Final Report

Triclosan

December 14, 2010

The 2010 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A Hill, Ph.D. James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by F. Alan Andersen, Ph.D., Director.

1101 17th Street, NW, Suite 412 ♢ Washington, DC 20036-4702 ♢ ph 202.331.0651 ♢ fax 202.331.0088 ♢
cirinfo@cir-safety.org

ABSTRACT

The available data relevant to the safety of the chlorinated aromatic compound, triclosan, were reviewed by the Cosmetic Ingredient Review Expert Panel. In cosmetics, triclosan functions as a cosmetic biocide, preservative, or deodorant agent. A wide variety of studies addressing purity, stability, general toxicity, carcinogenesis, endocrine disruption, and antimicrobial resistance were reviewed. The Panel concluded that triclosan was safe as a cosmetic ingredient in the present practices of use and concentration of this safety assessment, even were all products types to contain triclosan and used concurrently, on a daily basis.

Introduction

Triclosan[1] is a chlorinated aromatic compound with both phenolic and ether structural moieties.

CIR has relied extensively on triclosan reviews available from various governmental sources as an alternative strategy to summarizing the large volume of original literature.[1-6] Many of these reviews are available on the internet as shown below.

- European Centre for Disease Prevention and Control (ECDC), European Food Safety Authority (EFSA), European Medicines Agency (EMEA), Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). "Joint Opinion on Antimicrobial Resistance (AMR) Focused on Zoonotic Infections." October 2009[1].
  http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_026.pdf

- European Commission Directorate-General for Health & Consumers. Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). Assessment of the Antibiotic Resistance Effects of Biocides. January 2009[2].
  http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_021.pdf

- Australian Government Department of Health and Ageing (NICNAS). Priority Existing Chemical Assessment Report No. 30 – Triclosan. January 2009[3].
  http://www.nicnas.gov.au/Publications/CAR/PEC/Drafts/Triclosan.asp .

- United States Environmental Protection Agency (EPA). Office of Prevention, Pesticides and Toxic Substances. Reregistration Eligibility Decision (RED) for Triclosan, List B, Case No. 2340. EPA 739-RO-8009. September 2008[4]. http://www.epa.gov/oppsrrd1/REDs/2340red.pdf

- National Toxicology Program. FDA Nomination Profile - Triclosan [CAS 3380-34-5]. Supporting Information for Toxicological Evaluation by the National Toxicology Program. July 2008[5].
  http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/triclosan_508.pdf.

- United States Environmental Protection Agency (EPA). Memorandum. January 4, 2008. Triclosan: Report of the Cancer Assessment Review Committee. PC Code: 054901[6].
  http://www.epa.gov/pesticides/chemical/foia/cleared-reviews/reviews/054901/054901-2008-01-04a.pdf

- European Commission's SCCP opinion on triclosan [7] was issued (http://ec.europa.eu/health/archive/ph_risk/committees/04_sccp/docs/sccp_o_166.pdf)

- Scientific Committee on Consumer Safety (same committee, new name) issued its opinion on triclosan antimicrobial resistance[8] (http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_023.pdf).

In addition, a major review of triclosan safety published in Critical Reviews in Toxicology[9] was reviewed by the CIR Expert Panel as part of this assessement, as have other individual studies key to assessing the safety of triclosan.

Also reviewed were comments received during an open public comment period, included an unpublished evaluation of potential endocrine activity of triclosan[10] and a presentation to the CIR Expert Panel by Dr. Robert Finking representing BASF Scweiz AG.[11]

Regulation of Triclosan

FDA's Center for Drug Evaluation and Research (CDER) regulates triclosan as a drug, including personal care products with antibacterial/antimicrobial claims; and FDA's Center for Devices and Radiological Health (CDRH) is responsible for regulation of devices that may contain triclosan for antibacterial/antimicrobial purposes. As defined by FDA, an antimicrobial (active) ingredient is "a compound or substance that kills microorganisms or prevents or inhibits their growth and reproduction and contributes to the claimed effects of the product in which it is included," and an antimicrobial preservative (inactive) ingredient is defined as "a compound or substance that kills microorganisms or prevents or inhibits their growth and reproduction and is included in a product formulation only at a concentration sufficient to prevent spoilage or prevent growth of inadvertently added microorganisms, but does not contribute to the claimed effects of the product to which it is added." A topical antimicrobial agent is defined, in part, as "an antiseptic-containing drug product applied topically to the skin to help prevent infection in minor cuts, scrapes, and burns." Table 1 identifies regulatory decisions for triclosan-containing products regulated by FDA, as well as international health authorities in the European Union, Canada, Japan, Australia, and Norway.

EPA's Office of Prevention, Pesticides and Toxic Substances regulates triclosan when used as an antimicrobial (whether as a bacteriostat, fungistat, mildewstat, deodorizer and material preservative).[5] Such uses include commercial, institutional and industrial premises and equipment such as conveyor belts. As a material preservative, triclosan is used in many products including adhesives, fabrics, vinyl, plastics (toys, toothbrushes), polyethylene, polyurethane, polypropylene, floor wax emulsions, textiles (footwear, clothing), caulking compounds, sealants, rubber, and latex paints.

Triclosan in Cosmetics

In the EU and other countries, antimicrobial and antiseptic products may be considered to be cosmetics, and, as such, are controlled under cosmetic regulations which may not require pre-clearance or pre-market approval of active ingredients. In Japan, antimicrobial and antiseptic agents may be regarded as drugs subject to pre-approval. In Europe, Canada and Australia, the use of triclosan in cosmetics is limited to a maximum concentration of 0.3%; in Japan, triclosan in cosmetics is limited to a maximum concentration of 0.1%, and Norway has stated that the use of triclosan in cosmetics should be limited, but no maximum concentration is given.

FDA's Center for Food Safety and Applied Nutrition (CFSAN) is responsible for the regulation of triclosan in cosmetics. It was FDA's request for CIR to undertake a review of the safety of triclosan in cosmetics that initiated this safety assessment.

As given in the *International Cosmetic Ingredient Dictionary and Handbook*[12], triclosan may function in cosmetic formulations as a cosmetic biocide, deodorant agent, or preservative.

Cosmetic Biocides are ingredients used in cosmetic products to help cleanse the skin or prevent odor by inhibiting the growth of, or destroying microorganisms, such as bacteria, fungi or yeast. Cosmetic biocides may be cidal or static. Cidal agents kill microbiota and act as disinfectants. Static agents inhibit the growth of microorganisms but do not kill them. Ingredients used primarily for the protection of products against contamination are found in the listing of *Preservatives*. Ingredients used as active ingredients in OTC drug products that are intended to kill bacteria, fungi or yeast in order to treat, prevent or mitigate diseases are included in the listing of *Antimicrobial Agents*.

Deodorant agents are ingredients that reduce or eliminate unpleasant odor and protect against the formation of malodor on body surfaces. *Absorbents* can act as deodorants if they have the ability to absorb malodorous chemicals. Also, chemical reactions can be used to destroy the malodorous substance in selected cases. Perfumes and the like can be used to mask the perception of malodor by the process of reodorization. Unpleasant odors also may be the result of microbiological activity. Thus, *Cosmetic Biocides* are ingredients frequently used in skin-surface deodorants.

Preservatives are ingredients which prevent or retard microbial growth and thus protect cosmetic products from spoilage. Cosmetic products may support the growth of microorganisms. The use of preservatives is required to prevent product damage caused by microorganisms and to protect the product from inadvertent contamination by the consumer during use. The use of more than one preservative can sometimes increase efficacy due to synergism.

Report structure

Because this document departs from the approaches that CIR has used in the past to initiate a safety assessment of cosmetic ingredients, a brief overview of what is included and why is appropriate.

Section I addresses the relevant issues for triclosan as used in cosmetics.

Section II presents technical names and synonyms, physicochemical properties, information on methods of manufacturing, chemistry methods for identification and analysis, information on impurities and photostability.

Section III provides information on the extent of use of triclosan in cosmetics based on information provided by the industry to the FDA's Voluntary Cosmetic Registration Program (VCRP). Use concentrations based on a survey conducted by the Personal Care Products Council also are provided.

Section IV provides a limited overview of triclosan's absorption, distribution, metabolism, excretion, and toxicokinetics.

Section V presents an overview of assessments that have been made on triclosan's potential toxicological hazards, including endocrine hazards.

Section VI provides information on triclosan's putative mechanisms for the inhibition of bacterial growth and presents the key arguments that have been raised concerning triclosan's potential for causing antibiotic and antibacterial resistance.

Section VII includes rationales for benchmark doses and/or no-observable-adverse-effects-levels (NOAELs), consumer exposures, and accordant margins-of-safety for triclosan in consumer products.

Finally, Section VIII summarizes and integrates information in the preceding sections.

I. Issues to be resolved in safety substantiation of triclosan as used in cosmetics.

1. Triclosan exposure.

Issue: uses of triclosan in OTC drugs may present different exposure scenarios compared to use in cosmetics.

2 Triclosan sourcing and dioxin impurities.

Issue: triclosan imported from India and China reportedly may contain dioxin compounds.

3. Photostability and dioxin photoproducts

Issue: triclosan applied to the skin may photodegrade to dioxin compounds on exposure to light.

4. Carcinogenicity

Issue: data from one mouse carcinogenicity study did suggest a statistically significant increase in liver carcinomas and adenomas as a function of dose, above a threshold level.

5. Endocrine disruption

Issue: triclosan may bind to estrogen and/or androgen receptors and thus may act as an endocrine disruptor.

6. Potential for bacterial resistance

Issue: any antibiotic/antimicrobial agent potentially can be a selective agent for resistance in target organisms.

II. Chemistry

Definition and Structure

The *International Cosmetic Ingredient Dictionary and Handbook* has established triclosan as the International Nomenclature Cosmetic Ingredient (INCI) name (to be used in cosmetic product labeling) for the substituted organic ether that conforms to the structure shown in Figure 1.[12, 13]

In addition to being an INCI name, triclosan also is an INN name (International Nonproprietary Names for Pharmaceutical Substances, WHO). Although other CAS numbers have been used previously for triclosan, the current CAS number is 3380-34-5 and the EINECS number is 222-182-2.

As given in the *International Cosmetic Ingredient Dictionary and Handbook*[12], triclosan is sold under a variety of trade names and trade name mixtures by 16 different companies. Because triclosan is a powder, any trade name triclosan supplied as a liquid must be a mixture with a solvent. \

Physical and Chemical Properties

Triclosan is a white crystalline powder that is stable under normal storage conditions. Physical and chemical properties are presented in Table 2.

Method of Manufacture

Triclosan is produced by treatment of 2,4,4′-trichloro-2′-methoxydiphenyl ether with aluminum chloride in benzene under reflux[13]. Conversion to chlorinated dibenzo-*p*-dioxins (see Impurities below and Figure 2) can occur under extreme conditions such as high alkalinity and heat or by heating alone to 600°C. The type and purity of the starting materials in the synthesis of triclosan influenced the extent of contamination by the impurities dioxins and dibenzofurans.[3]

Methods of detection.

Triclosan may be separated from a wide variety of matrices ranging from water and biological fluids to cosmetics and fabrics, using using high performance liquid chromatography,[3] and detected by infrared and/or UV (peak absorption at 281 nm)[52] spectroscopy. Gas chromatography/mass spectroscopy methodology has a detection limit for triclosan of 0.5 ng/ml.[53]

Impurities

Commercial grade triclosan is reported to be >99% pure (w/w) as the powder, and 10 to <20% (w/v) pure as a liquid solution.[4] Technical grade triclosan produced by Ciba and Harmet/Vivimed is >99.0% and 99.9% pure, respectively.[6] Trace level impurities identified by the US Pharmacopoeia (USP) include mono- and di-chlorophenols, as well as di-, tri-, and tetra-chlorodibenzo-p dioxins and di-, tri-, and tetra chlorodibenzofurans, as shown in Table 4.[14]

Samples of triclosan from India and China were tested for the presence of dioxins.[15] Six samples of triclosan, each of which were manufactured by a different producer in India or China (5 samples and 1 sample, respectively, from each country), were analyzed for the presence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF). All six samples contained TCDD in excess of 1 pg/g, and 4 of the six samples contained TCDF in excess of 1 pg/g. TCDD and TCDF ranged from 17.2 pg/g to 1712.0 pg/g, and 0.43 pg/g to 207.30 pg/g, respectively, as shown in Table 3. The authors suggested that the presence of these two trace impurities may be due to the quality or purity of the starting material, the particular synthetic process, or the inability to tightly control physical synthetic parameters.

Ciba Specialty Chemicals (now part of BASF) has reported that Irgasan® DP 300 (which Ciba manufactures and distributes for topical use, specifically) and Irgasan® MP, meet USP's requirements[11] (see Table 4), but that generic triclosan made by other manufacturers does not necessarily comply with USP specifications.[16]

As noted earlier, impurities in triclosan that may be present in trace amounts include dioxins, and which are limited or not allowed by the U.S. Pharmacopoeia (USP). In addition, the government of Canada has established limits on dioxins. Both of these limits are presented in the Table 4. Triclosan is not on the work program of either the British Pharmacopoeia Commission or the European Pharmacopoeia Commission.[17]

FDA has stated that it is unaware of the purity, identity and concentration of impurities in triclosan used in cosmetics, or the sources of triclosan used in cosmetic formulations in the US.[18]

Chemical Reactivity

While chloroform may be produced if a soap containing triclosan comes into contact with chlorinated water, the concentration of chlorine in the water has to be on the order of 20%[19]. Two dish soaps, one containing triclosan (at 1.4 mg triclosan/g soap) and one without, were added to chlorinated water (20%

chlorine) at a concentration of 0.25 g/L. The measured chloroform level was 15 μg/L after 5 min and 49 µg/L after 120 min for the triclosan-containing soap. The chloroform levels for the non-triclosan formulation were near the detection limit.

Photostability

In FDA's nomination of triclosan for study by the National Toxicology Program,[5] the agency argued that 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.

