mothers who used products containing triclosan (0.4–38.0 ng per g in plasma and 0.022–0.95 ng per g in milk) than in those who did not (0.01–19 ng per g in plasma and <0.018–0.35 ng per g in milk).

The excretion of triclosan in urine has been reported at rates of 0.1–743 μg per day from 10 randomly selected Swedish men (17) and at concentrations of 2.4–3,790 μg per liter of urine from a random selection of 2,517 participants 6 years of age and older from the US general population (19).

ENVIRONMENTAL FATE

Triclosan is readily chlorinated with sodium hypochlorite (20), and its chlorinated derivatives are also formed during the disinfection and deodorization of water supplies and wastewater with sodium hypochlorite (21). Triclosan is converted to di- and trichlorodibenzo-*p*-dioxin upon heating to temperatures greater than 400°C to simulate incineration (22). Bleaching fabrics containing triclosan with sodium hypochlorite, followed by combustion, leads to the formation of di-, tri-, and tetrachlorodibenzo-*p*-dioxins (22).

Photodegradation appears to be one of the major routes of elimination of triclosan in aquatic environments (8, 23) and takes place at low light intensity under UV (254, 313, or 365 nm) light, simulated solar light, or artificial white light. Triclosan photodegradation with UV (365 nm) and simulated solar light irradiation exhibits first-order kinetic behavior, with both light sources giving similar kinetic parameters (24). The photodecomposition products of triclosan are illustrated in Figure 1. The photochemical formation of dichlorodibenzodioxin from triclosan in both solid phase and thin films of triclosan has been demonstrated (20, 25). More recently, several highly toxic photoproducts, including 2,8-dichlorodibenzo-*p*-dioxin (23, 24, 26–32), 2,4-dichlorophenol (24, 26, 28, 29), and possibly dichlorohydroxydibenzofuran (24, 29), were identified in water samples. The photodegradation of triclosan and formation of 2,8-dichlorodibenzo-*p*-dioxin occur over a wide range of pH levels (3.0–9.0), with the rate of formation being faster at basic pH (24, 29).

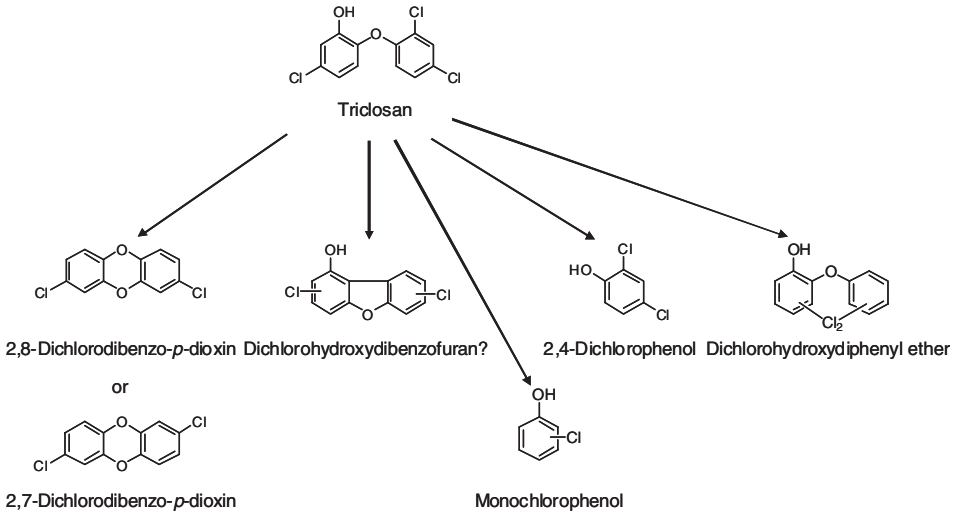


Figure 1: Simplified schematic of triclosan photodecomposition.

EFFICACY OF TRICLOSAN ON ANTI-MICROBIAL ACTIVITY

Mechanism of Action

Triclosan has been shown to intercalate into bacterial cell membranes and disrupt membrane activities, without causing leakage of intracellular components (33, 34). In addition, triclosan is an inhibitor of the enoyl-reductase of type II fatty acid synthase involved in the bacterial lipid biosynthesis (35–38).

At low doses, triclosan is bacteriostatic and, at higher doses, it becomes bactericidal (39, 40). Sub-lethal concentrations of triclosan favor a specific action against type II fatty acid synthase enoyl-reductase (FabI), while at bactericidal concentrations triclosan appears to act against multiple targets, including less specific targets such as the cell membrane (40). Triclosan also has anti-viral, anti-fungal (1), and anti-malarial activity (41).

