EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

OPINION ON

Triclosan

COLIPA n° P32

Scientific Committees
on consumer products
on emerging and newly identified health risks
on health and environmental risks

The SCCP adopted this opinion at its 19th plenary of 21 January 2009

About the Scientific Committees
Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCP
Questions concerning the safety of consumer products (non-food products intended for the consumer).
In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

Scientific Committee members
Claire Chambers, Gisela Degen, Ruta Dubakiene, Bozena Jazwiec-Kanyion, Vassilios Kapoulas, Jean Krutmann, Carola Lidén, Jean-Paul Marty, Thomas Platzek, Suresh Chandra Rastogi, Jean Revuz, Vera Rogiers, Tore Sanner, Günter Speit, Jacqueline Van Engelen, Ian R. White

Contact
European Commission
Health & Consumer Protection DG
Directorate C: Public Health and Risk Assessment
Unit C7 - Risk Assessment
Office: B232 B-1049 Brussels
Sanco-Sc6-Secretariat@ec.europa.eu

(ISSN)

http://ec.europa.eu/health/ph_risk/risk_en.htm

ACKNOWLEDGMENTS

Dr. C. Chambers
Prof. G. Degen (rapporteur)
Dr. B. Jazwiec-Kanyion
Prof. V. Kapoulas
Prof. J.-P. Marty
Prof. T. Platzek
Dr. S.C. Rastogi
Prof. J. Revuz
Prof. V. Rogiers
Prof. T. Sanner (chairman)
Dr. J. van Engelen
Dr. I.R. White

Keywords: SCCP, scientific opinion, preservative, triclosan, P32, directive 76/768/ECC, CAS 3380-34-5, EINECS 222-182-2

Opinion to be cited as: SCCP (Scientific Committee on Consumer Products), Opinion on triclosan, 21 January 2009

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1. BACKGROUND

Triclosan (CAS 3380-34-5) with the chemical name 5-chloro-2-(2,4-dichlorophenoxy)phenol or 2,4,4'-trichloro-2'-hydroxy-diphenyl ether has a long history of use as a preservative in cosmetic products. It is currently regulated in Annex VI, entry 25 with a maximum concentration of 0.3%.

An opinion on triclosan (SCCP/1040/06) was adopted by the SCCP at the 9th plenary meeting of 10 October 2006 with the following conclusions to the request:

1. "*On the basis of the available data, the SCCP is of the opinion that there is presently no evidence of clinical resistance and cross-resistance occurring from the use of triclosan in cosmetic products. Information is required on consumer exposure to triclosan from all sources, including cosmetic products.*

2. *For a toxicological assessment of the safe use of triclosan, the SCCP requires a dossier to be submitted in which data is provided to all relevant exposure and toxicological end-points and conforming to currently accepted standards. This should be regarded as a matter of urgency because triclosan has been identified in human milk of some European populations."*

The dossier provided by Industry consists of an update on the bacterial resistance issue (submission III) and of a toxicological dossier for triclosan (submission IV).

Furthermore the Norwegian authority on cosmetics has earlier this year submitted a report "Risk assessment on the use of triclosan in cosmetics; Development of antimicrobial resistance in bacteria – II".

2. TERMS OF REFERENCE

1. *Does SCCP consider a continued use of triclosan as a preservative in cosmetic products as safe for the consumer at the current concentration limit of maximum 0.3% taking into account the provided toxicological data?*

2. *Does SCCP consider a continued use of triclosan as a preservative in cosmetic products as safe taking into account the new provided documentation of resistance development by certain micro-organisms and cross-resistance?*

3. OPINION

This opinion only addresses possible toxicological effects of triclosan on human health (question 1 in Terms of Reference). It does not cover aspects of potential resistance induction in micro-organisms by triclosan, which will be treated in a separate opinion.