With exposure to UV radiation at 254 nm, triclosan can photodegrade to 2,7- and 2,8-dichlorodibenzo-*p*-dioxin (2,7/2,8-DCDD) [20]. In addition, 2,4-dichlorophenol (DCP), which is not a dioxin, has been identified as a major degradation product under artificial conditions - 93.8-96.6% of the applied triclosan degrades to DCP within 240 minutes post-treatment.

Since the pKa for triclosan is around 8.1, at physiological pH, the phenolic form (shown in Figure 1) would predominate while at pH of 9, for example, the phenolate form (shown in Figure 3) would predominate. Australia's National Industrial Chemicals Notification and Assessment Scheme (NICNAS) stated that the phenolic form of triclosan is relatively photostable, whereas the phenolate form is more photodegradable. [3]

NICNAS[3] included the proposal that triclosan photolysis products would include the three permutations of dichloro compounds; a dihydroxy compound (2,4'-dichloro-2',4-dihydroxydiphenyl ether), which could further degrade to a monochloro compound, 4-chloro-2,4'-dihydroxydiphenyl ether; or 4-chloro-2-hydroxyphenol, which is closely related to the DCP photodegradation product noted by above.

The absorption of triclosan in the UV region varies as a function of pH[21]. At pH 11.8, the absorption maximum was around 290 nm, but at pH 5.5, the maximum was seen at 280 nm with very little overlap of the absorption spectrum with the spectrum of solar radiation.

III. Extent of use and use concentrations for triclosan in cosmetics.

According to information supplied to FDA by industry as part of the Voluntary Cosmetic Registration Program (VCRP),[22] *personal hygiene products* is the category with the greatest number of products containing triclosan (226 triclosan-containing products, 162 of which are deodorant products). Since FDA does not verify labelers' product status with regards to its VCRP database,[18] it may be that some of these products are marketed with antibacterial claims, and could be considered drug products or both drug products and cosmetic products. The *skin care products* category has 162 triclosan-containing products. FDA VCRP data for triclosan are given in Table 5. FDA's VCRP is, as the name of the program implies, "voluntary." As a result, these data cannot be regarded as complete.

A Personal Care Products Council ("Council") survey reported that triclosan is used in cosmetics at concentrations ranging from 0.01 to 0.3%.[23] Use concentration survey responses are given in Table 5, as a function of product category.

To interpret the data in Table 5, consider that, of all baby shampoos reported (total of 56), only 1 contains triclosan (~2% of all baby shampoos reported), or conversely, the vast majority do not, and the situation is

similar for the baby lotions, powders, and creams category. While uses of triclosan were reported to FDA under the VCRP for each of these categories, no use concentrations were provided in the industry survey for these categories. And no uses of triclosan or use concentrations were reported for the 143 products in the "other" baby products category - usually this row would not be included in Table 5 because there are no data to provide, but in this case it was not deleted because information that there is a product category in which there are no products containing triclosan is important information.

Rodricks et al.[9] reported use concentrations that are consistent with the data from the Council survey, except that a use concentration range up to 0.45% was reported for a liquid hand soap. The SCCP [7] reported use concentrations up to 0.3%.

Table 5 presents all of the cosmetic product categories, so that a reader may see all categories in which triclosan is used and at what levels (depending on availability of those data), as well as the cosmetic product categories in which triclosan is ***not*** used.

Triclosan-containing rinse-off and leave-on cosmetics uses may include products that result in triclosan exposure by the dermal, inhalation, and oral routes. Dermal exposure appears to include rinse-off and leave-on cosmetics applied to adults, as well as to children.

Cosmetic aerosols

Safety of inhaled aerosols depends on the ingredient, the concentration, the duration of the exposure and where they are deposited within the respiratory system.[24] The site of deposition is associated most with the particle size and density of the particle being inhaled. In general, the smaller the particle, the further into the respiratory tree the particle will deposit and the greater the impact on the respiratory system.

The parameter most closely associated with this regional deposition is the aerodynamic diameter, d_a, defined as the diameter of a sphere of unit density possessing the same terminal settling velocity as the particle in question. In humans, particles with an aerodynamic diameter of ≤ 10 µm are respirable. Particles with a d_a from 0.1 – 10 µm settle in the upper respiratory tract and particles with a $d_a < 0.1$ µm settle in the lower respiratory tract. [25,26]

Particle diameters of 60-80 µm and ≥80 µm have been reported for anhydrous hair sprays and pump hairsprays, respectively.[27] In practice, aerosols should have at least 99% of their particle diameters in the 10 – 110 µm range and the mean particle diameter in a typical aerosol spray has been reported as ~38 µm.[28] Therefore, most aerosol particles are deposited in the nasopharyngeal region and are not respirable.

IV. <u>Absorption, distribution, metabolism and excretion</u>

NICNAS[3] presented a review of triclosan's absorption/toxicokinetics, distribution, metabolism and excretion. These data were supplemented with data reviewed by FDA, [5] specifically for data that NICNAS[3] did not address, or had discounted because of shortcomings in reporting.

Absorption

Most reviews have suggested that triclosan is slowly and not extensively absorbed by the dermal route, consistent with its low water solubility and log $P_{o/w}$ of 4.8, but is rapidly and well absorbed by the oral route.

Rodricks et al.[9] suggested that dermal absorption would likely be <10%, but that oral absorption would be complete. In human subjects, for example, daily use of triclosan-containing toothpaste for up to 65 weeks resulted in increased blood levels compared to pre-use levels, but those increased levels remained steady and returned to baseline after use. Using full thickness human skin, total absorption of triclosan, at 24 h, was vehicle specific, with dishwashing liquid at 12%, water/oil emulsion at 11.3%, deodorant at 7.65%, and soap solution at 7.2%.

Similar dermal absorption figures were reported in the SCCP opinion on triclosan.[7]

Distribution

Triclosan measured in rodent radioactivity studies (following oral and dermal exposures) indicates distribution at highest levels to the liver, lung, kidney, gastrointestinal tract, and gall bladder.[3]

Rodricks et al.[9] suggested that differences in distribution between mice, rats, and hamsters (plasma levels are higher than liver or kidney levels in rats and hamsters, but not mice) implies that triclosan can accumulate in the mouse liver.

Metabolism

Oral and dermal routes (humans and rodents): Triclosan absorbed from the gastrointestinal tract undergoes extensive first-pass metabolism, which primarily involves glucuronide and sulfate conjugation. In both humans and rodents, at high triclosan plasma concentrations, metabolism shifts from the generation of predominantly glucuronide conjugates to sulfate-conjugates. The bioavailability of unconjugated triclosan may be limited after oral exposure because of triclosan's extensive first-pass metabolism. Triclosan is also metabolized to its glucuronide and sulfate conjugates by the skin.[4] FDA concluded that > 90% of absorbed triclosan is metabolized.[5]

Rodricks et al.[9] noted that the glucuronide metabolite predominates in humans while the sulfate conjugate is the dominant metabolite in mice.

The SCCP[7] also emphasized the extensive first-pass metabolism and the almost-total conversion to glucuronide and sulfate metabolites. Based on results from oral studies (e.g., toothpaste use) and dermal studies (e.g., washing with soap), there was no evidence of accumulation of triclosan in the human body.

Excretion

Oral and dermal routes (humans and rodents): Triclosan glucuronide is predominantly excreted in the urine, and triclosan is predominantly excreted in the feces. Triclosan that is administered orally and dermally is excreted in greater concentrations in the urine than in the feces in humans, hamsters, rabbits, and monkey. In rats, mice, and dog, the reverse is true. Up to 87% of triclosan that is administered to humans (by an unspecified route) is excreted in the urine, most of it within 72 h after dose.[4]

Rodricks et al.[9] noted that elimination half-lives following repeated dermal application of triclosan (1.4 to 2.1 days) are greater than those following oral administration (10 to 20 hours).

Biomonitoring Data

According to Rodricks et al.[9], several studies have reported triclosan in plasma and urine in the general population and in human breast milk in nursing mothers. Triclosan amounts in breast milk were reported to range from <20 to 300 μg/kg lipid in one study and <5 to 2100 μg/kg lipid in another. In a study that

compared triclosan levels in women who used triclosan-containing products with those who did not, levels in breast milk were 0.022 to 0.95 μg/kg lipid compared to 0.018 to 0.35 μg/kg lipid, respectively.

The largest bio-monitoring study was conducted as a subset of the National Health and Nutrition Examination Survey (NHANES) in which urine samples were taken from a random ⅓ of the 9643 subjects yielding data on 2514 individuals.[29] For the entire sample, the geometric mean triclosan level in urine was 13 μg/l. There were age differences in the findings as well as sex differences for urine concentrations, as shown in Table 6.

V. Toxicology/Safety

This section presents an overview of studies performed in experimental animals models or in vitro systems as well as in humans.

Acute toxicity

Triclosan has low acute toxicity by all evaluated routes and in all evaluated species.[5,6] Oral rat and mouse LD_{50} values were > 3700 mg/kg. Rabbit dermal LD_{50} values were > 9000 mg/kg and the rabbit inhalation LC_{50} is > 0.15 mg/L. The rat subcutaneous and intraperitoneal, and intravenous LD_{50} values were > 14,700 mg/kg, >1090 mg/kg, and 29 mg/kg.

Repeat dose toxicity

Triclosan repeat dose toxicity has been evaluated in the baboon and hamster (oral), rat (oral and inhalation), mouse (dermal) and rabbit (dermal). Triclosan NOAELs based on local irritation effects tended to be $\leq$ 10 mg/kg/day by all routes, except inhalation, which had a reported No-Observed-Adverse-Effect-Concentration (NOAEC) of 5×10^{-5} mg/m^3. LOAELs and NOAELs based on systemic toxicity tend to be <1000 mg/kg/day, with no obvious common toxicity among studies and species. NICNAS,[3] EPA,[4,6] and FDA[5] toxicology data summaries are presented in Table 8. NICNAS[3] or FDA[5,30] summary statements were not included if such statements did not provide duration of exposure or information on doses, or were fundamentally flawed (e.g., LOAEL lower than the NOAEL). Table 7 is organized hierarchically by route of exposure, duration of dosing (subacute → subchronic → chronic), species (monkey → rat/mouse → rabbit). Unless otherwise noted, NOAEL units were not standardized.

Rodricks et al.[9] summarized findings from repeated dose dermal exposures of triclosan in propylene glycol or acetone vehicles using CD-1 mice (doses from10 to 200 mg/kg/day) and Crl:CD BR rats (doses from 1.2 to 24 mg/kg/day). Responses varied as a function of vehicle, dose, species, and sex of the exposed mice. For example, in mice, liver weights were increased in males at all doses >10 mg/kg/day, independent of vehicle, but only at 200 mg/kg/day in females for the propylene glycol vehicle exposures. Pale foci were noted in the livers of male mice from the 100 and 200 mg/kg/day groups with both vehicles, but not in females. No significant changes in liver weights were reported in rats, nor were there any effects seen on macroscopic or microscopic examination, in either sex.

Genotoxicity

Triclosan has been evaluated in a number of standard (and other) genotoxicity assays, including bacterial reverse mutation tests, *in vitro* mammalian cell gene mutation test, and *in vitro* mammalian chromosome aberration tests, a mammalian bone marrow chromosomal aberration test, and an unscheduled DNA synthesis assay in mammalian cells in culture. As FDA[5] noted: the preponderance of data suggested that

triclosan is not genotoxic. NICNAS[4] drew a similar conclusion. EPA[4] provided a detailed review of seven genotoxicity tests. It concluded that each test, except one (an *in* vitro cytogenetic assay with Chinese hamster lung fibroblasts), was negative. Therefore, the consensus on the weight of evidence on triclosan's genotoxicity potential is that it is not genotoxic.

Carcinogenicity

NICNAS[3] and EPA[6] concluded that triclosan was not carcinogenic based on the available data. FDA, however, concluded that the available data are not adequate to resolve the question of triclosan carcinogenicity via the dermal route of exposure seen in skin cleansing preparations and requested that a dermal carcinogenicity study be conducted under the auspices of the NTP.[6]

EPA[6] specifically evaluated rat and hamster oral chronic toxicity/carcinogenicity studies and concluded that triclosan exhibited no carcinogenic potential in rats at $\leq$ 3000 ppm (in the diet) and in hamsters at $\leq$ 250 mg/kg/day (in the diet). However, EPA reviewed a mouse oral chronic/carcinogenicity bioassay and found it positive for carcinogenicity based on an increased incidence of liver neoplasms in male and female mice at $\geq$30 mg/kg/day.[6] Nevertheless, EPA concluded that this study did not support triclosan carcinogenicity, and that triclosan is "not likely to be carcinogenic in humans". This conclusion was based on the weight of evidence that supports activation of peroxisome proliferator activated receptor alpha (PPARα) as the primary mode of action for triclosan-induced hepatocarcinogenesis in mice. Also, EPA stated that, while the data did not support either a mutagenic mode or cytotoxic mode of action, that the mode of action for liver tumors in mice is theoretically plausible in humans. Based on differences in the PPARα responses in humans compared to mice, however, EPA suggested that such a mode of action was unlikely.

In referring triclosan to the NTP for study, FDA specifically commented on oral toxicity data using albino rats submitted to the agency in 1977, suggesting that the presence of test material in control animals invalidated the results and on an oral rat study conducted in 1986, suggesting that the study was inadequate based on a high rate of mortality, absence of significant body weight differences between treated and control animals, and the presence of hepatocellular lesions not consistent with the morbidity/mortality.[5] In spite of the agency describing the latter study as inadequate, one FDA reviewer concluded that Triclosan was oncogenic at 3,000 ppm at 104 weeks. In 1999, FDA reviewed another carcinogenicity study (hamsters) and apparently formed no conclusion on the merits of the study because the sponsor did not respond to the Agency's request for histopathology slides of kidneys, liver, lungs, adrenals and all tumors from all animals on study for review.