While triclosan has in vitro activity against a broad spectrum of bacteria, it is generally more effective against gram-positive than gram-negative bacteria (1, 42). Triclosan is particularly effective against *Staphylococcus aureus* (1, 42, 43). However, some clinical isolates of *Staphylococcus aureus* are not as susceptible to triclosan due to the overexpression of FabI (44). In addition to its overexpression, the FabI from these isolates carries a single amino acid change that prevents the formation of a stable triclosan-NAD^+-FabI complex (44). In gram-negative bacteria, such as *Pseudomonas aeruginosa,* there are several multi-drug efflux pumps that remove a number of drugs, including triclosan, from the cells (45, 46). In addition, some strains of a *Pseudomonas aeruginosa* contain a triclosan-resistant enoyl-acyl carrier protein reductase FabV (47).

In Soaps

The efficacy of triclosan in soaps is equivocal. 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 (48). A review of studies on the efficacy of triclosan in soap, however, revealed that it does not reduce bacterial counts on hands to a greater extent than plain soap unless it is used repeatedly and at relatively high concentrations ($\geq$1%) compared to the 0.1–0.45% found in consumer anti-bacterial soaps (49).

There have been few appropriately designed studies to assess the impact of the use of triclosan-containing products on infection rates. One randomized double-blind study evaluated the effect of the use of the anti-microbial products by consumers on the occurrence of infectious disease symptoms over a one-year period in 228 inner-city households. The study found no statistical difference between use of "anti-microbial" household cleaners, detergents, and hand washes compared to the use of identically packaged products lacking

anti-microbial ingredients (50). This study used several types of anti-microbial household cleaners, such as a liquid hand washing soap containing triclosan, a liquid kitchen spray, an "all-purpose" hard-surface cleaner containing a quaternary-ammonium compound, and a laundry detergent containing oxygenated bleach, and focused primarily on symptoms consistent with a viral illness as a measure of efficacy.

Studies using bacterial counts to determine the efficacy of triclosan-containing soaps have produced conflicting results. An epidemiological study of household use over 11 months found that a hand washing soap containing 0.2% triclosan was not significantly better at reducing bacterial levels on hands than a similar plain hand washing soap without triclosan (51). When overall bacterial counts were used to determine the efficacy of soaps containing triclosan, those with less than 1% triclosan were not significantly more effective than plain soap (49), except in 2 studies, one in which 0.3% triclosan soap was used 18 times daily for 5 days (52), and the other in which 0.75% triclosan was used in 2-min hand washes 3 times daily for 2 days (53). When 1% triclosan soaps were compared to plain soap using bacterial counts as the measure of efficacy, one study found no significant difference when hands were washed using a standard surgical technique (54), but another study found that 1% triclosan significantly reduced bacterial counts when hands were washed for 30 sec or for 3 min (55).

In studies employing artificial contamination of hands with *Serratia marcescens*, 10 hand washings for 10 sec each using 1.0% triclosan soap (56) or as little as one hand washing with 1.5% triclosan soap (57) reduced bacterial counts significantly more than washing with plain soaps, as measured by a $\log_{10}$ bacterial reduction. In a study using artificial contamination of fingertips with *Escherichia coli*, when hands were washed for 30 sec with 1.5% or 2.0% triclosan soaps, reduction of bacterial counts was not significantly greater than when plain soap was used (58). A soap containing 2.0% triclosan exhibited residual anti-microbial activity on the forearm skin of 20 volunteers for up to at least 2 hr after three applications, as compared to plain soap (59).

In Deodorants

When used ad libitum for 6 months, deodorant sprays containing 0.15% triclosan and anti-perspirant deodorant sprays containing 0.25% triclosan reduced bacterial counts per cm^2 of skin from 5.2×10^5 to 1.4×10^3 and 3.74×10^2, respectively (60). Bacterial levels returned to pre-test levels within 4 to 7 days after stopping the use of triclosan-containing deodorants (60).

In Dentifrices

There have been a number of reports of the anti-plaque and anti-gingivitis efficacy of toothpaste containing triclosan (61–66). Volpe et al. (61) reviewed

the caries-preventive benefits of a triclosan/copolymer/fluoride dentifrice from 13 independent, double-blind studies. Individuals using a dentifrice containing triclosan/copolymer/fluoride had, on average, 27% less plaque ($p < 0.01$) than subjects using a fluoride dentifrice. The improved level of plaque control was accompanied by a 57% reduction in gingivitis severity index ($p < 0.01$) (61). Recently, a double-blind clinical study also demonstrated that a single evening's use of a dentifrice containing 0.3% triclosan/2.0% polyvinylmethyl ether/maleic acid copolymer/0.243% sodium fluoride in a 17% dual silica base provided a 28.4% reduction in oral malodor scores ($p < 0.05$) and 49.5% reduction in microbial colony forming unit scores ($p < 0.05$) when compared to a commercially available dentifrice containing 0.243% sodium fluoride in a silica base (63). When triclosan (0.3%) formulated with 2.0% Gantrez® in dentifrices, there was effective anti-plaque and anti-gingivitis activity (65). Dentifrices with combinations of triclosan and soluble pyrophosphate or zinc citrate, however, were not effective against plaque and gingivitis (65). Following the use of toothpaste containing triclosan, approximately 36% of the triclosan dose was retained in the saliva and bacterial plaque (67, 68).