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Triclosan (INCI)

3.1.1.2. Chemical names

2,4,4'-trichloro-2'-hydroxy-diphenylether

3.1.1.3. Trade names and abbreviations

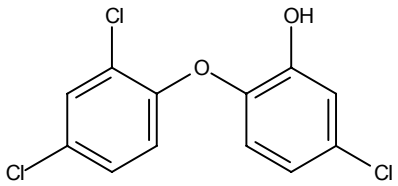
Irgasan® DP300, Irgasan® PG60, Irgacare® MP, Irgacare® CF100, Irgacide® LP10

Triclosan is also referred to as Irgasan, DP300, FAT 80'023), CH 3565, and GP 41-353 in a number of toxicology studies

3.1.1.4. CAS / EINECS number

CAS: 3380-34-5
EINECS: 222-182-2

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{12}H_7Cl_3O_2$

3.1.2. Physical form

White crystalline powder

3.1.3. Molecular weight

Molecular weight: 289.5

3.1.4. Purity, composition and substance codes

Identification: IR Spectroscopy

The purity of batches of triclosan used in personal care products since the 1970s has been as described in the following table:

Purity Specifications for triclosan since 1970						
Test Point	**Effective from 1970**	**Effective from 26.09.1985**	**Effective from 1.1.1990**	**Effective from 31.12.1994**	**Effective from 26.6.2000**	**Effective from 06.11.2003**
Triclosan Active Substance[1]	99.0 - 100.0%	99% min	99% min	99.0-100%	97.0-103.0%	97.0 - 103.0%

[1] Analysis by gas chromatography

The purity of different batches of triclosan used in the toxicology studies is described in the following table.

Purity of triclosan Batches Used in Toxicology Studies	
Triclosan Batch Number/Information	**Purity[1]**
FAT 80'023/A Mischung 652	99.3%
FAT 80'023/B CH3565, Mg 120	99.3
FAT 80'023 P4-11-210 , Package Nr. RP68118	99.9
FAT 80'023 BA Irgasan DP300, Batch #5.2.0211.0	99
Lot No. S 15155 T01 = Unilever No. S15155 T01	≥99%
FAT 80'023/H 5/0/0194/0, Batch No. EN46856.02	Within specifications[2]
FAT 80'023/Q Batch No. EN 91390.76	99.6
Triclosan R&D name: GP41353 PBS 5357.0, Lot No. 800187	100.3
C-P sample No 39317 Lot 19851206, Irgacare MP	101
C-P sample No. 38328 Lot 19851206, Irgacare MP	101
FAT 80'023/R Batch No. EN 275927.26	99.5%
FAT 80'023/S Batch No. 505017	99.5%
Lot 020750A7	99.8
P&G No. D1063.01 D1063.02R	99.7

[1] Analysis using gas chromatography
[2] A compiled analytical report is not available, but data shows the batch was within specifications.

Water content: ≤ 0.1%

3.1.5. Impurities / accompanying contaminants

Individual related compound (Gas Chromatography)	≤0.1%
Total related compounds (Gas Chromatography)	≤ 0.5%
2,4 Dichlorophenol	≤10 mg/kg
Sum of 3- and 4-Chlorophenol	≤10 mg/kg
2,3,7,8 Tetrachlorodibenzo-p-dioxin	<0.001 µg/kg
2,3,7,8-Tetrachlorodibenzo-furan	<0.001 µg/kg
2,8-Dichloroldibenzo-p-dioxin	≤0.5 mg/kg
1,3,7-Trichlorodibenzo-p-dioxin	≤0.25 mg/kg
2,8-Dichlorodibenzo-furan	≤0.25 mg/kg
2,4,8-Trichlorodibenzo-furan	≤0.5 mg/kg
Ash	≤0.1%
Mercury	≤1 mg/kg
Arsenic	≤2 mg/kg
Antimony	10 mg/kg
Lead	≤10 mg/kg
Cadmium	≤5 mg/kg
Nickel	≤10 mg/kg
Copper	≤10 mg/kg
Chromium	≤2 mg/kg
Sum of heavy metals as lead sulfide precipitation	≤20 mg/kg