FDA,[5] in its presentation of the rationale for NTP study of triclosan, also suggested that the only available dermal toxicity data (90 day dermal rat study) could be interpreted to suggest dose-dependent abnormalities which need subsequent study with a 2-year dermal carcinogenicity bioassay.

Rodricks et al.[9] reviewed chronic toxicity studies using rats, hamsters, and mice in which the incidence of tumors was evaluated. Rats were fed triclosan in the diet at 0, 300, 1000, and 3000 ppm for up to 104 weeks, with an additional group at 6000 ppm for 52 weeks. No evidence of tumors or preneoplastic lesions was found.

In its discussion of these data, the SCCP [7] noted that the exposures were calculated to yield doses of 0, 12, 40, and 127 mg/kg/d for males and 0, 17, 56, and 190 mg/kg/d for females. The additional dose for the 52-week animals was calculated to be 247 mg/kg/d for males and 422 mg/kg/d for females. The SCCP did not

disagree that no evidence of tumors or preneoplastic lesion was found, but did determine that there were significant reductions in red blood cell counts in males and females, including low-dose males at 104 weeks. Increases in mean corpuscular hemoglobin were observed in mid- and high-dose females and in males at all doses. Other hematologic parameters were also different from controls, but only in the high-dose groups.

The SCCP also noted decreased absolute and relative spleen weights in mid-dose females.

The SCCP considered that these hematotoxicity results and the spleen weight changes as indications of an adverse effect and established the NOAEL from this study at 12 mg/kg/d. This is the calculated lowest dose for male animals, but the SCCP did not comment on the red blood cell count changes or the increases in mean corpuscular hemoglobin that were observed in low-dose males. Finking[11] suggested that the absence of hemolytic anemia in the animals means that a NOEAL should not be established based on hematological parameters and spleen weight changes.

Rodricks et al.[9] suggested that the statistically significant hematological changes were slight and transient (red blood cell counts were down at 13, 26, and 52 weeks, but not at 78 or 104 weeks) and that there were no other indications that the animals were anemic. These authors also noted an absence of macroscopic or microscopic evidence of an effect on the hematopoietic system and no apparent effects on homeostasis.

Rodricks et al. described a study in which hamsters were fed triclosan in their diet at 0, 12, 75, or 250 mg/kg/day for 90-95 weeks. Deaths in male hamsters in the high-dose group were significantly higher than in the control group. No evidence of liver damage was seen at any dose, but body weight gain was significantly reduced and nephropathy was significantly increased in both sexes at the highest dose compared to controls. In addition, hyperplasia in the fundic region of the stomach, abnormal spermatogenic cells, reduced spermatozoa, and germ-cell depletion were noted in high-dose males.

The SCCP [7] review of these data also noted the positive findings in high-dose hamsters and set the NOAEL at 75 mg/kg/d.

Rodricks et al.[9] reviewed the study in which CD-1 mice were fed triclosan in the diet at doses of 0, 10, 30, 100, or 200 mg/kg/d for 6 months or 18 months. In the 18-month study, statistically significant increases in liver adenomas and carcinomas were seen at several dose levels compared to controls as shown in Table 8.

The SCCP [7] did note an increased incidence in liver tumors at doses of 30 mg/kg/d, and commented that triclosan is a peroxisome proliferator in mouse liver. The SCCP described dose-related increases in liver weights at 30, 100, and 200 mg/kg/d in males and females, and hepatocyte hypertrophy in males at all doses. The LOAEL was established at 10 mg/kg/d.

While triclosan in the diet appears to be linked to the adenomas and carcinomas in the liver of the exposed mice, the questions that arise from this finding are: (1) why did those tumors occur, and (2) is the finding relevant to human health? For example, since mice accumulate triclosan in the liver and humans don't, might this explain the causation (high accumulated levels of triclosan) and be an adequate basis for discounting the effect for human exposure?

Rodricks et al.[9] used the International Programme on Chemical Safety (IPCS) framework[31] to assess the relevance of the mode of action (MOA) of tumor formation in the mouse study to humans. This approach posits three questions:

1. Is the weight of evidence sufficient to establish an MOA in mice for tumor formation?

2. Is the MOA relevant to human health (i.e., can it even happen in humans)?

3. Even if the MOA can happen in humans, is the MOA inconsequential on the basis of quantitative differences in either kinetic or dynamic factors between mice and humans?

The first step in addressing these questions, obviously, is to postulate the MOA of triclosan in the mouse liver that produces tumors. While hepatic tumors are the most common spontaneous tumors in mice, the mouse liver is a frequent target of chemically induced tumors. MOAs for chemically induced liver tumors include genome mutation in liver cells, or non-genotoxic gene activation/deactivation, and/or receptors.

As discussed earlier, the preponderance of evidence is that triclosan is not genotoxic, so the MOA is likely non-genotoxic. Activation of peroxisome proliferator-activated receptors (PPARs) is a well-characterized, non-genotoxic mechanism by which a cascade of events can lead to tumor formation. Three types of PPARs have been identified: α, β/δ, and γ, with the α form expressed in the liver. In concept, a ligand binds to a retinoid X-receptor (RXR) in the cytoplasm, is transported to the nucleus, where the combination ligand/RXR binds to promoter sequences of peroxisome proliferation genes, activating PPARα. That alters the transcription of genes involved with peroxisome proliferation, apotosis, and lipid metabolism. Those changes increase fatty acid β-oxidation which can lead to oxidative stress. In turn, increased stimulation of nonparenchymal cells and inhibition of gap junction intercellular communication can occur. Increased cell proliferation and decreased apotosis, leads to hyperplasia and hepatic tumors.

So, for the mice in the triclosan carcinogenicity study, is there evidence of triclosan-related PPARα activation, cell proliferation, fatty acid β-oxidation, etc? Rodricks et al.[9] reviewed the available data and concluded that there was no direct evidence of PPARα activation, but there was evidence of triclosan-related PPARα-dependent up-regulation of CYP3A and CYP4A, testosterone hydroxylation, and lauric acid 11-12 hydroxylation, and PPARα-dependent expression of nonperoxisomal fatty acid metabolism genes (cyanide-independent palmitoyl CoA oxidation). Peroxisome proliferation was supported by the findings of triclosan-related hypertrophy due to an increase in the number and size of peroxisomes and an increase in smooth endoplasmic reticulum. While there was no evidence of PPARα-dependent expression of cell-cycle growth and apotosis, there were triclosan dose-dependent increases in Proliferating Nuclear Cell Antigen (PNCA) labeling index, indicative of perturbation of cell proliferation and/or apotosis. Hepatocyte oxidative stress was suggested by the triclosan dose-related increases in lipofuscin in the Kupffer cell region. Kupffer cell-mediated events were suggested by triclosan-related Kupffer-cell activation. And finally, selective clonal expansion is suggested by the finding of triclosan-related hepatic adenomas and carcinomas.

The authors considered the possibility that hepatic cytotoxicity could be the MOA. In concept, triclosan would be cytotoxic, resulting in a hyperplastic response, during which hepatic cells with DNA damage proliferate, produce preneoplastic foci, and then tumors. While cell proliferation was linked to triclosan treatment, necrosis was not.

If PPARα activation is the MOA of triclosan in mice, then how would it translate to a human health risk? Expression of PPARα in the human liver is $1/10^{th}$ of that in the mouse. A study of a known liver carcinogen in mice containing the gene for human PPARα compared to mice containing the normal mouse gene produced no tumors in the mice containing the human gene and the expected tumors in the mice containing the normal mouse gene.

Is there something about how humans metabolize, distribute, and excrete triclosan that suggests the mouse MOA would not be applicable? Certainly, excretion is different. In mice, triclosan is mostly excreted in the feces as unchanged parent chemical. In humans, the primary excretion route is in the urine as the glucuronide conjugate. Also, the method of excretion in mice supports the finding that triclosan can accumulate in the mouse liver.

Overall, Rodricks et al.[9] concluded that the hepatic tumors produced by a PPARα activation MOA are not relevant to predicting human health outcomes.

Reproductive and Developmental Toxicity

NICNAS[3] reported a developmental and maternal toxicity NOAEL of 50 mg/kg/day for no specific species, but the basis for that NOAEL was unclear.

The effect of triclosan, at larval exposure levels up to 32.3±9.43 µg/ml (measured 21-day mean±SEM), on frog metamorphosis was examined.[32] A small marginally-significant acceleration in premetamorphic development was reported, but the effect was not thyroid-mediated. Overall there was no effect on metamorphosis. The authors suggested that the effect that was seen would be consistent with the reduced bacterial stressors that would be found in the 50 L tanks used for the study.

Rodricks et al.[9] summarized findings on developmental toxicity studies using mice, rats, hamsters, and rabbits. Only in rats and mice were significant findings reported.

In a two-generation reproductive and developmental study using CRL:CD (SD)Br rats given triclosan at doses of 0, 300, 1000, and 3000 ppm (in the diet), body weights were significantly decreased in F_1 animals on postnatal days 14 and 21 in the high-dose group compared to controls. The viability index for high-dose F_1 animals was decreased, but the difference was not significant.

The SCCP [7] reviewed this same study and noted an absence of reproductive toxicity at the 3000 ppm dose (~200 mg/kg/day for both sexes combined), but concluded that the NOAEL for developmental effects would be 65 mg/kg/d (both sexes combined) because of pup body weight decreases at the high dose.

Rodricks et al.[9] reviewed a study in which CD-1 mice were given 0, 10, 25, 75, or 350 mg/kg/day on gestation days 6 – 15, fetal body weights were significantly decreased in the two highest dose groups. The incidence/litter of irregular skull ossification was significantly increased in high-dose litters and the litter averages for ossified forepaw and hind paw phalanges per fetus, possibly linked to developmental delay due to the reduced fetal weights.

The SCCP [7] stated that taking maternal toxicity and fetal toxicity both into consideration, there is no evidence of triclosan developmental toxicity (teratogenicity).

Endocrine Disruption

In vitro studies

Ahn et al.[33]reported results from a series of receptor-based bioassay systems for triclosan. Three receptors were stably transfected: aryl hydrocarbon receptor (AhR – activates gene expression in a ligand-dependent manner); estrogen receptor (ER); and androgen receptor. In each case the reporter gene was firefly luciferase. In addition, the ryanodine receptor type 1 (RyR1) assay for compounds with potential to alter Ca^{2+} homeostasis was performed using primary cultures of skeletal myotubes from wild-type mice.

In the AhR assay, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was used as a positive control. Triclosan at 10 µM was found to induce luciferase activation at 40.6±6.1% of that of TCDD, suggesting weak agonist activity of triclosan. With both 1 nM TCDD and 10 µM triclosan (10^4 molar excess of triclosan over TCDD) added, induction reached only 70.4±2.1%, also suggesting weak antagonist activity of triclosan. Overall, the authors suggested triclosan would be a partial agonist of AhR.

In the ER assay, triclosan exhibited no direct estrogenic activity alone, suggesting no agonist activity. When combined with estradiol, a 10^3 excess of triclosan reduced estradiol activity to 50% and a 10^5 excess of triclosan reduced estradiol activity to 20%, suggesting antagonist activity.

Ryanodine binding to microsomes enriched in RhR1 was significantly increased by 1.2 µM triclosan, suggesting to the authors that triclosan is a dysregulator of cell Ca^{2+} homeostasis.

The estrogenic and androgenic activity of triclosan using MCF7 human breast cancer cells in culture and other in vitro assays was examined.[34] In MCF7 breast cancer cells in vitro, estradiol binding remained >90% at 10^4 molar excess of triclosan. Half of estradiol binding to estrogen receptors (ER) was displaced at a 10^6 molar excess of triclosan. A 10^5-fold excess of triclosan over 17β-estradiol effectively inhibited activation of the ERE-CAT reporter gene and inhibited 17β-estradiol-induced cell growth stimulation.

In a seeming contradiction, the authors suggested that there was a small but not statistically significant increase in MCF7 cell growth in the presence of triclosan alone. In a follow-up assay in which the cultures were maintained for 21 days (the assay is normally done over 8 days), a statistically significant increase in cell growth (but still less than was reported for 17β-estradiol at 10^{-6} M and at 4 x 10^{-6} M, but not at lower (2 x 10^{-7} , 6 x 10^{-7}) or a higher concentration (8 x 10^{-6}).

The authors also performed a competitive binding assay between triclosan and testosterone to rat recombinant androgen receptor (AR) protein, with the result that triclosan, at a 10^3 molar excess over testosterone, reduced testosterone binding by around half and the decrease was linear when binding was plotted versus the log of the molar ratio. They also determined growth stimulation in the presence of triclosan in S115+A mouse mammary tumor cells and T24 human breast cancer cells. At a testosterone concentration of 10^{-9} M, S115+A cells are stimulated to grow and undergo a doubling in number. Triclosan at 2 x 10^{-5} M prevented that growth.

Activation of the LTR-CAT reporter gene in stably transfected S115+A cells and in transiently transfected T24 human breast cancer cells also was studied. In S115+A cells, 10^{-9} M testosterone triggered activation. Triclosan at molar ratios to testosterone of 1 and 10 had no effect, but at a molar excess of 100, reduced the activation by 20%. A molar excess of triclosan of 10^4 reduced activation by 40%. At a testosterone concentration of 10^{-8} M, triggered activation in T24 cells. At a 10^2 molar excess of triclosan, the activation was reduced by 30% and by 75% at a molar excess of 10^3.

James et al.[35] postulated that triclosan is structurally related to inhibitors of estrogen sulfotransferase, such as polychlorobiphenylols. To test potential enzyme inhibition, the authors harvested placental tissue from almost term fetal sheep, homogenized the tissue and incubated the cellular material with triclosan for ~15 min. Estrone and 17-beta-estradiol were substrates and the effect of 4-hydroxy-3,3',4',5-tetrachlorobiphenyl and 2'-hydroxytriclocarban on estradiol sulfonation was used for comparison. Triclosan was a very potent inhibitor of both estradiol and estrone sulfonation suggesting competitive binding of triclosan for estradiol sites on the sulfotransferase enzyme. The authors suggested that the effect of

triclosan as an inhibitor of estrogen sulfotransferase activity raised concern about the possible effects of triclosan on the ability of the placenta to supply estrogen to the fetus, and in turn on fetal growth and development.