In Sutures

Sutures impregnated with triclosan have efficacy in vitro against gram-positive and gram-negative bacteria, including isolates that are methicillin resistant (6). An in vivo challenge test was conducted to mimic a clinically relevant environment. In this assay, control and test sutures were implanted subcutaneously in the dorsal-lateral regions (control on the left side, test on the right side) of the same animal, and inoculated with a known number of bacteria. After a direct in vivo challenge, sutures with triclosan produced a nearly 3-log reduction in the growth of *Staphylococcus aureus* in guinea pigs and a 1-log reduction in the growth of *E. coli* in mice compared to control sutures (6, 69).

In Plastics

There is no clear evidence available to demonstrate the efficacy of triclosan as an anti-microbial agent when combined with plastics. A low-density polyethylene film containing triclosan (1 g triclosan per kg polyethylene film) had a strong anti-microbial effect in in vitro simulated vacuum-packaged conditions against the psychrotrophic food pathogen *L. monocytogenes*, but did not effectively reduce spoilage bacteria or the growth of *L. monocytogenes* on refrigerated vacuum-packaged chicken breasts stored at 7°C (70). A plastic wrap incorporating 1,500 ppm of triclosan did not effectively reduce bacterial numbers on refrigerated and vacuum packed meat surfaces (71). Triclosan-incorporated plastics desorbed insufficient amounts of triclosan to inhibit bacterial growth

when used in cutting boards (72). An anti-bacterial toothbrush containing triclosan-coated tufts also failed to inhibit the bacterial growth when compared to the regular toothbrush without the coated tufts (73).

ABSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION

Triclosan reaches the systemic circulation via absorption through the mucous membranes of the oral cavity (74) and gastrointestinal tract after oral exposure (17, 74, 75), through the skin after dermal exposure (76, 77), and through mucosal tissues following intra-vaginal administration (78).

Several studies have reported the blood levels of total triclosan in humans following use in either mouth rinses or dentifrices (17, 74, 75, 79). After 10 healthy volunteers were exposed to a single dose of 4 mg triclosan by swallowing an oral mouthwash solution, the triclosan levels in plasma increased rapidly from a median baseline of 0.4 ng per ml to a maximum concentration of 218 ng per ml within 1 to 3 hrs (17). In another study, nine subjects who ingested 20 ml of a 0.01% triclosan (2 mg) aqueous solution twice daily for 21 days had blood triclosan levels between 150 and 174 ng per ml at 4 hrs after the morning dose. Nine other subjects who brushed twice daily with 1 g of dentifrice containing 0.2% triclosan (2 mg) had blood levels between 15 and 21 ng per ml, which was approximately 9%–14% of that from subjects who ingested an equivalent dose level of the aqueous solution (75). Measurable amounts of triclosan appeared in the plasma of 21 healthy individuals at 15 min after a single tooth brushing plus full ingestion of 1.25 g of dentifrice containing 0.3% triclosan. The peak plasma triclosan concentration occurred at 2–6 hrs after the dosing, and the mean peak plasma triclosan concentration was 243 ng per ml. When this application was repeated three times daily for 12 days, the mean plasma triclosan concentrations ranged between 252 and 402 ng per ml, with an overall mean of 352 ng per ml (75). Other studies using a dentifrice containing triclosan at a concentration of 0.2%, 0.3%, or 0.6% daily for 2–12 weeks resulted in the blood levels of triclosan between 16 and 25 ng per ml (79). 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 μg per ml, and it was estimated that about 2%–4% of the daily triclosan dose (9.0 mg) was absorbed and circulated in the blood (74). When absorbed through the oral mucous membrane from twice daily oral rinse with a mouth rinse containing 0.03% triclosan for 21 days, triclosan levels in human blood plasma returned to baseline approximately 8 days following the final exposure (74), and no apparent accumulation of triclosan in blood was observed (17, 74).

Dermal absorption of triclosan in humans and animals has been reported (79–82). Triclosan was detected in blood (0–189 ng per ml) and urine (0–5,600 ng per ml) following daily use of a dermal spray containing triclosan by 4 healthy males for 4 weeks or a soap bar containing 1% triclosan for

bathing and showering by 25 leukemic patients for 4 weeks and 6 healthy males for 48 weeks.