3.1.6. Solubility

Solubility of triclosan in some selected solvents and chemicals	
Solvent	**Solubility at 25°C (g triclosan/100 g solvent)**
Distilled water (20°C)	0.001
Distilled water (50°C)	0.004
1 N caustic soda	31.7
1 N sodium carbonate	0.40
1 N ammonium hydroxide	0.30
Triethanolamine	>100
Acetone	>100
Ethanol 70% or 95%	>100
Isopropanol	>100
Propylene glycol	>100
Polyethylene glycol	>100
Methyl cellosolve (Union Carbide Corp.)	>100
Ethyl cellosolve (Union Carbide Corp.)	>100
Dipropylene glycol	~40
Glycerine	0.15
n-Hexane	8.5
Petroleum jelly (white, USP)	~0.5
Tween 20 (ICI America Inc.)	>100

Solubility of triclosan in some selected solvents and chemicals	
Solvent	**Solubility at 25°C (g triclosan/100 g solvent)**
Tween 80 (ICI America Inc.)	>100
Triton X-100 (Rohm & Haas)	>100
Olive oil	~60
Castor oil	~90

3.1.7. Partition coefficient (Log P_{ow})

Log Po/w: 4.8

3.1.8. Additional physical and chemical specifications

Melting point: 57 ± 1°C
Boiling point: /
Relative density: 1.55 ± 0.04 g/cm^3
Vapour pressure: 4 x 10^{-6} mmHg (20°C)
Viscosity: /
pKa: 8.14 (20°C)
Refractive index:
UV_Vis spectrum (200-800 nm): /

3.1.9. Homogeneity and Stability

The stability of triclosan under normal storage conditions (ambient temperature) has been assessed with a batch produced in 1973 and re-analyzed 4 and 9 years after manufacturing. The study showed that triclosan does not decompose under normal storage conditions; the quality has remained constant over the 9 years of storage.

Storage time	Starting time (1973)	After 4 years (1977)	After 9 years (1982)
Appearance	White, fine crystalline	White, fine crystalline	White, fine crystalline
Content active substance	99%	99.7%	99.5%

[1] Irgasan Dp 300, Batch No. EN 30142 storage conditions: ambient temperature

General Comments to physico-chemical characterisation

- Stability of triclosan in marketed products is not reported.

3.2. Function and uses

Triclosan is an antibacterial ingredient that has been used in consumer products for over 30 years. It is widely used as a non-ionic antibacterial agent in personal care products (*e.g.*, bar and liquid soaps, deodorants (Danish EPA, AR5), skin-care products, foot-care products oral care products, and make-up products). Triclosan was approved in 1986 by the European Community Cosmetic Directive for preservative in cosmetics products at concentrations up to 0.3%. Triclosan was evaluated also by SCF [SCF, 2000, AR8] and EFSA [EFSA, 2004, AR6] for use in food contact materials and classified in SCF_List 3 with a restriction of 5 mg/kg of food.

Other fields of applications are textiles (*i.e.*, sport wear) and plastic materials (*i.e.*, plastic containers, brushes), with a triclosan concentration of up to 0.3%.
In the EU, about 85% of the total volume of triclosan is used in personal care products, compared to 5% for textiles and 10% for plastics and food contact materials. The quantity used within the EC reached approximately 450 tons (as 100% active) in the year 2006.
At or near typical usage levels, triclosan appears to intercalate into the membrane of bacteria, resulting in destabilised structure/function. This level of exposure results in disruption of nutrient uptake, inhibition of amino acid incorporation, inhibition of uracil incorporation, as well as membrane lysis.

3.3. Toxicological Evaluation

A number of the animal toxicology studies were conducted prior to the publication of GLP standards and the establishment of OECD guidelines for the conduct of such studies; of these studies, many were still considered relevant, based on comparability to OECD guidelines. However, other non-GLP/non-OECD animal studies provided limited supportive information. The pivotal repeated-dose, sub-chronic and chronic studies were conducted pursuant to GLP regulations and generally followed the OECD guidelines. These pivotal studies are described in more detail.

3.3.1. Acute toxicity

A number of acute toxicity studies have been conducted for triclosan using the oral, dermal, intraperitoneal or intravenous routes of administration in mice, rats, rabbits, and dogs. The study designs were not always consistent with OECD guidelines for acute toxicity studies and there were no GLP compliance statements. A number of the acute toxicity studies were conducted prior to the establishment of GLP regulations.