Environ International Corporation[10] in its analysis, reasoned that, were this phenomenon to be of any significance, then administration of triclosan in vivo should have an impact on successful pregnancies. In a two-generation rat reproductive and developmental toxicity study of triclosan at doses up to 3000 ppm (described earlier), there was no evidence of an impact on reproductive performance, nor were there any data to demonstrate that the ability to carry fetuses to term was compromised.

In vivo studies

A no-effect level of 5 mg/kg/day (or higher) triclosan in a 60-day study of male rats treated daily was reported[36]. Endpoints studied included body weights (no significant change at any dose); decreased testis, prostate, seminal vesicle, vas deferens and cauda epididymis weights (at 10 and 20 mg/kg/day); down-regulation in the testicular levels of mRNA for cytochrome P450SCC, cytochrome P450C17, 3β-HSD, 17-βHSD, StAR and AR as compared to control (at 10 mg/kg/day); decreased testicular 3β-HSD and 17β-HSD levels in vitro (at 20 mg/kg/day); and decreased serum hormone levels (at 20 mg/kg/day).

Weanling rats were exposed to 0, 3, 30, 100, 200, or 300 mg/kg/day of triclosan by oral gavage from postnatal day (PND) 23 to 53[37]. Predicated on the idea that the separation of the foreskin of the penis from the glans penis, so-called preputial separation (PPS), is an early reliable marker of the progression of puberty in the male rat, this gross endpoint was examined beginning on PND 33. Triclosan did not affect growth or the onset of PPS. Serum testosterone and triiodothyronine (T3) were not different in a dose-effect manner. Total serum thyroxine (T4) decreased in a dose-dependent manner at 30 mg/kg and higher. Thyroid stimulating hormone was not statistically different at any dose. Liver weights were significantly increased at 100 mg/kg triclosan and above, but not in a dose-effect manner and other tissue weights were not different from controls, exemplifying the difficulty in identifying a cohesive body of work on Triclosan's potential as an endocrine disruptor (or as an endocrine toxicant).

A follow-up study examined the hypothesis that triclosan upregulates rat hepatic catabolism and alters expression of cellular transport proteins[38]. The authors measured total serum T4, T3, and thyroid stimulating hormone (TSH). Cytochrome P450 isoforms (*Cyp1a1*, *Cyp2b1/2* and *Cyp3a1/23*) were determined enzymatically and as mRNA expression levels using quantitative reverse transcriptase. Uridine diphosphate glucuronyltransferase (UGT) activity, mRNA expression of UGT isoforms and sulfotansferase (SULT) isoforms, and mRNA expression of hepatic transporters, including *Oatp1a1*, *Patp1a4*, *Mrp2*, and *Mdr1*, were also measured.

T4 levels decreased as expected as a function of triclosan concentration, with doses of 100, 300, and 1000 mg/kg/day producing significant decreases, but not at 10 and 30 mg/kg/day. T3 levels were decreased at 300 and 1000 mg/kg/day, but not at the three lower exposure levels. No significant differences in TSH were found at any exposure, but the authors suggested that this may relate to T3 glucuronidation.

Cyp2b1/2 (at triclosan levels of 300 mg/kg/day) and *Cyp3a1/23* (at Triclosan levels of 100 and 300 mg/kg/day) gene expression were increased, No significant effect was seen at lower doses. Liver microsomal UGT activity was increased significantly only at 1000 mg/kg/day triclosan. UGT gene

expression was not significantly increased for *Ugt1a6* or *Ugt2b5* genes, but was increased at 100 and 300 mg/kg/day for *Ugt1a1* genes.

SULT isoform expression was not dose-related for *Sult1b1* (significantly reduced at 10 and 30 mg/kg/day, but not at 100 or 300 mg/kg/day triclosan), but *Sult1c1* expression was increased significantly at 100 and 300 mg/kg/day only.

No statistically significant changes were reported for mRNA expression of hepatic transporters.

The authors cautioned that, while these findings support a role for hepatic catabolism of T4 in the rat as a likely mechanism of observed triclosan-induced hypothyroxinemia in the rat, the relevance to humans is not established.

The potential for everyday exposure to triclosan via triclosan-containing toothpaste for 14 days in 12 adult humans to cause an increase in plasma 4β-hydroxycholesterol, indicative of CYP3A4 induction, and/or alterations in thyroid hormonal status was investigated[39]. Plasma triclosan concentrations increased from 0.009–0.81 ng/g to 26–296 ng/g. The authors noted that the 296 ng/g plasma triclosan level is in the range of triclosan plasma levels that could be attained with an oral dose of 4 mg triclosan. No significant changes in plasma levels of either plasma 4β-hydroxycholesterol or thyroid hormones were reported during the exposure. The authors concluded that triclosan-containing toothpaste use was not likely to alter metabolism of drugs via CYP3A4 induction or cause adverse events because of thyroid disturbances in humans.

Other studies suggested weak estrogenic and androgenic effects, and antiestrogenic and antiandrogenic effects (reported in fish/frogs or *in vitro*). Summary statements from Triclosan reviews already available from various governmental sources on the import of those data are presented in Table 9.

The SCCP[7] did comment that data from a study using Japanese medaka fry exposed to concentrations of triclosan up to 100 g/l for 14 days showed no effect on sex ratios in the developing fish.

Clinical studies

FDA[5] summarized several studies reviewed by DeSalva et al.[40] and by Lyman & Furia.[41] None of the clinical studies indicated that triclosan at concentrations of $\leq$ 0.25% causes sensitization, or that triclosan at concentrations at $\leq$ 0.5% causes irritation. In contrast, NICNAS[3] stated that it had reviewed several studies that had shown evidence of skin irritation, although applicable doses or references were not identified. NICNAS[4] stated that there is very limited evidence for triclosan causing photosensitization in healthy volunteers or those with dermatological conditions. NICNAS[3] also reported that humans orally administered triclosan at $\leq$ 30 mg/day for 15 or 42 days showed no evidence of any treatment effect.

Using data from the 2003-2006 National Health and Nutrition Examination Survey (NHANES), a comparison of urinary levels of triclosan with diagnoses of allergies or hayfever in U.S. adults and children.[42] Levels of triclosan in the urine were associated with allergy/hay fever diagnoses in individuals less than 18 years of age, but not in those individuals 18 years old or older. The authors speculated that endocrine disruption properties of triclosan may be linked to immune function and hence to diagnoses of allergy and hayfever.

Dermal and eye irritation; phototoxicity, respiratory irritation, sensitization

In rabbits, triclosan was a moderate dermal irritant (Primary Irritation Index score of 3.5 @ 72 hours), as well as a moderate eye irritant.[6] Rodricks et al.[9] stated that concentrations of 1 to 100% produced reversible eye irritation in rabbits.

Triclosan in various formulations at $\leq$ 0.25%, when tested dermally in a rabbit primary dermal irritation test and acute dermal lethality test did not cause dermal toxicity.[40] EPA[6] did not consider triclosan a sensitizer in guinea pigs. NICNAS[3] considered triclosan at most a very weak sensitizer in guinea pigs, although a review of the studies considered in the NICNAS report each concluded that there was no sensitization. NICNAS considered triclosan to be a respiratory irritant.[3] Triclosan was not a phototoxicant in guinea pigs.[3]

Rodricks et al.[9] reviewed the available dermal irritation and sensitization studies. Dermal irritation was concentration dependent. For example, a single application of triclosan at 0.3% was not irritating in any animal species, but dermal concentrations of 5% were irritating to guinea pig skin. Repeated dosing dermal studies (14-day) have consistently found a threshold of around 1.5% for irritation. Sensitization studies were negative in guinea pigs at 0.1% when administered subcutaneously, or topically at concentration up to 10%. Triclosan at concentrations up to 1% were not photosensitizers in guinea pigs, mice, or pigs with irradiation in either the UVA, UVB, or UVC region.

The SCCP[7] also reviewed the available photosensitization data in guinea pigs (exposed to UVB and UVA radiation), and mice and pigs (exposed to UVC, UVB, and UVA radiation) and, while noting that all of the available studies predated good laboratory practices, suggested that there was no evidence for photosensitization.

VI. Antibacterial/antimicrobial resistance

FDA[5] described two triclosan mechanisms of action for the inhibition of bacterial growth: 1) intercalation into bacterial cell membranes and disruption of membrane activities (without causing leakage of intracellular components, and 2) inhibition of bacterial type II fatty acid synthase enoyl-reductase (FabI gene). Triclosan is bacteriostatic at low doses and bactericidal at high doses.

In a 2002 report of the Scientific Steering Committee (SSC) of the European Commission Health & Consumer Protection Directorate-General,[43] the conclusion was reached that, at high (biocidal) concentrations, triclosan is very effective and unlikely to produce a major problem of anti-microbial resistance (e.g., all the microorganisms are dead). However, at sub-biocidal and bacteriostatic, concentrations (MIC's ranging from 0.1 mg/ml to 33 mg/ml), triclosan is capable of penetrating bacteria and initiating changes related to important mechanisms of antimicrobial resistance including possibly transferable mechanisms of resistance, though the scientific evidence for transferability has been disputed. Sound scientific laboratory evidence exists for the development of triclosan related mechanisms for antimicrobial resistance, but the evidence as to whether these mechanisms are shared by other antimicrobial agents or whether they are transferable to micro-organisms other than those used in the laboratory is limited and contradictory. Overall the SSC noted that no evidence of such resistance has been seen in clinical isolates, and there is no epidemiological evidence to suggest a problem in clinical practice.

No relationship between the use of triclosan and other biocides and antibiotic resistance was found in another study[44].

Minimum inhibitory concentrations (MICs) from clinical strains of *S. aureus* (both methicillin resistant (MRSA) and sensitive strains (MSSA)) and *P. aeruginosa* for changes from 1989 to 2000 were analyzed[5]. While MRSA strains developed biocide resistance, MRSA antibiotic resistance has remained the same. The same was true for MSSA strains. Overall this suggested to the author that any acquisition of biocide resistance does not alter antibiotic resistance. For *P. aeruginosa*, the MICs for triclosan were actually reduced in 2000 compared to 1989, although the difference was not statistically significant.

The information that is available from studies of manufacturing sites[45] and clinical follow up studies of dental plaque flora which have failed to show biologically significant changes in MIC values to commonly used antibiotics in patients using triclosan long term[46,47] points to resistance patterns being stable over periods of three to ten years.

Aiello et al.[48] reviewed triclosan efficacy data along with data their laboratory developed on antimicrobial resistance in use situations. Two studies reported findings from a randomized and masked intervention trial of 238 households using either 0.2% triclosan–containing liquid hand soap or plain soap over one year. Neither of these studies demonstrated the emergence of antibiotic resistance associated with use of triclosan-containing liquid hand soap, compared with plain soap.

50 vancomycin-resistant *Enterococcus faecium* (VRE) isolates from human wastewater effluents in Texas were characterized[49]. These VRE isolates were also resistant to 8 fluoroquinolone antibiotics and most of the National Antimicrobial Resistance Monitoring System gram positive antibiotics. The VRE isolates were sensitive to quinupristin/dalfopristin and to linezolid antibiotics and they were sensitive to triclosan and other biocides. No cross-resistance or co-resistance between antibiotic resistance and biocide susceptibility was found.

These results contrast with the results of studies of triclosan susceptibility to 732 pathogenic *Acinetobacter baumanii* clinical isolates from hospitals in China. MIC values for triclosan ranged between 0.015 and 16 mg/l. These MIC values were lower than the in-use concentrations of triclosan of 2000 to 20000 mg/l. 20 (out of the 732) isolates for which the MIC was greater than 1 mg/l were identified; the authors declared those to have reduced susceptibility to triclosan.

The authors then further examined those 20 isolates for antibiotic resistance and compared the results with 20 isolates with triclosan MIC values of 0.5 mg/l down to 0.03 mg/l. All 20 of the isolates with reduced susceptibility to triclosan were resistant to amikacin, tetracycline, levofloxacin, and imipenem. Among the 20 isolates with triclosan MIC values < 0.5 mg/l, 11 were resistant to amikacin and tetracycline, and 8 were resistant to levofloxacin and imipenem.

These authors further examined the potential that mechanism for triclosan reduced susceptibility, including efflux pump over expression (in concept, if triclosan is removed from the bacterial cell, it is no longer available to function as a biocide), but were unable to correlate expression of efflux pump genes with triclosan reduced susceptibility. They identified mutations in the FabI (NADH dependent, enoyl-[acyl-carrier-protein] reductase) gene in all 12 of the isolates with triclosan MIC values >4 mg/l and postulated that triclosan resistance was linked to mutations in that gene.

Clinical isolates of *Proteus mirabilis* were studied in vitro to determine if exposure to triclosan could result in decreased sensitivity (higher MIC values) to triclosan itself and/or increased antibiotic resistance[50]. Five strains of *Proteus mirabilis* were exposed in culture to triclosan at concentrations from 0.5 to 10 mg/l for 5

days. Viable colonies (mutated to reduced triclosan susceptibility) were subcultured and tested to determine triclosan MIC values and MIC values for trimethoprim, ampicillin, ciprofloxacin, nitrofurantoin, norfloxacin, cephalexin, nalidixic acid, and gentamicin. The parental strains and 2-3 mutant strains for each were tested. While mutant isolates were found with MIC values for triclosan up to 60 mg/l, none of the mutated strains showed resistance to any antibiotic that was different from the parent strain.

In 2009, SCENIHR[2] stated that triclosan at low concentrations acts by both inhibition of enoyl acyl reductase mechanism, inhibition of energy-dependent uptake of amino acids, and possibly discharge of membrane potential (as demonstrated in *E. faecalis*). The SCENIHR report concluded that current scientific evidence (including bacteriological, biochemical and genetic data) does indicate that the use of certain active substances in biocidal products in various settings may contribute to the increased occurrence of antibiotic resistant bacteria. Some mechanisms of resistance are common to both biocides and antibiotics (e.g. efflux pumps, permeability changes and biofilms). The selective pressure exerted by biocides may favor the expression and dissemination of these mechanisms of resistance.