There is evidence that triclosan can be absorbed through the intact skin of guinea pigs (80). In another study using ddY mice, Kanetoshi et al. (81) applied 50 μl (32 mg triclosan per ml) [^{3}H]triclosan (in ethanol: olive oil) to the mouse skin and quantified the absorption 6, 12, and 18 hrs later. Maximum levels of [^{3}H]triclosan occurred between 12 and 18 hrs, with the greatest concentrations in the gall bladder, liver, body fat, lungs, kidneys, blood, heart, testes, spleen, and brain. The levels in the tissues were approximately 14%–67% the levels achieved in a comparable study where [^{3}H]triclosan was given orally (82).

In in vitro studies, triclosan was shown to penetrate rat skin more rapidly and extensively than human skin. Twenty-three percent of the dose penetrated completely through rat skin into receptor fluid by 24 hrs, whereas penetration through human skin was only 6.3% of the applied dose (77) or about 0.7% of the dose administered in a transdermal adhesive formulation patch model (76).

A shampoo containing 0.05% (w/v) [^{3}H]triclosan or an aerosol deodorant containing 0.1% (w/v) [^{3}H]triclosan was applied to Wistar rat skin in a manner similar to consumer use, and the dermal penetration was calculated from the amount of radioactivity excreted by the rats. The penetration of triclosan from shampoo was 197 ng per cm^2 (3.3% of the applied amount) compared to 6.85 μg per cm^2 from the aerosol deodorant (35.3% of the applied dose). These data indicate that the composition and mode of use of different products containing triclosan may be very important in determining the extent of penetration (83).

Triclosan is readily metabolized to glucuronide and sulfate conjugates (Figure 2). The conjugation of triclosan in the presence of human liver microsomes or cytosol (84) and in skin (77) was demonstrated in vitro. Dermal metabolism of triclosan in diffusion cells fitted with human skin following application of 7 μl of 64.5 mM [^{3}H]triclosan showed that triclosan sulfate was the only metabolite in the skin at 4 hrs after application, whereas both the sulfate and glucuronide were present at 8 and 24 hrs after application (77). At all times, there was more unchanged triclosan than either of the conjugates. Similar results were found in diffusion cells with rat skin (77). Tulp et al. (85) reported that both aromatic hydroxylation and cleavage of the ether bond of triclosan occurred in Wistar rats, as indicated by the presence of monohydroxylated triclosan, 2,4-dichlorophenol, and 4-chlorocatechol in the urine and feces, after oral administration a single dose of 500 mg triclosan per kg body weight (bw) (Figure 2). Although triclosan is readily photodegraded into dichlorodibenzo-*p*-dioxin by heat and UV irradiation (20, 22, 23, 28, 31), there have been no studies to investigate the metabolic formation of dichlorodibenzo-*p*-dioxin or chlorodibenzofurans on the skin in vivo.

Triclosan is excreted in the feces and urine. Rats and mice show predominantly biliary excretion into the feces, whereas guinea pigs excrete the majority of the dose via the kidney. In humans, urinary excretion is the major route

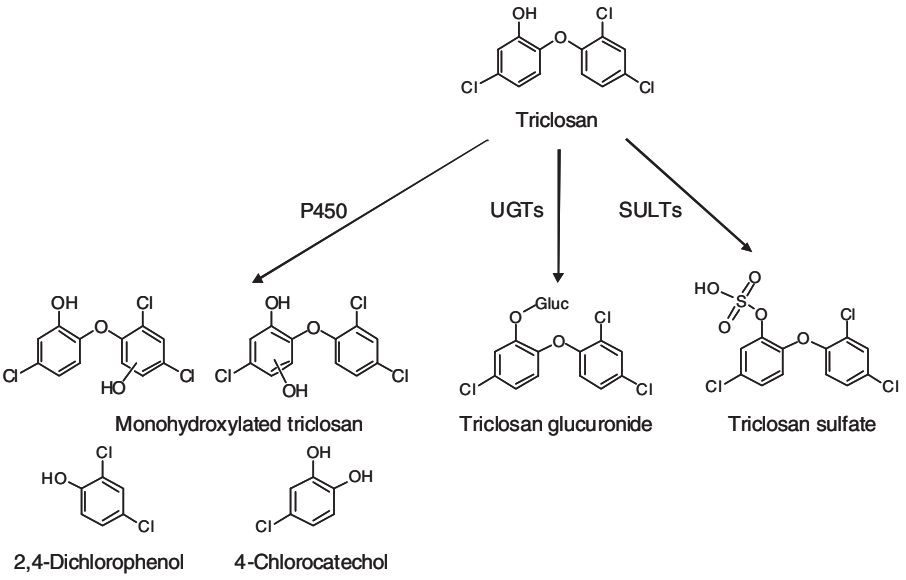


Figure 2: Simplified schematic of triclosan metabolism. P450; Cytochrome P450; UGTs; UDP-glucuronosyltransferases; SULTs; Sulfotranserases.