3.3.1.1. Acute oral toxicity

Triclosan is not acutely toxic *via* the oral route of administration, with high oral intubation LD_{50} values in the range of 3,750 to 5,000 mg/kg bw in mice and rats, and an oral capsule LD_{50} value of greater than 5,000 mg/kg bw in dogs.

Ref.: 1

3.3.1.2. Acute dermal toxicity

The dermal LD_{50} value for triclosan was reported to be greater than the high dose of 9,300 mg/kg bw tested in rabbits. These data indicate that triclosan is not acutely toxic *via* the dermal route of administration.

Ref.: 1

3.3.1.3. Acute inhalation toxicity

No acute inhalation toxicity studies were available.

3.3.1.4. Additional acute toxicity studies

Mice and rats administered triclosan intravenously at 10, 20 and 30 (rats only) mg/kg bw in a vehicle solution of triethyleneglycol/water (1/2) showed signs of slight cramps, exopthalmos (mice only), mydriasis (rats only), dyspnoea, and ventral decubitus, with recovery by 24 to 48 h after dosing.

Ref.: 2, 3

Mice in the intraperitoneal studies showed signs of general lethargy, lassitude, close huddling, lack of response to tactile stimuli and no increase in respiration rate and hyperactivity with death occurring between 6 and 72 hours.

Ref.: 4, 5

Data from the study by Kanetoshi *et al.* (1992) were also mentioned in a review by Bhargava and Leonard (1996).

Additional References Bhargava, Leonard 1996

Table 1: summary of LD_{50} values from toxicity studies in mice, rats, rabbits, and dogs

Species	**Route of Administration**	**LD_{50}** (mg/kg)	**Reference; GLP and OECD Status**
Mouse (NMRI)	Intravenous	19	Walther, 1968a (2); Predates GLP and OECD
Mouse	Oral Gavage	4,350	DeSalva *et al.*, 1989 (1); Not reported, but some studies likely predate GLP and OECD
Mouse (CD-1) (Female)	Intraperitoneal	184	Miller *et al.*, 1982 (4); GLP: not reported OECD: no comparable guidelines
Mouse (ddy) (Male)	Intraperitoneal	1,090	Kanetoshi *et al.*, 1992 (5); GLP: not reported OECD: no comparable guidelines
Rat (Wister CFE)	Intravenous	29	Walther, 1968b (3); Predates GLP and OECD
Rat	Oral Gavage	3,750 to 5,000	DeSalva *et al.*, 1989 (1); Not reported, but some studies likely predate GLP and OECD
Rabbit	Dermal	>9,300	DeSalva *et al.*, 1989 (1); Not reported, but some studies likely predate GLP and OECD
Dog	Oral Capsule	>5,000	DeSalva *et al.*, 1989 (1); Not reported, but some studies likely predate GLP and OECD

3.3.2 Irritation and corrosivity

3.3.2.1. Skin Irritation

Table 2: Findings from Non-GLP Skin Irritation Studies with triclosan

Species (Strain)	Application Details	Major Findings	Reference, GLP and OECD Status
Guinea pig (Pirbright white)	0.1 mL single application, test site: 2 cm^2 area on the shaven back, concentrations of 0.1, 0.5, 1.0, and 5.0% (duration of exposure and occlusion status unknown)	Erythema reactions were only observed in the highest dose group (4/10 positive responses) at 24 hours post-application. No positive responses were reported at 48 hours.	Thomann and Maurer, 1978 (7) Predates GLP and OECD
Rabbit (Russian)	Triclosan-soaked 2.5 cm^2 gauze patches with occlusive dressings applied to shaved intact skin or abraded skin sites on the backs and flanks of the rabbits for 24 h (concentration of triclosan unknown).	Erythema reactions were observed at 24 h after the start of exposure [3/6 positive responses in intact skin (mean score: 2.5); 5/6 positive in abraded skin (mean score 2.8)]. Oedema reaction at 24 h was positive in 1/6 animals. Erythema reactions improved at 72 h (mean scores: 1.3 and 2.5 in intact and abraded skin, respectively). Triclosan did not induce corrosion effects. Triclosan was classified as a moderate irritant based on an overall score of 3.58 (irritance scores of greater than 6 would have been considered "severe").	Sachsse and Ullmann, 1975 (6) Predates GLP and OECD

The results of the guinea pig study suggests that triclosan is not a skin irritant at concentrations below 5%, while both studies show that the erythema reactions are reversible.