Most recently, the European Commission's Scientific Committee on Consumer Safety (SCCS) issued an opinion on triclosan antimicrobial resistance[8] with the conclusion that the available data have failed to demonstrate an increase in antibiotic resistance following triclosan use in situ. Because in vitro studies have demonstrated that resistance to triclosan in bacteria is possible (see, for example, Stickler and Jones[50] above) and that there are mechanisms in bacterial resistance that can result in cross-resistance to biocides and antibiotics (see for example, Chen et al.[51] above), the opinion went on note that it was not possible to draw an overall conclusion on whether the continuous use of triclosan is involved in the development of resistance. The SCCS recommended prudent use of triclosan, for example, in applications where a health benefit can be demonstrated and that additional research, for example, on mechanisms of resistance, transfer of resistance, and translational studies from in vitro to in situ situations.

VII. Exposure Assessment and Margins-of-Safety

A determination of triclosan exposures and margins-of-safety presumes that a hazard has been identified. Rodricks et al.[9] suggested that the statistically significant increases in nephropathy and stomach pathology seen in male and female hamsters and the statistically significant effects in epididymides and testes in male hamsters, all at the high-dose level of 250 mg/kg/day but not at 75 mg/kg/day could establish a no-observable-adverse-effect-level (NOAEL) for repeated dose systemic toxicity. Further, in CD-1 mice given 350 mg/kg/day on gestation days 6 – 15, fetal body weights were significantly decreased in the two highest dose groups. The incidence/litter of irregular skull ossification was significantly increased in high-dose litters and the litter averages for ossified forepaw and hind paw phalanges per fetus. None of these effects were seen at 75 mg/kg/day, a presumptive NOAEL for developmental toxicity.

In addition, Rodricks et al.[9] modeled the high-dose levels at which significant effects were seen using the U.S. EPA's benchmark dose approach. The lowest benchmark dose (BMDL) that provided the best fit to the available male hamster nephropathy data was 46.91 (~47) mg/kg/day. All other hamster endpoints for which there were statistically significant effects yielded BMDLs higher than that. The BMDL that provided the best fit to the rat developmental toxicity data (body weight decreases in F_1 animals) was 75.65 (~76) mg/kg/day. Noting that these two BMDLs were not inconsistent, the BMDL of 47 mg/kg/day was recommended.

The SCCP[7] relied solely on a NOAEL value of 12 mg/kg/d based on hematotoxicity in a chronic exposure study using rats. The SCCP noted that the mean plasma level of 28,160 ng/ml (± 12,928) from the 12 mg/kg/d dose group could be compared to human plasma levels, were they available.

Exposure Assessment

Rodricks et al.[9] noted that, for products that may be ingested, the daily triclosan intake is determined by the amount of product used per day, the percentage of triclosan in the product, the amount of triclosan absorbed by the GI tract, and the body weight of the subject. The corresponding calculation for use of dermal products varies only in replacing GI tract absorption with dermal absorption. Combined oral and dermal intake for adult males and females were determined. Intake for children was also determined, but utilized scaling to convert use rates for liquid body washes and body lotions from available adult usage.

For toothpastes, the adult male intake was 0.005 mg/kg/day; adult female, 0.006 mg/kg/day; and children, 0.023 mg/kg/day. For mouthwashes, the adult male intake was 0.003 mg/kg/day; adult female, 0.004 mg/kg/day; and children, considered to be zero.

For rinse off products such as liquid hand soap, liquid body washes, dish detergents, the adult male intake was 0.007 mg/kg/day, combined; adult female, 0.009 mg/kg/day, combined; children, 0.011 mg/kg/day, combined.

For leave-on products such as body lotions, moisturizers, and deodorants, the adult male intake was 0.033 mg/kg/day, combined; female, 0.046 mg/kg/day, combined; children, 0.042 mg/kg/day.

Were all the products to be used on a daily basis, the intake estimates for adult males, adult females, and children, respectively, would be 0.047, 0.064, and 0.074 mg/kg/day. For comparison purposes, data from biomonitoring levels were converted from urine concentrations to intake estimates. For the 95th percentile level (largest urine concentration levels reported), the intake estimates for adult males, adult females, and children, respectively were 0.009, 0.007, and 0.004 mg/kg/day (5 – 20 times less than the product usage based estimates).

The SCCP[7] determined systemic doses for oral products assuming 100% availability of whatever triclosan was present, toothpaste use levels of 2,750 mg/d, mouthwash use levels of 30,000 mg/d, and triclosan content of 0.3% for toothpaste and 0.3% or 0.2% for mouthwashes. The resulting systemic dose for toothpaste was 0.0234 mg/kg/d and for mouthwash was either 0.10 or 0.15 mg/kg/d for mouthwashes.

For leave-on cosmetics, the SCCP determined systemic doses using dermal absorption based on in vitro data, triclosan content (ranged from 0.15 to 0.3%), surface area exposed (e.g., deodorant stick = 200 cm^2 and body lotion = 15670 cm^2), and one application per day. The resulting systemic dose for deodorant stick was 0.0015 mg/kg/d, for body lotion at 0.15% and 0.3% triclosan was 0.0823 and 0.1646 mg/kg/d, respectively. Face powder systemic doses ranged from 0.004 to 0.006 mg/kg/d and blemish concealer doses ranged from 0.0003 to 0.0006 mg/kg/d.

For rinse-off cosmetics, the SCCP determined systemic doses using dermal absorption based on in vitro data, a 10x dilution of triclosan in the product in use situations, and an 860 cm^2 exposure area for hand soap or 17500 cm^2 for shower gel/body soap. The resulting systemic dose for hand soap was 0.0066 mg/kg/d and, for shower gel/body soap, was 0.0268 mg/kg/d.

Table 10 presents a list of the systemic doses determined by the SCCP as a function of product type.

Margin of Safety

Rodricks et al.[9] determined a margin of safety (MOS) by dividing the BMDL by the daily intake estimate. For such a determination to be the most conservative, the daily intake should be the largest value supportable by the available data and the BMDL should be the lowest value supportable by the available data. For example, the MOS for body lotion usage for adult males is l808, for adult females is 1237, and for children is 1119. These were the lowest MOSs reported. Were the biomonitoring levels (95 percentile) used instead, the MOS for body lotion usage for adult males would be 5222, for adult females would be 6714, and for children would be 11750.

Consideration was also given to the use of multiple products on a daily basis. Were each of the oral care products, rinse-off products, and leave-on products applied on a daily basis, and were each of them preserved with triclosan (unlikely, given the data in Table 5, where only 2% of baby shampoos contain triclosan), then the MOS for adult males would be 1000, for adult females would be 732, and for children would be 634. Rodricks et al.[9] concluded that exposure to triclosan in consumer products is not expected to result in adverse health effects in children or adults who use these products as intended.

The SCCP[7] used the rat NOAEL (which SCCP determined to be 12 mg/kg/d) divided by the systemic dose delivered by products containing triclosan as given in Table 9 to determine an MOS. For toothpaste, the MOS was 513 and for combined use of toothpaste, deodorant sticks, and hand soap, the MOS was 381. For all products usage, which includes body lotion, the MOS values ranged from 49 to 32. The SCCP concluded that use of triclosan up to a maximum concentration of 0.3% in toothpastes, hand soaps, shower gels/body soaps, and deodorant sticks is safe, any additional use in face powders and blemish concealers at concentrations up to 0.3% also is considered safe, but use in other leave-on products (e.g., body lotions) and in mouthwashes is not considered safe for consumer use.

Table 11 provides a side-by-side comparison of MOS determinations by Rodricks et al.[9] and the SCCP [7].

Summary

Triclosan is a chlorinated aromatic compound with functional groups representative of both phenols and ethers. Its IUPAC name is 5-Chloro-2-(2,4-dichlorophenoxy)phenol. Triclosan may function in cosmetic formulations as a cosmetic biocide, deodorant agent, or preservative. At ambient temperatures, triclosan is a crystalline powder, so any material supplied as triclosan in a liquid form, must, by definition, be a mixture with a solvent. Triclosan is supplied to cosmetic formulators under several trade names and in several trade name mixtures.

Information on the frequency of use of triclosan in cosmetics as a function of cosmetic product type is available from the VCRP maintained by the FDA based on voluntary reports from industry. Use concentration data as a function of product type is limited (not all reported uses have use concentrations), but use concentrations in cosmetics appear to be in the 0.01 - 0.3% range. Triclosan also is used in some product categories that raise the possibility of user exposure to aerosols, such as the category “suntan gels, creams, liquids and sprays.” Most aerosol particles from cosmetic products, however, are sufficiently large such that they are deposited in the nasopharyngeal region and are not respirable.

Analysis of triclosan imported from India and China uncovered the presence of dioxin and difuran impurities. USP and the government of Canada have established limits for such impurities.

Independent of the presence of dioxin impurities in triclosan as supplied to cosmetics formulators, there is a question regarding the possibility that triclosan in cosmetic formulations applied to the skin may photodegrade to dioxin compounds on exposure to light. Triclosan can photodegrade to 2,7- and 2,8-dichlorodibenzo-*p*-dioxin, and 2,4-dichlorophenol on exposure to UV radiation at 254 nm. While triclosan absorption of UV is pH dependent, all absorption peaks are below the atmospheric cut-off for UV reaching the earth's surface (290 nm).

Triclosan is slowly and not extensively absorbed by the dermal route, but is rapidly and well absorbed by the oral route. Triclosan measured in rodent radioactivity studies (following oral and dermal exposures) indicate distribution to the liver, lung, kidney, gastrointestinal tract, and gall bladder. Triclosan absorbed from the gastrointestinal tract undergoes extensive first-pass metabolism, which primarily involves glucuronide and sulfate conjugation. Triclosan is also metabolized to its glucuronide and sulfate conjugates by the skin. Triclosan glucuronide is predominantly excreted in the urine, and triclosan is predominantly excreted in the feces. Triclosan administered orally and dermally is excreted in greater concentrations in the urine than in the feces in humans.

Triclosan has low acute toxicity by all routes and species evaluated.

Repeat dose toxicity has been evaluated in the baboon (oral route), rat (oral, and inhalation routes), mouse (dermal route), rabbit (dermal), and hamster (oral). Triclosan NOAELs based on local irritation effects tend to be $\leq$ 10 mg/kg/day by all routes, except inhalation, which has a reported NOAEC of 5×10^{-5} mg/m^3. LOAELs and NOAELs based on systemic toxicity tend to be <1000 mg/kg/day, with no obvious common toxicity among studies and species. Statistically significant increases in nephropathy and stomach pathology were reported in male and female hamsters and statistically significant effects were reported in epididymides and testes in male hamsters, all at the high-dose level of 250 mg/kg/day but not at 75 mg/kg/day.

Triclosan does not appear to have significant reproductive/fertility/developmental toxicity. Triclosan has been linked to hypothyroxinemia in rats and has been suggested as having potential to disrupt the thyroid axis in amphibians. In rats, hypothyroxinemia via a hepatic catabolism mechanism has been suggested, but the implications for human exposure is unclear. One recent study in frogs reported a marginal acceleration of pre-metamorphic development by a non-thyroid mechanism in amphibians, with no overall alteration in metamorphosis. In CD-1 mice given 350 mg/kg/day on gestation days 6 – 15, fetal body weights were significantly reduced in the two highest dose groups. The incidence/litter of irregular skull ossification was significantly increased in high-dose litters and the litter averages for ossified forepaw and hind paw phalanges per fetus. None of these effects were seen at 75 mg/kg/day, a presumptive NOAEL for developmental toxicity.

In various assays for endocrine disruption effects, triclosan gave weak responses, although one study did report competitive binding to the estrogen receptor sufficient to support growth of an estrogen-dependent cell line. A study using frogs concluded that exposure to low levels of triclosan disrupted thyroid hormone-associated gene expression.

Triclosan has been evaluated in a number of standard (and other) genotoxicity assays, including bacterial reverse mutation tests, *in vitro* mammalian cell gene mutation test, and *in vitro* mammalian chromosome aberration tests, a mammalian bone marrow chromosomal aberration test, and an unscheduled DNA synthesis assay in mammalian cells in culture --- except in one (an *in* vitro cytogenetic assay with Chinese

hamster lung fibroblasts), the findings were negative. Based on the weight of evidence, triclosan is not genotoxic.

Rat, mouse, and hamster carcinogenicity studies are available. Rat and hamster oral chronic toxicity/carcinogenicity studies found no carcinogenic potential for USP triclosan in rats at $\leq$ 3000 ppm (in the diet) and in hamsters at $\leq$ 250 mg/kg/day (in the diet). However, a mouse oral chronic/carcinogenicity bioassay was positive for carcinogenicity based on an increased incidence of liver neoplasms in male and female mice at $\geq$30 mg/kg/day. Presuming that activation of peroxisome proliferator activated receptor alpha is the primary mode of action for triclosan-induced hepatocarcinogenesis in mice, these findings did not support either a mutagenic mode or cytotoxic mode of action. FDA has nominated triclosan for dermal carcinogenicity study under the NTP.

In rabbits, triclosan is a moderate dermal irritant as well as a moderate eye irritant, but when tested clinically at concentrations $\leq$ 0.5% in various formulations did not cause dermal irritation. Triclosan in clinical tests and in guinea pig studies was not a sensitizer at concentrations of $\leq$ 0.25% in formulation. Triclosan is not a phototoxicant in guinea pigs.