of elimination, with fecal elimination being the secondary route (17, 75, 79). Administration of a [^{14}C]triclosan soap solution to rats at 400 mg triclosan per kg bw using an occluded dermal patch resulted in 14.7% and 0.5% of the dose being excreted in the feces and urine, respectively (79). After topical application of a 100 μl (18.7 mg triclosan per ml) alcoholic solution of [^{3}H]triclosan to the skin of CD rats, 12% radioactivity was recovered in the feces, 8% in the carcass, 1% in the urine, 30% in the stratum corneum, and 26% was rinsed from the skin surface at 24 hrs after application (77). In humans, the urinary excretion of triclosan increased after exposure, and the major fraction was excreted within the first 24 hrs. Between 24% and 83% (median 54%) of the original 4 mg dose was excreted within the first 4 days, and the excretion approached baseline levels (0.1–91 μg per day) within 8 days after exposure (17). Triclosan was excreted predominantly as its conjugates (75, 77, 79, 80), with the only exception being a study by Tulp et al. (85), who showed that triclosan was present in the urine and feces essentially unchanged, with only limited evidence of conjugation. No triclosan was present in the expired breath of guinea pigs and rats (80).

The pharmacokinetics of triclosan has been studied in experimental animals (76, 78–82, 85) and is consistent with a two-compartment open-system model (17, 78, 85). After oral administration, the apparent volume of distribution was 42% of the body weight, which is more than the extracellular water, suggesting a rapid transfer of triclosan from blood to tissues (78). The blood

half-life ($t_{1/2}$) of triclosan following intraperitoneal injection to guinea pigs and rats was 13 and 18 hrs, respectively (83), whereas the blood $t_{1/2}$ of triclosan during the β-phase was 8.8 hrs after intravenous administration of 5 mg triclosan per kg bw to Wistar rats (78). Oral intubation of 5 mg triclosan per kg bw in a 0.66% triclosan sodium lauryl sulfate solution or 0.2% triclosan toothpaste to rats gave a $t_{1/2}$ of 7–65 hrs for triclosan and its metabolites (glucuronide and sulfate conjugates) (79). After oral administration, the $t_{1/2}$ of triclosan in ddY mouse liver was approximately 8 hrs (82). The $t_{1/2}$ terminal blood/plasma concentration phase for humans ranged between 6 and 63 hrs (17, 75). Triclosan does not appear to sequester into a long-term compartment (e.g., fat) in the body following single or repeated administration (74, 75, 79).

Due to its structural resemblance to polychlorinated biphenyls, triclosan has been proposed to affect the hepatic mixed function oxidase system. Triclosan has been shown to increase the activities of aminopyrine *N*-demethylase, biphenyl 2-hydroxylase, biphenyl 4-hydroxylase, *p*-nitroanisole *O*-demethylase, *p*-nitrophenetole *O*-deethylase, and 7-ethyoxycoumarin *O*-deethylase in male Wister rats (81, 86). The increase in the activities of these P450-dependent monooxygenases was associated with an elevation of cytochrome P450 content (81) and the induction of cytochromes CYP2B1/2, 3A2/1, and 4A1 (86). In male ddY mice, only aminopyrine *N*-demethylase activity was induced by triclosan (81). A subsequent study using rat hepatocytes cultured on Matrigel demonstrated that triclosan preferably induced cytochromes CYP2B1/2, along with a slight increase in CYP3A (87). The study also showed that triclosan produced an accumulation of hydroxymethylbilane, and consequently uroporphyrin I, in rat hepatocytes by inhibiting uroporphyrinogen III synthetase (87). Recently, triclosan was shown to be a selective inhibitor of the glucuronidation and sulfonation of phenolic xenobiotics in human liver preparations in vitro (84).

Triclosan has been demonstrated to be a slow binding inhibitor of human and goose type I fatty acid synthase and to inhibit partially enoyl-reductase activity type I fatty acid synthase with IC_{50} values between 10 and 50 μM (88). Triclosan, at similar concentrations, also inhibited cell growth of MCF-7 and SK-BR-3 human breast cancer cells (88).

ENDOCRINE DISRUPTOR EFFECTS

Triclosan has been associated with endocrine disruption effects. In one study, triclosan was weakly androgenic, causing changes in the fin length and sex ratio of Japanese Medaka fish when exposure started at 2 days post-hatching for 14 days (89), while in another study, triclosan was toxic and a weak estrogen, with the potential to induce vitellogenin in male Medaka (90). In male frogs, intraperitoneal injection of triclosan (4, 40, and 400 μg per g bw) resulted in no significant reduction in the levels of plasma vitellogenin or testosterone (91).