Skin irritation data were also available from 14-day repeated-dose and 90-day sub-chronic dermal toxicity studies of triclosan in the rat, mouse, and newborn monkey (Tables 10, 14 and 15). In the 14-day mouse and rat studies, conducted as dose range finding studies for longer-term repeated dose toxicology studies [Burns, 1997a (8); Burns, 1996 (9); Burns, 1997b (10)], erythema and scaling were observed in the mouse at doses of 1.5 mg/animal/day and higher (applied as solutions of 1.5 to 6%). Erythema, oedema, fissuring, eschar, alopecia, thickening and discoloration of the test site were noted in the rat at doses of 3.0 and 6.0 mg/animal/day (applied as solutions of 1% and 2% in 0.3 mL acetone). These studies show that repeated topical application of triclosan (up to 6%) resulted in moderate to severe skin irritation in the rat and mouse. In the 90-day sub-chronic toxicity study in rats, reversible skin irritation was observed starting at the lowest dose tested of 10 mg/kg bw/day (a concentration of approximately 0.5% triclosan in propylene glycol applied in a volume of 2 mL/kg body weight) [Trimmer, 1994 (11)]. In an early non-GLP study, dermatitis was observed in weanling dogs treated for 90 days with 200 mg/kg body weight/day, but not in dogs treated with the lower doses of 20 or 2 mg/kg bw/day [Dorner, 1973 (12)]. Limited and selective reporting of findings made the interpretation of the data from this dog study unreliable. In an early non-GLP study in newborn Rhesus monkeys bathed using a 15 mL of a 0.1% soap solution containing triclosan, no signs of dermal irritation were observed after 90 days of daily bathing [Hazleton Laboratories, 1979a (13)].

Additional skin irritation data are available from studies in humans (Section 3.3.11.3-1).

3.3.2.2. Mucous membrane Irritation

Table 3: Findings from a Mucosal Irritation Study with Triclosan in Hamsters

Species (Strain)	Application Details	Major Findings	Reference; GLP and OECD Status
Hamster (Syrian golden)	1.5% sodium lauryl sulphate (SLS), 1.5% SLS and 0.3% triclosan, or 0.3% triclosan in paste, 1 cm^2 area of the right cheek pouch, 4 applications for 15 seconds at 24-hour intervals.	The histological structure of the mucosa of the triclosan-treated animals (Group 3) was similar to that of the control specimens. Structural changes, including basal hyperplasia, acanthosis, hypergranulosis, and orthokeratotic hyperkeratosis, were present in animals treated with SLS, or with SLS and triclosan. No signs of inflammation were observed in the subepithelial connective tissue.	Baert *et al*., 1996 (14); GLP: not specified

The results of this study show that the application of triclosan in a paste (0.3% triclosan) does not result in mucosal irritation in the hamster cheek pouch.

Table 4: Findings from a GLP Eye Irritation Study with Triclosan

Species (Strain)	Application Details	Major Findings	Reference, GLP and OECD Status
Rabbit (New Zealand White)	0.1 g of pure solid triclosan was instilled into the conjunctival sac of the left eye (control: right eye). Examinations were on Days 1, 2, 3, 4, and 7.	Based on mean irritation scores in the cornea, iris, and conjunctiva, triclosan was found to cause slight primary eye irritation when applied to the rabbit eye mucosa.	Ullmann, 1980 (15); GLP: comparable OECD 405 consistent