Triclosan is bacteriostatic at low concentrations and bactericidal at high concentrations (MIC's ranging from 0.1 mg/ml to 33 mg/ml). At high (biocidal) concentrations, Triclosan is very effective and unlikely to produce a major problem of anti-microbial resistance (e.g., all the microorganisms are dead). However, at sub-biocidal and bacteriostatic, concentrations, triclosan is capable of penetrating bacteria and initiating changes related to important mechanisms of antimicrobial resistance including possibly transferable mechanisms of resistance. In actual usage, however, no evidence of such resistance has been seen so far in clinical isolates, and there is no epidemiological evidence to suggest a problem in clinical practice. Although, the stability and persistence of triclosan biocidal resistance has not been widely studied, the limited information available points to resistance being stable over a three to ten year period. One study found no relationship between the use of triclosan and other biocides and antibiotic resistance in homes where biocidal products were or were not being used.

Different approaches have been described for determining the systemic dose that would result from use of triclosan-containing products, although the maximum use concentration of triclosan in those products is given by 0.3% by most. Resulting systemic doses from use of triclosan-containing products have been compared to the dose determined in animal studies to be a NOAEL, but the NOAEL value chosen in one case was 12 mg/kg/d based on hematotoxicity in rats and in another case was 47 mg/kg/d based on nephropathy in hamsters. Resulting MOS values have ranged from a low of 32 for use of all products by one analysis to a high of 47000 for hand soap use by another analysis.

Discussion

The CIR Expert Panel noted that information on the number and types of personal care products and OTC drugs that contain triclosan, and at what use concentration, was now available from a number of sources. For example, there was consistent information suggesting that triclosan was used in body washes at 0.3%. Information on the daily usage of the specific types of products that contain triclosan also were available. For body washes, again by example, the daily use of product for adult males and females was on the order of 12 g/day (a bit over 0.4 oz of body wash) and for children, was 7 g/day (0.25 oz of body wash).

Triclosan absorption through the skin is consistently low and blood levels increased immediately after application and were proportional to dose applied. Because triclosan is metabolized and both glucoronide and sulfate conjugates occur in vivo in humans, the majority of circulating triclosan is in conjugated form, ready for excretion.

Combining the exposure data with estimates of dermal absorption (7.2 – 10.8% for a body wash, considered a rinse-off cosmetic product) yields systemic exposures to triclosan from use of individual products. Similar determinations were available for products used orally (assuming 100% absorption) and for leave-on products applied to the skin.

While different analyses used different estimates for each of these parameters, there was a range of concentrations at which triclosan was used (0.15 – 0.3%) and the amount of product used on a daily basis which varied within high and low limits, so that the systemic exposure to triclosan that resulted could be estimated for each product category. The triclosan exposure range for body washes, for example, was from 0.03 mg/kg bw/d (all ages) to 0.005 mg/kg bw/d (adult female), a factor of 10 difference, between two estimates.

When this exposure information was combined and compared to the exposure level considered to be a no-observable-adverse-effect-level (NOAEL) for triclosan, an MOS ratio was determined.

The CIR Expert Panel considered that an acceptable NOAEL for triclosan was 48 mg/kg bw/d based on male hamster nephropathy data. For body washes, then, the MOS ranged from 1600 to 9600. The CIR Expert Panel considered such MOS values to represent an adequate margin of safety.

Using that same approach, the Panel considered the impact of multiple cosmetic use as well as use of triclosan containing cosmetics in combination with other products known to containing triclosan. The MOS of the combined use of the following product categories: oral products, including mouthwashes and toothpastes; dermal rinse-off products, including hand and body washes; and dermal leave-on products, including deodorants and body lotions, was calculated. A MOS at the low end of the range of 128 was determined for the daily use of all products combined, using the appropriate NOAEL and the most conservative exposure values, with the MOS extending up to 1000 for adult males, 732 for adult females, and 634 for children exposed to all product types, daily.

Were fewer products used that contained triclosan, the exposure estimates would be lower and MOS values would be even higher. For bath products, for example, only 1 out of approximately 700 products on the market actually contained triclosan as reported to the U.S. FDA. Overall, the assumptions made were conservative and would overestimate any risk of exposure.

The Panel also addressed the other issues identified earlier in the document. Impurities data do indicate the potential for low levels of dioxins to be present. Limits on the levels of dioxin compounds in triclosan have been established by the United States Pharmacopeial Convention, suggesting that the technology exists to produce triclosan with minimal levels of dioxins. Accordingly, the Panel expects that dioxins in triclosan supplied for use in cosmetics will be as low as reasonably achievable, and no greater than 1 μg/g (1 ppm).

Available data suggest that photodegradation to produce dioxin takes place at 254 nm, a wavelength of light from the sun that does not reach the earth's surface. Accordingly, in normal use, triclosan would not photodegrade to produce dioxin. The available mouse data that demonstrated statistically significant

increases in liver adenomas and carcinomas seen at several dose levels compared to controls were carefully considered. It was noted that no such effect was seen in rats or hamsters.

The Panel accepted that activation of peroxisome proliferator-activated receptors (PPARs) is a well-characterized, non-genotoxic mechanism by which a cascade of events can lead to tumor formation. Considering PPARα activation as the mode of action (MOA) of triclosan in mice, expression of PPARα in the human liver is $1/10^{th}$ of that in the mouse. A study of a known liver carcinogen in mice containing the gene for human PPARα compared to mice containing the normal mouse gene produced no tumors in the mice containing the human gene and the expected tumors in the mice containing the normal mouse gene. Overall, then, hepatic tumors produced by a PPARα activation MOA in mice are not relevant to predicting human health outcomes.

The Panel also considered a recent study of NHANES data that found a link between triclosan levels in the urine and incidence of allergy/hayfever diagnoses in individuals less than 18 years of age. This link was not demonstrated to be causal and limitations in study design suggested the need for further studies before any conclusion could be reached.

The Panel is aware that additional dermal carcinogenesis work is ongoing under the auspices of the National Toxicology Program (NTP), but believes that completion of this safety assessment should not be delayed in anticipation of those findings. When NTP dermal carcinogenicity data become available, the Panel will consider if the results of this assessment should be reconsidered.

Endocrine disruption is a concern that increasingly has been considered by the Panel. An endocrine disruptor is a substance or mixture that alters function(s) of the endocrine system and consequently produces adverse health effects in an intact organism, or in its progeny, or (sub) populations.

Several endpoints that may be considered to relate to endocrine disruption by triclosan have been studied.

These data include studies that found no effect, and other studies suggesting weak estrogenic and androgenic effects, anti-estrogenic and anti-androgenic effects, and thyroid hormone-associated gene expression. So, while weak or no perturbations of the endocrine system are reported with triclosan exposure, no adverse consequence of those effects is apparent.

Most bacteriocidal agents will exert selective pressure on any bacterial strain exposed to those agents, allowing cells with a mutation making them resistant to the effects of those agents to thrive, and triclosan is no exception. The Panel noted, however, that while bacterial strains with resistance to triclosan can be developed in vitro, this phenomenon is not seen in surveillance studies of organisms in most use situations. Another issue relating to triclosan resistance is the potential that bacteria that acquire triclosan resistance would have the additional resistance to other bacteriocidal agents, such as antibiotics. This phenomenon occurs most frequently when the mode of action of the bacteriocidal agents that makes them effective in killing bacteria is the same or similar. While some data are available that suggested that emergence of triclosan resistance is accompanied by resistance to common antibiotics, many more studies failed to find this effect and one study suggested that triclosan resistant bacteria were more sensitive to aminoglycoside antibiotics.

Overall, the Panel found that the available safety data across a wide variety of studies addressing purity, stability, general toxicity, carcinogenesis, endocrine disruption, and antimicrobial resistance, support a conclusion that triclosan may be used safely in a wide variety of products in the present practices of use and concentration, even if all products types to contain triclosan were used concurrently, on a daily basis.

Conclusion

The CIR Expert Panel concluded that triclosan is safe for use in cosmetics in the practices of use and concentration described in this safety assessment.

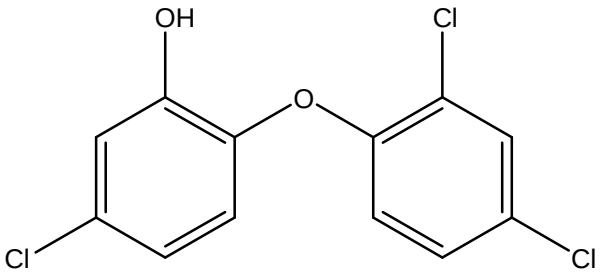

Figure 1. Triclosan (5-Chloro-2-(2,4-dichlorophenoxy)phenol (IUPAC name))

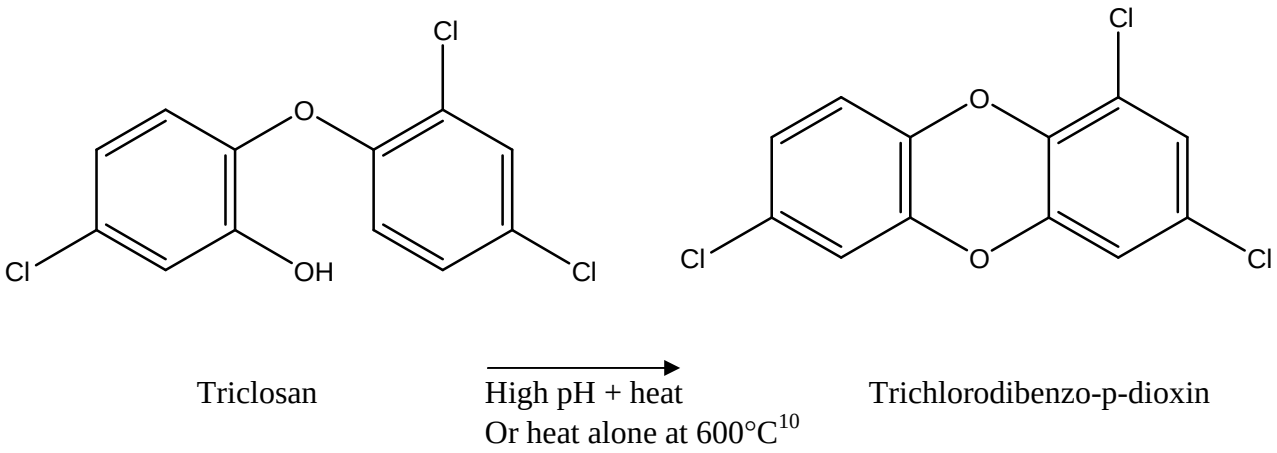

Figure 2. Conversion of triclosan to trichlorodibenzo-p-dioxin
(for illustrative purposes, the structure of triclosan is pictured in Figure 2 with the phenol moiety rotated around the –O bond so that the proximity of the hydroxyl group and conversion to a dioxin compound can be more readily seen).

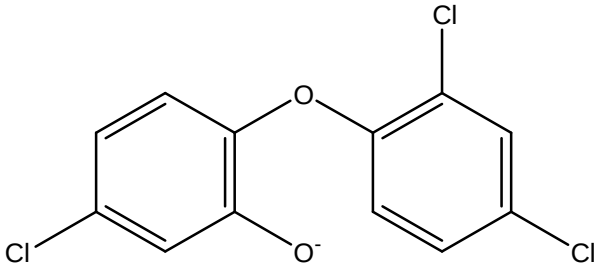

Figure 3. Phenolate form of Triclosan at pH > 8.1.

Table 1. Regulatory Decisions on Triclosan.

Authority	Product Name or Category	Concentration Limit/Restriction	Comment
U.S. (FDA)[44-47]			
Drugs	*Total Toothpaste* (Colgate-Palmolive Co.)	0.30%	OTC dentifrice to treat gingivitis[52]
	Acne therapeutic	Proposed: leave-on: 0.2-0.5%rinse-off: 0.3-1.0%	Under review as an OTC [53]
Other	Soap and deodorant	Antibacterial soaps generally contain ≤ 0.3% triclosan.[53]	Antibacterial claim
Devices	*TempBond Clear with Triclosan* (Sybron Dental Specialties, Inc.)	NR	Regulated as a temporary dental cement. [54]
	MONOCRYL Plus (Poliglecaprone 25) Antibacterial Suture* (Ethicon Inc.)	≤ 2360 µg/m	Regulated as an absorbable surgical suture[55]
Europe[56]	Cosmetic products	≤ 0.3%	
Canada[57]	Cosmetic products (mouthwashes excluded)	≤ 0.3% in other cosmetic products	
	All oral products	≤ 0.03% in mouthwashes; not to be used by children < 12 years of age and labeled "do not swallow"	
		polychlorinated dibenzo-p-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) impurities <0.1 ng/g 2,3,7,8-tetra-chlorodibenzo-p-dioxin and 2,3,7,8-tetra-chlorodibenzofuran; and <10 µg/g total other PCDD/PCDF impurities, with no individual impurity greater than 5 µg/g	
Japan[58]	Cosmetic products as a preservative	≤0.10 g/100 g product (≤0.1%)	
Australia[3]	Cosmetic products	≤ 0.3%	Provisional recommendation. Eye, skin, respiratory system irritant. Recommendation for compliance with USP limits for dioxins and dibenzofurans (synthesis impurities).
Norway[59]	Cosmetic products	"… should be restricted."	Concern about bacterial resistance to triclosan and to clinically important antimicrobial agents.

NR = not reported

Table 2: Triclosan Physicochemical Properties

Property	Value and Conditions	Reference
Molecular weight	289.541	14
Physical state	white crystalline powder	3,16
Specific Gravity	1.55 X 10^3 kg/m^3	14
Density	1.55 g/cm^3 at 22° C	3
Acid Dissociation Constant (pKa)	8.14 at 21° C	14
pH	Not available	14
Stability	Neat triclosan is stable to UV radiation	3
	Triclosan solutions are not stable to chlorine	3
	Stable under normal storage conditions (ambient temperature) when tested after 4 and after 9 years	7
Melting point	56.5° C	14
	54 – 57.3° C	16
Boiling point	280 – 290° C (decomposes)	16
Water solubility	0.012 g/l at 20° C	14
	20 mg/l at 20° C	16
Octanol-water partition coefficient (Log $P_{o/w}$)	4.8 at 25° C	14
	4.76	16
Vapor Pressure	5.2 x 10^{-6} mm Hg at 25° C	14
	2.2 x 10^{-6} mm Hg at 20° C	
	4 x 10^{-6} mm Hg at 20° C	16

Table 3. Measured 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-Tetrachloro-dibenzofuran (TCDF) impurities in Triclosan from India and China.[60]

Sample #	***Country***	***TCDD (pg/g)*** [a]	***TCDF (pg/g)*** [a]
1	India	17.2	0.70
2	China	95.4	7.13
3	India	111.8	3.43
4	India	41.5	8.51
5	India	1712.0	0.43
6	India	18.9	207.3

[a] those values in excess of USP specifications[14] are highlighted.