Gee et al. (92) demonstrated that triclosan possesses intrinsic estrogenic and androgenic activity. In terms of estrogenic activity, triclosan could displace estradiol from estrogen receptors of MCF-7 human breast cancer cells and from recombinant human ERα/ERβ. Triclosan, at 10 μM, completely inhibited the induction of an androgen-responsive ERE-CAT reporter gene in MCF7 cells by 10^{-4} μM 17β-estradiol and the stimulation of growth of MCF-7 human breast cancer cells by 10^{-4} μM 17β-estradiol. Triclosan, by itself (1.0 μM), increased the growth of MCF-7 cells over 21 days. With regard to androgenic activity, triclosan displaced testosterone from binding to the ligand-binding domain of the rat androgen receptor. Triclosan (0.1 μM) was able to inhibit the induction of an androgen-responsive LTR-CAT reporter gene in S115+A mouse mammary tumor cells by 10^{-3} μM testosterone; 1.0 μM triclosan also inhibited the induction of the same reporter gene in T47D human breast cancer cells by 10^{-2} μM testosterone. Triclosan, at 20 μM, antagonized the stimulation of the growth of S115+A mouse mammary tumor cells by 10^{-3} μM testosterone (92). Studies conducted in rats revealed that triclosan exposure did not alter androgen-dependent tissue weights or the onset of preputial separation (93).

In North American bullfrogs, triclosan exposure during the premetamorphic stage altered the rate of triiodothyronine-induced metamorphosis and thyroid hormone receptor mRNA expression (94). In a 4-day oral study using weanling female rats, a dose-dependent decrease in serum thyroxine was observed (at 100 mg triclosan per kg bw and higher), with a no-observable-effect-level of 30 mg triclosan per kg bw per day (95). In a similar study using weanling male rats exposed to triclosan by gavage from postnatal days 23 to 53, triclosan significantly decreased total serum thyroxine in a dose-dependent manner at 30 mg triclosan per kg bw and higher; triiodothyronine was deceased only at 200 mg triclosan per kg bw, and thyroid-stimulating hormone was not affected at any dose of triclosan (93). The decrease in circulating thyroxine was associated with an up-regulation of hepatic catabolism (96).

TOXICITY

Acute Toxicity

Triclosan demonstrated a high threshold for severe toxicity in acute studies. The LD_{50} following oral administration is from 3,750 to >5,000 mg triclosan per kg bw in rats, 4,350 mg triclosan per kg bw in mice, and >5,000 mg triclosan per kg bw in dogs (42, 79, 97). The acute LD_{50} in neonatal rats is 580 mg triclosan per kg bw, which is lower than that for adult rats (79). The route of administration has a significant influence on the toxicity of triclosan. Intravenous administration displayed a greater degree of toxicity, with an LD_{50} of 19 mg triclosan per kg bw in mice and 29 mg triclosan per kg bw in rats (79, 97), while intraperitoneal injection led to LD_{50} values ranging from

184 to 1,090 mg triclosan per kg bw in mice (81, 98). The dermal LD_{50} of triclosan applied to rabbit skin as a slurry in propylene glycol under an occluded patch was ≥9,300 mg triclosan per kg bw (97). Subcutaneous administration of triclosan at ethanol led to an LD_{50} of 14,700 mg triclosan per kg bw in rats (97). Body powders and soaps containing triclosan have been formulated as aqueous slurries and administered orally to rats (8 to > 250 mg triclosan per kg bw). No deaths were recorded (79). Likewise, there has been no mortality when body lotions and shower gels containing triclosan have been administered orally to rats (79).

Subchronic Toxicity

Several human safety studies have been conducted in a total of 1,246 volunteers using dental products (toothpaste, mouth rinses, or aqueous slurry) containing triclosan at concentrations ranging from 0.01% to 0.6%, for durations of <1 week to >12 weeks. No adverse effects were noted at any time period, in any product, or at any triclosan concentration (79). In several studies, pre- and post-treatment blood chemistry tests for liver and kidney function as well as hematological measurements were conducted. There was no difference between the control and treated populations (79).

The subchronic toxicity of triclosan has been investigated in rats, rabbits, dogs, and baboons, with time frames ranging from 3 days to 52 weeks, using both oral and dermal dosing (42, 79, 97). In general, toxicity was more evident following oral intubation or when administered by capsule than when mixing triclosan in the diet. The administration of triclosan in the diet to rabbits (125 mg triclosan per kg bw per day) and beagle dogs (25 mg triclosan per kg bw per day) for 13 weeks caused no symptoms or pathological changes. When given in similar fashion to male rats at 150 and 300 mg triclosan per kg bw per day, hepatic and hematopoietic changes were observed (42, 79). Oral intubation of triclosan to rabbits for 13 weeks at 30 and 150 mg triclosan per kg bw per day induced mortality and hematologic changes. Baboons receiving up to 300 mg triclosan per kg bw daily by oral capsule for 52 weeks showed no pathological findings, although emesis and diarrhea were reported at 100 and 300 mg triclosan per kg bw per day. Administration of triclosan in capsules to 5-month-old dogs for 13 weeks induced hepatic morphologic and functional changes at 25, 50, and 100 mg triclosan per kg bw per day and nephric and hematopoietic dysfunction at 100 and 200 mg triclosan per kg bw per day (42, 79).