Table 5: Findings from a Non-GLP Eye Irritation Study with Triclosan

Species (Strain)	Application Details	Major Findings	Reference; GLP and OECD Status
Rabbit (strain not reported)	Triclosan was applied either in gum Arabic (1, 2, 5, 10, or 20% concentrations) into the conjunctival folds (observations up to 24 h), or as an undiluted substance (observations up to 11 days) without rinsing, or with rinsing after 2 or 4 seconds of exposure.	Triclosan at concentrations of 1, 2, 5, and 10% produced only slight to moderate reddening of the conjunctiva as observed 2 hours after exposure, with recovery within 24 hours. Triclosan at a 20% concentration caused reddening and slight swelling of the conjunctiva as observed 24 hours after the start of exposure. The undiluted (pure) triclosan caused fully reversible eye irritation effects, as observed in rinse experiments, with a return to normal eye state between 7 and 11 days after exposure.	Lyman and Furia, 1969 (16); Predates GLP and OECD

The results of eye irritation studies in rabbits showed that triclosan causes slight eye irritation when tested in its undiluted form. Triclosan did not produce either severe irritation or corrosive effects. Minimal to slight irritation effects to the cornea, iris, and conjunctivae were observed in tests using pure triclosan, resulting in an overall irritation score that indicated slight primary eye irritation [Ullmann, 1980 (15)]. Similarly, when tested at concentrations of 1, 2, 5, and 10% in gum Arabic, triclosan was shown to cause only

reddening of the conjunctivae as observed at 2 hours, which was fully reversed within 24 hours after the start of exposure [Lyman and Furia, 1969 (16)]. Triclosan at the high concentration of 20% caused slight to pronounced reddening and slight swelling of the conjunctiva that was still observed after 24 hours.

Summary of Irritation/Corrosivity Data

Triclosan was a skin irritant at a level of 5% but not at 1% in the guinea pig skin irritation study. Repeated topical application of 1 to 6% triclosan for 14 days in dermal toxicity dose range-finding studies resulted in moderate to severe skin irritation in the rat and mouse. Lower concentrations were without effect in these studies. However, all of the doses in the 14- and 90-day dermal toxicity studies are considerably higher than exposures expected from the use of triclosan-containing personal care products. Effects were also observed in a skin toxicity study of 90 days duration in the rat at a concentration of 0.5%. In summary, the irritation/corrosivity data from either irritation studies in the hamster, guinea pig, and rabbit, or skin toxicity studies conducted in the mouse, rat, monkey, and dog suggest that triclosan may cause slight reversible skin irritation at concentrations of 0.5 to 5% under experimental conditions.
Triclosan was not an irritant to mucous membranes in the hamster cheek pouch assay at a level of 0.3%.
Triclosan at concentrations of 1 to 10% produced only slight, reversible irritation in the rabbit eye.

3.3.3. Skin sensitisation

Table 6: Findings from Sensitisation Studies with Triclosan

Species (Strain)	Application Details	Major Findings	Reference; GLP and OECD Status
Guinea pig, Hartley (albino)	Guinea pig Buehler test. N=5 treated animals, 6 control animals. 1% triclosan in a cream/gel (occluded dermal application for both induction and challenge). Induction: 9 5-hour exposures on backs of animals. No adjuvant. Challenge: 14 to 21 days after induction.	Induction: Slight primary irritation was observed after the first few treatments but was alleviated with regular wash-off procedures. Challenge: Treated sites showed slight irritation (redness) 24 and 48 hours after the challenge. Previously untreated sites did not show any significant oedema or erythema after challenge. Skin contact sensitization did not occur.	Toxicological Resources, 1974 (17); Predates GLP and OECD
Guinea pig, Hartley (albino)	Split Adjuvant method. N=20 animals/treated or control group. Induction: triclosan (10% in petrolatum) applied 3 times. Complete Freund's adjuvant administered intradermally between 2nd and 3rd induction doses. Challenge: triclosan (3% in petrolatum) applied once 13 days after induction.	Induction: Slight erythema without oedema or vesiculation was observed in 7 of 20 triclosan-treated animals. Erythema disappeared 1 or 2 days after the last application. Challenge: Bright pink and moderately elevated reaction in 1 of 20 animals at 24 and 48 hours post-challenge. At 72 hours, erythema was still present but without oedema. There were no reactions in any of the control group animals. The authors concluded that triclosan has a very low sensitisation index.	Lachapelle and Tennstedt, 1979 (18); Predates GLP and OECD