Table 4. USP and Health Canada's Limits on Triclosan Impurities

Impurity	**USP 32 (2009)[14]**	**Canada[57]**
2,4 Dichlorophenol	< 10 µg/g (< 10 ppm or 0.001%)	NA
3-Chlorophenol	< 10 µg/g (< 10 ppm or 0.001%))	
4-Chlorophenol	< 10 µg/g (< 10 ppm or 0.001%)	
2,3,7,8 Tetrachlorodibenzo-p-dioxin	<1 pg/g (1 ppt)[a]	≤ 0.1 ng/g
2,3,7,8 Tetrachlorodibenzo-furan	<1 pg/g (1 ppt)[a]	≤ 0.1 ng/g
2,8-Dichlordiobenzofuran	< 0.25 µg/g (< 0.25 ppm or 0.000025%)	≤ 10 µg/g total other PCDD/PCDF impurities, with no individual impurity greater than 5 µg/g
2,8-Dichlorodibenzo-p-dioxin	< 0.5 µg/g (< 0.5 ppm or 0.00005%)	
1,3,7 Trichlorodibenzo-p-dioxin	< 0.25 µg/g (< 0.25 ppm or 0.000025%)	
2,4,8 Trichlorodibenz-furan	< 0.5 µg/g (< 0.25 ppm or 0.000025%)	
Other	Contains not less than 97.0% triclosan calculated on the anhydrous basis. Not more than 0.5% of total impurities. Not more than 0.1% of any individual impurity.	manufacturers must possess: raw material specifications for triclosan; identification of analytical method used to determine PCDD and PCDF levels; and finished product specifications

NA = Not applicable or not specified

PCDD/PCDF = polychlorinated dibenzo-p-dioxin / polychlorinated dibenzofuran

[a] Calculated as follows: (1 pg/µl) x (10 µl) x (1/30 g) x (30 µl) = 1 pg/g = 1 ppt[60]

Table 5. Frequency of use and use concentrations of Triclosan in cosmetics.

Product Category	Total number of products in each product category[22]	Number of products containing Triclosan in each product category[22]	Personal Care Products Council Survey Concentration of Use (%) [23]
Baby Products[a]			
Shampoos	56	1	None reported
Lotions, oils, powders, and creams	137	3	None reported
Other	143	None reported	None reported
Baby products subtotal	**336**	**4**	**None reported**
Bath products			
Oils, tablets, and salts	314	1	None reported
Bubble baths	169	None reported	None reported
Capsules	4	None reported	None reported
Other	234	None reported	None reported
Bath products subtotal	**721**	**1**	**None reported**
Eye Makeup			
Eyebrow pencils	144	None reported	None reported
Eyeliners	754	None reported	None reported
Eye shadow	1215	18	0.05%
Eye lotions	254	2	None reported
Eye makeup remover	128	None reported	None reported
Mascara	499	2	None reported
Other	365	6	None reported
Eye makeup subtotal	**3359**	**28**	**0.05%**
Fragrance products			
Colognes and toilet waters	1377	27	0.1%
Perfumes	666	None reported	None reported
Powders	221	5	None reported
Sachets	12	None reported	None reported
Other	566	10	None reported
Fragrance products subtotal	**2842**	**42**	**0.1%**

Product Category	Total number of products in each product category[22]	Number of products containing Triclosan in each product category[22]	Personal Care Products Council Survey Concentration of Use (%) [23]
Non-coloring hair care products			
Conditioners	1226	1	0.05%
Sprays/aerosol fixatives	312	None reported	None reported
Straighteners	178	None reported	None reported
Permanent waves	69	None reported	None reported
Rinses	33	None reported	None reported
Shampoos[c]	1361	None reported[c]	0.04 - 0.2%[c]
Tonics, dressings, etc.	1205	1	0.1%
Wave sets	51	None reported	None reported
Other	807	1	None reported
Non-coloring hair care products subtotal	**5242**	**3**	**0.04 – 0.2%**
Hair coloring product[b]			
Dyes and colors	2393	None reported	None reported
Tints	21	None reported	None reported
Rinses	40	None reported	None reported
Shampoos	40	None reported	None reported
Color sprays	7	None reported	None reported
Lighteners with color	21	None reported	None reported
Bleaches	149	None reported	None reported
Other	168	None reported	None reported
Hair coloring products subtotal	**2839**	**None reported**	**None Reported**

Product Category	Total number of products in each product category[22]	Number of products containing Triclosan in each product category[22]	Personal Care Products Council Survey Concentration of Use (%) [23]
Makeup			
Blushers (all types)	434	1	0.2%
Face powders[c]	661	None reported[c]	0.2%[c]
Foundations	589	5	0.1%
Leg and body paints	29	None reported	None reported
Lipsticks	1883	None reported	None reported
Makeup bases	117	1	None reported
Rouges	102	None reported	None reported
Makeup fixatives	45	None reported	None reported
Other	485	3	0.3%
Makeup subtotal	**4345**	**10**	**0.1 – 0.3%**
Nail care products			
Basecoats and undercoats	79	None reported	None reported
Cuticle softeners	27	None reported	None reported
Creams and lotions	14	None reported	None reported
Extenders	2	None reported	None reported
Nail polishes and enamels	333	None reported	None reported
Nail polish and enamel removers	24	None reported	None reported
Other	138	1	None reported
Nail care products subtotal	**617**	**1**	**None Reported**
Oral hygiene products			
Dentifrices	59	None reported	None reported
Mouthwashes and breath fresheners [c]	74	None reported [c]	0.04%[c]
Other	86	None reported	None reported
Oral hygiene products subtotal	**219**	**None reported**	**0.04%**

Product Category	Total number of products in each product category[22]	Number of products containing Triclosan in each product category[22]	Personal Care Products Council Survey Concentration of Use (%) [23]
Personal hygiene products			
Bath soaps and detergents	1665	45	None reported
Underarm deodorants	580	162	0.2 - 0.3%
Douches	14	None reported	None reported
Feminine deodorants	19	None reported	None reported
Other	792	19	0.3%
Personal hygiene products subtotal	**3070**	**226**	**0.2 - 0.3%**
Shaving Products			
Aftershave lotions	367	2	None reported
Beard softeners	3	None reported	None reported
Mens talcum	3	None reported	None reported
Preshave lotions	22	None reported	None reported
Shaving cream	122	3	None reported
Shaving soap	10	None reported	None reported
Other	134	6	None reported
Shaving products subtotal	**661**	**11**	**None Reported**
Skin care products			
Skin cleansing creams, lotions, liquids, and pads	1446	31	0.01-0.3%
Depilatories	42	Not reported	None reported
Face and neck creams, lotions, etc.	1583	30	0.1%
Body and hand creams, lotions, etc.	1744	27	0.1%
Foot powders and sprays	47	6	None reported
Moisturizers	2508	28	None reported
Night creams, lotions, powder and sprays	353	14	None reported
Paste masks/mud packs	441	12	None reported
Skin fresheners	259	3	None reported
Other	1308	11	None reported

Skin care products subtotal	**9731**	**162**	**0.01-0.3%**
Product Category	**Total number of products in each product category[22]**	**Number of products containing Triclosan in each product category[22]**	**Personal Care Products Council Survey Concentration of Use (%) [23]**
Suntan products			
Suntan gels, creams, liquids and sprays	107	2	None reported
Indoor tanning preparations	240	1	None reported
Other	62	None reported	None reported
Suntan products subtotal	**409**	**3**	**None reported**
Total usage/concentrations-of-use range across all product categories	**34391**	**491**	**0.01-0.3%**

[a] For baby shampoos reported, only ~2% contain Triclosan, or conversely, the vast majority do not, and the situation is similar for the baby lotions, powders, and creams category. While uses of Triclosan were reported to FDA under the VCRP for each of these categories, no use concentrations were provided in the industry survey. And no uses of Triclosan or use concentrations were reported for the 143 products in the "other" baby products category.
[b] None of the 2839 hair coloring products were reported to contain Triclosan in the VCRP and no use concentrations were reported by industry.
[c] While no reported uses were submitted to the VCRP, a use concentration was reported in the Council survey, so it must be presumed there is at least one use.

Table 6. Triclosan in urine from NHANES subjects.[29]

		Urine concentrations (μg/L)		
Group	Sample size	Geometric mean	50th percentile	95th percentile
All	2514	13.0	9.2	459.0
6 - 11 years	314	8.2	5.9	148.0
12 - 19 years	713	14.5	10.2	649.0
20 - 59 years	950	14.7	10.3	491.0
≥60 years	537	10.3	6.5	386.0
All male	1228	16.2	11.7	566.0
All female	1286	10.6	7.4	363.0

Table 7. Triclosan route-specific and species-specific repeat-dose NOAELs.[3-6]

Route, Duration, Species	NOAEL	Comments
Dermal		
Subacute		
Rat	3.0 mg/kg/day	LOAEL = 6.0 mg/kg/day (local effects at application sites)
Rat	7.5 mg/kg/day, males 3.5 mg/kg/day, females	Basis for NOAEL selection: irritation
Mouse	0.6 mg/animal (100 µg/cm^2)	LOAEL = 1.5 mg/kg/day (dermal irritation and increased absolute and relative liver weights)
Rabbit	15%	None.
Subchronic		
Rat	40 mg/kg	NOAEL based on systemic toxicity, characterized as occult blood in urine (EPA) or lack thereof (NICNAS). Each Agency may have drawn a different conclusion from the data, which NICNAS stated were unreliable evidence of systemic toxicity.
Rat	80 mg/kg	
Rat	10 mg/kg/day	NOAEL from above study, but based on local irritation – reversible after a 20 day recovery period.
Rat	2.5%, 5%	None
Inhalation		
"All Durations"		
Rat	None assigned	LOAEL = 3.21 mg/kg/day, males, 9.91 mg/kg/day females. Based on increased total leucocyte count and serum alkaline phosphatase.
Subacute		
Rat	NOAEC (irritation): 5 x 10^{-5} mg/m^3 [b]	LOAEL (systemic): 1300 mg/m^3, clinical signs of toxicity and death after 2 days of dosing
Oral		
Subchronic		
Dog	12.5 mg/kg	Effects observed at all doses; NOAEL based on reversal of serum alkaline phosphatase elevations after 28-day recovery period.
Rat	1000 ppm (52.4 mg/kg/day)	LOAEL: 3000 ppm (168.0 mg/kg/day) based on liver histopath.
Mouse	None assigned	LOAEL: 25 mg/kg/day. Based on hematology parameters, relative liver weights and total cholesterol. NICNAS excluded this study from its risk assessment due to mechanistic differences between humans and mice.
Chronic		

Route, Duration, Species	NOAEL	Comments
Baboon	30 mg/kg	Based on diarrhea at LOAEL of 100 mg/kg/day
Rat	40 mg/kg/day, males 56 mg/kg/day, females	NOAEL based on histopath. changes in male liver and a trend for reduced body weight in females (LOAEL not reported).
Rat	1000 ppm (52.4 mg/kg/day)	LOAEL of 3000 ppm (168.0 mg/kg/day) based on significant body weight decreases (both sexes) and non-neoplastic liver changes in males.
Mouse	10 mg/kg/day (systemic)	Based on neoplasms (both sexes) at 30 mg/kg/day [interpreted as mouse-specific and PPARα-,mediated]
Hamster	75 mg/kg/day	Based on LOAEL of 250 mg/kg/day (both sexes) based on decreased body weight gains, mortality, nephropathy, and histopathology (stomach and testes).

Table 8. Hepatic carcinomas and adenomas in mice as a function of triclosan dose.

	Number of tumor bearing mice[a]					
	Carcinoma		Adenoma		Combined	
Dose (mg/kg/day)	Males	Females	Males	Females	Males	Females
0	2	0	5 (5)	0	6 (6)	0
10	3 (3)	0	7 (7)	1	10 (10)	1
30	6 (3)	1 (1)	13 (16)[b]	3 (3)[b]	17 (17)[c]	3 (3)[b]
100	11 (9)[c]	1 (1)	22 (24)[c]	6 (6)[c]	32 (32)[c]	6 (6)[c]
200	24 (22)[c]	14 (14)[c]	26 (26)[c]	11 (11)[c]	42 (42)[c]	20 (20)[c]

[a] results in parentheses are based on a pathology peer review conducted after the study using the liver slides prepared during the study.

[b] statistically significant at $p \leq 0.05$.

[c] statistically significant at $p \leq 0.01$.

Table 9. Observations made in various governmental reviews regarding triclosan endocrine disruption effects in fish, frog, and *in vitro* preparations.

Fish	Weakly androgenic, weakly estrogenic, toxic (altered fin length, sex ratio, etc.) [4]
	Preliminary data indicate that triclosan (or metabolite) is not potently estrogenic to freshwater fish but it may be weakly estrogenic, anti-androgenic, or androgenic. [3]
Frog	Induces estrogen antagonism following intraperiotoneal injection of high doses; reduced testosterone levels at lower doses. Exposure also resulted in decreased T3-mediated TRβ mRNA expression in the tadpole tail fin and altered thyroid hormone receptor α transcript levels in the brain of pre-metamorphic tadpoles.[4]
In vitro	Competively binds to estrogen receptor and supports growth of estrogen-dependent MCF-7 cell line. Binds to rat androgen receptor [4]

Table 10. Systemic triclosan doses determined as a function of product type containing triclosan [7].