DeSalva et al. (79) reviewed the potential toxic effects of the subchronic dermal administration of triclosan. There were four such dermal toxicity studies in rats, three in rabbits, and one in 11-day-old beagle pups. Dermal application did not induce systemic toxicity, although skin irritation was observed in one of the rabbit studies at the doses of 15 and 30 mg triclosan per kg

bw per day (42, 79). In addition, the manufacturer of triclosan (BASF; formerly Ciba) conducted two 14-day dermal toxicity studies in CD1 mice (99, 100) and one 14-day dermal toxicity study in Cr1:CD®BR rats (101) where triclosan was applied at doses of 0, 0.3, 0.6, 1.5, 3.0, or 6.0 mg triclosan per animal per day in propylene glycol (99) or acetone (100, 101). There were no dose-related clinical signs of toxicity in any of the studies; however, in both mice and rats, high doses of triclosan induced severe skin effects, including eschar and/or fissuring. Ulceration was also noted in mice. No-observable-adverse-effect levels for 14 daily dermal doses of triclosan were 0.3 mg triclosan per day for mice, 0.6 mg triclosan per day for female rats, and 1.5 mg triclosan per day for male rats (99–101).

In a 90-day dermal toxicity study conducted by Colgate-Palmolive in 1998, triclosan was applied at doses of 0, 10, 40, or 80 mg triclosan per kg bw per day in propylene glycol for at least 6 hrs under gauze. There were no treatment related systemic effects, although dermal erythema and/or edema were observed in all treatment groups, especially in the high-dose group. Histopathological examinations indicated the presence of eschar and desquamation, hyperplasia/hyperkeratosis of epidermis, dermal inflammation, and focal necrosis at the treated site among treated animals (102). The effects of chronic dermal exposure (i.e., 2 years) to triclosan are currently not known.

Skin Sensitization

Triclosan is considered to have a low skin sensitizing potential. Skin sensitization tests have been conducted in guinea pigs, using several modalities, such as intracutaneous injection of 0.1% triclosan in physiological saline and gum Arabica according to the method of Draize (103); intradermal injection of 0.1 ml of 1% triclosan in 5% polyethylene glycol three times per week for a total of 10 injections followed by a challenge injection 2 weeks later; and dermal application of 50 ppm triclosan in a water-isopropanol mixture for 6 days per week for a period of 3 weeks followed by dermal challenge 3 weeks later. In all cases, there was no consistent triclosan-related sensitization (97). When the split adjuvant technique of Maguire (104) was used, 1 of 20 guinea pigs was sensitized by the triclosan treatment (105).

Triclosan presented no evidence for skin sensitization in male human subjects tested using the Draize test at concentrations up to 20% of triclosan (106). Skin sensitization was not observed in 20 human subjects who were assessed using the maximization test of Kligman and Epstein (107). Consumer dermatological products containing triclosan have been tested in human volunteers. Application of body powder (0.1% triclosan), body lotion (0.1% triclosan), or soap (0.1%–0.25% triclosan), formulated as slurry or solution, to the skin of human volunteers did not induce skin sensitization using a repeated insult patch test or prophetic patch test (79). Several other studies conducted in human

volunteers also indicated that triclosan was not a sensitizer (97). In addition, triclosan was neither a phototoxicant nor a photosensitizing agent (97, 106).

There have been rare reports of contact dermatitis from triclosan-containing formulations. Two patients were reported to have allergic contact dermatitis, one caused by a deodorant foot-powder containing 0.2% triclosan and the other by a deodorant stick containing 0.12% triclosan (108). Both individuals had positive patch tests to triclosan. Shortly afterward, a similar case was documented (109). In addition, two patients were positive to triclosan after routine patch testing among 1,100 patients (110). In 1986, three cases of contact dermatitis were reported from exposure to 2.0% triclosan in petrolatum after previous use of Logomel, a steroid/anti-microbial cream containing 3.0% triclosan (111). In a recent study of 103 patients patch and photopatch tested with 2% triclosan in petrolatum, three had allergic contact reactions and none had photoallergic reactions (112).

Reproductive/Developmental Toxicity

A two-generation reproduction study has been reported in rats and four developmental studies have been conducted in mice, rabbits, and rats. All five studies were sponsored by triclosan manufacturers. The two-generational reproduction study in rats was conducted at doses of 0, 300, 1,000, and 3,000 ppm triclosan in the diet (equivalent to 0, 15, 50, and 150 mg triclosan per kg bw per day). There were no adverse effects on reproduction activity at any dose tested, although neonatal toxicity, which was indicated by reductions in survival in the F1 and F2 litters and a slight increase in the incidence of dilated kidneys, occurred in litters of dams administered 3,000 ppm triclosan (79).

Triclosan was found to have no teratologic effects in mice or rats (50 and 100 mg triclosan per kg bw per day) or rabbits (10, 25, 50, and 100 mg triclosan per kg bw per day) following oral administration during organogenesis. Oral administration of triclosan to pregnant mice (gestation days 1 to 16) resulted in maternal and fetal toxicity as represented by the death of dams, a reduction in the litter size, and a decrease in pup weights at 50 and 100 mg triclosan per kg bw per day (79).

Genotoxicity and Mutagenicity

There have been 18 independent studies to assess the mutagenic potential of triclosan (79). These studies included in vitro test systems (bacterial reverse mutation assays, genetic mutation in yeast, gene mutation in mouse lymphoma cells, chromosomal aberration test in Chinese hamster bone marrow cells, and sex-linked recessive lethal test in *Drosophila melanogaster*) and in vivo assays (mouse-dominant lethal and spot tests, chromosomal aberration

test in Chinese hamster, micronucleus test in Chinese hamster bone marrow cells, and chromosomal aberration test in mouse germinal epithelium). Sixteen of these studies showed no evidence of mutagenicity. Of the two positive tests, one was a weak positive, which could not be replicated. In a mouse in vivo somatic mutation test (mammalian spot test), a positive response was seen at a dose of 50 mg triclosan per kg bw, which could not be reproduced in a subsequent study by other investigators (113). The Comet assay has indicated that triclosan can lead to dose-dependent DNA damage in *Closterium ehrenbergii;* 0.86 μM triclosan caused significant genotoxic effects and higher concentrations resulted in irreversibly altered the DNA strands (114). More recently, the genotoxicity of triclosan was evaluated using the somatic mutation and recombination test in *Drosophila melanogaster*, using flies with normal bioactivation (a standard cross) and flies with increased cytochrome P450-dependent biotransformation capacity (a high bioactivation cross). In this assay, triclosan produced negative responses in both types of flies (115). Taken together, the preponderance of data indicates triclosan is neither genotoxic nor mutagenic.

Chronic Toxicity/Carcinogenicity

The chronic oral toxicity/carcinogenicity of triclosan has been studied in rats, mice, and hamsters. Recently, Rodricks et al. (116) summarized the results and indicated that there was a significant increase in hepatocellular adenomas and carcinomas in mice, but not in rats or hamsters. These studies were conducted by the triclosan industry to support the chronic oral use of marketed products under the new drug application path and the over-the-counter monograph path. While the conducted studies were deemed to be appropriate for the oral route of administration, the impact of chronic dermal route of exposure is not yet known.

Lyman and Furia (97) summarized an 18-month dermal carcinogenicity study in Swiss white mice. The study consisted of five groups: an untreated control group, a vehicle control group using acetone, a positive control group using 0.01% 7,12-dimethylbenz[*a*]anthracene in acetone, and two test groups treated with 0.5 or 1% triclosan in acetone. One hundred microliters of the test solutions were applied to the shaved intrascapular region of mice 3 times weekly. The positive control group exhibited an increased mortality and all the mice had skin squamous cell carcinomas varying in the extent of differentiation as well as severe erythema, eschar formation, and edema at the site of the tumor development. In addition to these findings, there were changes in body weight, food consumption, behavior, skin reactions, mortality, gross and microscopic pathology, and tumor formation in all groups, which compromised interpretation of the results (97). As such, a properly designed and conducted dermal carcinogenicity study is still needed.

REGULATORY POSITION

Under the monograph rulemaking, the FDA first issued a notice on the need for toxicological data on triclosan in 1972 (117). Upon reviewing the available data in 1978, the FDA classified triclosan as a Category III product (insufficient information on the safety and effectiveness). As of today, triclosan is still a Category III product due to insufficient data on the dermal carcinogenicity potential of triclosan as a result of dermal application (118).

The FDA has recommended that a properly designed dermal carcinogenicity study be conducted with triclosan to provide reliable data on the effects of long-term dermal triclosan exposure. The primary reasons for this recommendation include: (a) the high volume of dermal exposure to triclosan worldwide; (b) a significant level of exposure from various triclosan-containing products in all age groups resulting in life-time durations of exposure; and (c) the lack of published data on the carcinogenic effects of long-term use of triclosan by the dermal route. In addition, the FDA has recommended that studies be conducted to address the phototoxicity of triclosan because of (a) the photoactivation to dioxin derivatives and (b) the use of triclosan on solar exposed skin.

In order to obtain dermal toxicity data on triclosan, the NTP is currently conducting several dermal toxicological studies to determine the carcinogenic potential of triclosan, evaluate its endocrine and developmental-reproductive effects, and investigate the potential UV-induced formation of chlorinated phenols and dioxins of triclosan on skin.

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