Product	Triclosan content (%)	Systemic triclosan dose (mg/kg/d)
Toothpaste	0.3	0.0234
Hand soap	0.3	0.0066
Shower gel/body soap	0.3	0.0268
Deodorant	0.3	0.0015
Mouthwash	0.2	0.1000
	0.3	0.1500
Face powder	0.2	0.0040
	0.3	0.0060
Body lotion	0.15	0.0823
	0.3	0.1646
Blemish concealer	0.15	0.0003
	0.3	0.0006
Toothpaste, hand soap, shower gel/body soap, deodorant combined	0.3	0.0583
Mouthwash, body lotion, face powder, blemish concealer combined	0.15 - 0.3	0.1866
	0.3	0.3212
All products	0.15 - 0.3	0.2449
	0.3	0.3795

Table 11. Comparison of margin of safety (MOS) determinations.

Product	MOS from Rodricks et al. (2010)[9]			MOS from SCCP[7]
	adult male	adult female	child	
toothpaste	9400	7834	2043	513
mouthwash	15667	11190	ND	80 - 120
hand soap	47000	47000	9216	1118
body washes	11750	9400	7833	448
body lotion	1808	1237	1119	73 - 146
deodorant	15667	15667	ND	8000
combined exposures	1000	732	634	32 – 49

ND = not determined

References

1. European Centre for Disease Prevention and Control (ECDC), European Food Safety Authoirty EFSA European Medicines Agency EMEA Scientific Committee on Emerging and Newly Identified Health Risks SCENIHR. Joint Opinion on Antimicrobial Resistance (AMR) Focused on Zoonotic Infections. 2009. http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_026.pdf. Date Accessed 10-15-2009.

2. European Commission Directorate-General for Health & Consumers.Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). Assessment of the Antibiotic Resistance Effects of Biocides. 2009. http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_021.pdf. Date Accessed 10-15-2009.

3. Australian Government, Department of Health & Ageing (NICNAS). Priority Existing Chemical Assessment Report No. 30. "Triclosan". 1-1-2009. http://www.nicnas.gov.au/Publications/CAR/PEC/PEC30/PEC_30_Full_Report_PDF.pdf. Date Accessed 10-15-2009.

4. United States Environmental Protection Agency (EPA). Office of Prevention, Pesticides and Toxic Substances. Reregistration eligibility decision (RED) for Triclosan, List B, Case 2340. 9-1-2008. http://www.epa.gov/oppsrrd1/REDs/2340red.pdf. Date Accessed 10-15-2009. Report No. EPA 739-RO-8009.

5. National Toxicology Program. United States Food and Drug Administration (FDA). Department of Health and Human Services. Nomination Profile. Triclosan. [CAS 3380-34-5]. Supporting Information for Toxicological Evaluation Toxicology Program. 2008. http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/triclosan_508.pdf. Date Accessed 10-15-2009.

6. United States Environmental Protection Agency (EPA). Memorandum. January 4, 2008. Triclosan: Report of the Cancer Assessment Review Committee. PC Code: 054901. http://www.epa.gov/pesticides/chemical/foia/cleared-reviews/reviews/054901/054901-2008-01-04a.pdf

7. European Commission. Directorate General for Health and Consumer Protection. Scientific Committee on Consumer Products (SCCP). Opinion on Triclosan. 1-21-2009. http://ec.europa.eu/health/archive/ph_risk/committees/04_sccp/docs/sccp_o_166.pdf. Date Accessed 6-22-2010.

8. European Commission. Directorate General for Health and Consumers. Scientific Committee on Consumer Safety (SCCS) Opinion on triclosan antimicrobial

resistance. 6-22-2010. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_023.pdf. Date Accessed 7-20-2010.

9. Rodricks JV, Swenberg JA, Borzelleca JF, Maronpot RR, and Shipp AM. Triclosan: A critical review of the experimental data and development of margins of safety for consumer products. Early online. *Crit Rev.Toxicol*. 2010.

10. Environ International Corporation. 5-17-2010. Written communication - investigation of potential endocrine activity of triclosan.

11. Finking R. 8-30-2010. Triclosan: Current Issues. Presentation to CIR Expert Panel (power point presentation available on request).

12. Gottschalck TE and Bailey JE. International Cosmetic Ingredient Dictionary and Handbook. Washington, DC: Personal Care Products Council, 2010.

13. Fiege, H, Voges, H-W, Hamamoto, T, and et al. Phenol derivatives. In: *Ullmann's Encyclopedia of Industrial Chemistry*. 6 ed. John Wiley & Sons Inc; 2002:

14. United States Pharmacopeia. USP 32. The National Formulary. NF 27. Official Monographs. "Triclosan.". 2009.

15. Menoutis, J and Parisi, Al. Testing for dioxin and furan contamination in triclosan. *Cosmetics and Toiletries Magazine*. 2002;117(10):75-78.

16. Ciba.United States Pharmacopeia (USP): Standards of Quality and Purity. http://www.ciba.com/ind-pc-triclosan-global-steward-ups.htm. Date Accessed 10-14-2009.

17. Turner, Richard British Pharmacopeia Secretariat. Written communication. 11-2-2009.

18. Havery, Don. Personal communication. 11-2-2009.

19. Rule, KL, Ebbett, VR, and Vikesland, PJ. Formation of chloroform and chlorinated organics by free-chlorine-medicated oxidation of triclosan. *Environ Sci Technol.* 2005. 39:(9): pp.3176-3185.

20. Lorres, M and et al. Short Communication: Confirmation of the formation of dichorodibenzo-*p*-dioxin in the photodegradation of triclosan by photo-SPME. *Anal Bioanal Chem*. 2005;3811294-1298.

21. Wong-Wah-Chung P, Rafqah S, Voyard G, and Sarakha M. Photochemical behavior of triclosan in aqueous solutions: Kinetic and analytical studies. *J.Photochem.Photobiol.A: Chemistry*. 2007;191(2-3):201-208.

22. US Food and Drug Administration (FDA).Uses of triclosan as a function of product category form the Voluntary Cosmetic Registration Program database.

23. Bailey, John. Written Communication. 10-26-2009.

24. Jensen PA and O'Brien D. Industrial Hygiene. Willeke K and Baron PA.In: *Aerosol Measurement: Principles Techniques and Applications*. New York: John Wiley and Sons, Inc.; 1993:538-540.

25. Oberdorster G, Oberdorster E, and Oberdorster J. Nanotoxicology: An Emerging Discipline Evolving from Studies of Ultrafine Particles. *Environmental Health Perspectives*. 2005;113(7):823-839.

26. James AC, Stahlhofen W, Rudolf G, and et al. Annexe D. Deposition of inhaled particles. *Annals of the ICRP*. 1994;24(1-3):231-232.

27. Bower D. Unpublished information on hair spary particles provided at the September 9, 1999 CIR Expert Panel Meeting.

28. Johnson MA. The influence of particle size. *Spray Technology and Marketing*. 2004;(November):24-27.

29. Calafat AM, Le X, Wong L-Y, Reidy JA, and Needham LL. Urinary Concentrations of Triclosan in the U.S. Population: 2003-2004. *Environ Health Perspect*. 2008;116(3):303-307.

30. National Toxicology Program. FDA Nomination Profile - Triclosan [CAS 3380-34-5]. Supporting Information for Toxicological Evaluation by the National Toxicology Program. 2008. http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/triclosan_508.pdf. Date Accessed 10-15-2009.

31. Boobis AR, Cohen SM, Dellarco V, and et al. IPCS framework for analyzing the relevance of a cancer mode of action for humans. *Crit Rev Toxicol.* 2006;36781-792.

32. Fort DJ, Rogers RL, Gorsuch JW, Navarro LT, Peter R, and Plautz JR. Triclosan and Anuran Metamorphosis: No Effect on Thyroid-Mediated Metamorphosis in Zenopus laevis. *Toxicological Sciences*. 2010;114392-400.

33. Ahn KC, Zhao F, Chen J, Cherednichenko G, Sanmarti E, Denison MS, Lasley B, Pessah IN, Kultz D, Chang DPY, Gee S, and Hammock BD. In vitro biologic activities of the Antimicrobials triclocarban, its analogs, and triclosan in bioassay screen: receptor-based bioassay screens. *Environ Health Perspect*. 2008;1161203-1210.

34. Gee RH, Charles A, Taylor N, and Darbre PD. Oestrogenic and androgenic activity of triclosan in breast cancer cells. *J Appl.Toxicol*. 2008;2878-91.

35. James MO, Li W, Summerlot D, Rowland-Faux L, and Wood CE. Triclosan is a potent inhibitor of estradiol and estrone sulfonation in sheep placenta. 2009 epub. *Environment International*. 2009.

36. Kumar V, Chakrabortya A, Kural MR, and Roya P. Alteration of testicular steroidogenesis and histopathology of reproductive system in male rats treated with triclosan. *Reprod Toxicol.* 2009;27(2009):177-185.

37. Zorrilla LM, Gibson EK, Jeffay SC, Crofton KM, Setzer WR, Cooper RL, and Stoker TE. The effects of triclosan on puberty and thyroid hormones in male wistar rats. *Toxicological Sciences*. 2009;107(1):56-64.

38. Paul KB, Hedge JM, DeVito MJ, and Crofton KM. Short-term exposure to triclosan decreases thyrozine in vitro via upregulation of hepatic catabolism in Young Long-Evans rats. *Toxicological Sciences*. 2010;113367-379.

39. Allmyr M, Panagiotidis G, Sparve ED, Diczfalusy U, and Sandborgh-Englund G. Human Exposure to Triclosan via Toothpaste does not change CYP3A4 Activity or Plasma Concentrations of Thyroid Hormones. *Basic & Clinical Pharmacology and Toxicology*. 2009;105(5):339-344.

40. DeSalva, SJ, Kong, M, and Lin, Y-J. Triclosan: a safety profile. *American Journal of Dentistry*. 1989;2186-196.

41. Lyman, FL and Furia, T. Toxicology of 2,4,4'-trichloro-s'hydroxy-diphenyl ether. *Industrial Medicine*. 1969;3845-52.

42. Clayton EMR, Todd M, Dowd DB, and Aiello AE. The Impact of Bisphenol A and Triclosan on Immune Parameters in the US Population, NHANES 2003-2006. 11-9-2010. Doi: 10.1289/ehp.1002883.

43. Scientific Steering Committee of the European Commission Health & Consumer Protection Directorat-Gengeral, 27-28 June 2002. 2002. http://ec.europa.eu/food/fs/sc/ssc/out269_en.pdf

44. Cole EC, Addison RM, Rubino JR, and et al. Investigation of antibiotic and antibacterial agent cross-resistance in target bacteria from homes of antibacterial product users and nonusers. *Journal of Applied Microbiology*. 2003;95664-676.

45. Lear et al. *Journal of Pharmacy and Pharmacology*. 2001;52126S.

46. Walker C, Borden LC, Zambon J, Bonta CY, DeVizio W, and Volpe AR. The effects of a 0.3% triclosan-containing dentifrice onthe microbia composition of supragingival plaque. *J Clin Periodontol*. 1994;21334-341.

47. Dunford RG. Efficacy of a triclosan/NAF dentifriceint he control of plaque and gingivitis and concurrent oral microflora monitoring. *Am J Dent*. 1998;11259-270.

48. Aiello, AE, Larson, EL, and Levy, SB. Consumer antibacterial soaps: effective or just risky? *Clin Infect Dis.* 2007. 45: pp.S137-S147.

49. Beier RS, Duke SE, Ziprin RL, and et al. Antibiotic and disinfectant susceptibility profiles of vancomycin-resistant *Entercoccus faecium* (VRE) isolated from community wastewater in Texas. *Bull.Environ.Contamin.and Toxicol.* 2008;80(3):188-194.

50. Stickler DJ and Jones GL. Reduced Susceptibility of Proteus mirabilis to Triclosan. *Antimicrobial Agents and Chemother.* 2008;53(3):991-994.

51. Chen Y, Phi B, Zhou H, Yu Y, and Li L. Triclosan resistance in clinical isolates of Acinetobacter baumannii. *J.Med.Microbiol.* 2009;581086-1091.

52. Draft Guidance for Industry on Gingivitis: Development and Evaluation of Drugs for Treatment or Prevention; Availability. *Federal Register*. 6-28-2005;7037102-37103.

53. ICIS Chemical News.Ciba Defends antibacterial triclosan in soap. http://www.icis.com/Articles/2008/06/23/9132724/ciba-defends-antibacterial-triclosan-in-soap.html. Date Accessed 11-18-2009.

54. Sybron Dental Specialties. Section III – 510(k) Summary of Safety and Effectiveness. 2-3-2006. http://www.accessdata.fda.gov/cdrh_docs/pdf5/K053565.pdf. Report No. K053565.

55. Ethicon Inc. Summary of Safety and Effectiveness. 6-29-2005. http://www.accessdata.fda.gov/cdrh_docs/pdf5/K050845.pdf. Report No. K050845.

56. European Commission. Volume 1. Cosmetics legislation. Cosmetic products. 1999 Edition. Directive 76/768/EEC, Annex VI, part 1. 1999.

57. Health Canada. List of Prohibited and Restricted Cosmetic Ingredients. Canada's Cosmetic Ingredient Hotlist. 9-1-2009. http://www.hc-sc.gc.ca/cps-spc/person/cosmet/info-ind-prof/_hot-list-critique/hotlist-liste-eng.php. Date Accessed 10-12-2009.

58. Japan Ministry of Health, Labour and Welfare. Evaluation and Licensing Division. Pharmaceutical and Food Safety Bureau and Ministry of Health and Welfare. "Standards for Cosmetics". Notification No.331 of 2000, 2006. 2010. http://www.mhlw.go.jp/english/topics/cosmetics/index.html. Date Accessed 11-18-2009.

59. Norwegian Scientific Committee for Food Safety. Risk Assessment on the Use of Triclosan in Cosmetics. 1-31-2005. Report No. #04/406-16.

60. Gilbert, Brian. Written communication. 11-10-2009.