Species (Strain)	Application Details	Major Findings	Reference; GLP and OECD Status
Guinea pig, Pirbright white	Modified Maximisation test (Optimization). N=10/sex/group. Induction: 0.1% triclosan in propylene glycol, 6 intracutaneous injections over 3 weeks. (Complete Freund's adjuvant was used during the 2nd and 3rd weeks.) 1st challenge: 14 days after last induction, intracutaneous (0.1% triclosan in 40% propylene glycol). 2nd challenge: 14 days after 1st induction, occluded patch (0.1% triclosan in petrolatum).	Induction: Erythema results were not reported for the induction phase. Challenge: The incidence of positive reactions was similar for triclosan-treated and control animals (4/20 after first challenge compared to 4/19 in the vehicle control group; 3/20 after second challenge compared to 1/19 in the vehicle control group). The investigators concluded that triclosan showed no skin-sensitising potential in this study.	Maurer *et al*., 1979 (19); Predates GLP and OECD
Guinea pig (Hartley albino)	Guinea pig Buehler test. N=10/group. Induction: 25% triclosan initially (reduced to 10%, then 2% (topically applied 3 times / week for 3 weeks). No adjuvant. Challenge: 5% triclosan in propylene glycol, considered the highest non-irritating concentration, applied to a naïve site. Positive control: DNCB treatment.	GLP-compliant. Conducted according to OECD Guideline No. 406. Skin reactions scoring >0.5 were considered to be positive (maximum score: 3). Induction: very faint to severe erythema, depending on the dose level (note that doses were continually reduced to reduce irritation). Challenge: After challenge, triclosan treatment induced weak, non-confluent reactions of very weak erythema (scores of 0.5) in 6/10 animals (not considered to indicate sensitisation). There were no scores greater than 0.5 among triclosan-treated animals. Negative controls showed similar faint erythema reactions in 2/5 animals (scores of 0.5). Positive controls showed strong reactions (scores of 1 to 3) in 10/10 animals (mean erythema scores of 1.8). The investigators concluded that triclosan is not a contact sensitizer.	Wnorowski, 1994 (20); GLP: compliant OECD: No.406 consistent

Triclosan as tested in 4 studies was found not to cause skin sensitisation. Both the 1974 and the 1978 studies used methods that were similar to those recommended by current OECD guidelines for sensitisation studies, and the more recent 1994 study was GLP-compliant and followed OECD (No. 406).

In a small Buehler test in guinea pigs, slight irritation was observed during the induction period with 1% triclosan, attributed to build-up of the cream formulation on the test site [Toxicological Resources, 1974 (17)]. The study investigators concluded that no sensitisation was observed, as previously untreated sites did not show any significant oedema or redness following the challenge dose. Slight irritation (erythema without oedema) also was observed during induction in a much larger sensitisation study using the "split adjuvant" method in guinea pigs [Lachapelle and Tennstedt, 1979 (18)]. In this study, a positive sensitisation reaction following challenge was observed in only 1 of 20 animals. Thus, these authors also concluded that triclosan has a very low sensitisation index. In the third study, a modified maximisation test in guinea pigs, there was no significant difference in sensitisation reactions following challenge in animals treated with 0.1% triclosan in propylene glycol compared to animals treated with the vehicle alone [Maurer *et al*., 1979 (19)]. In the GLP study, a Buehler assay conducted following OECD guidelines, triclosan showed no evidence of sensitisation potential, in contrast to the positive control substance [Wnorowski, 1994 (20)]. Altogether, the results of these studies suggest that triclosan is not a sensitising agent as tested in the guinea pig.

Summary of Sensitisation Data

Triclosan has been tested in skin sensitisation studies in guinea pigs, with both adjuvant and non-adjuvant methods used.
In summary, the results of these GLP and non-GLP studies indicate that there is no evidence for sensitisation with triclosan in various formulations and concentrations (up to 10% in petrolatum) in the guinea pig.

Results of related studies in Humans are reported in section 3.3.11.

3.3.4. Dermal / percutaneous absorption

In Vitro Studies of Dermal/Percutaneous Absorption

A single *in vitro* percutaneous absorption study with triclosan was conducted in rat skin [Moss *et al*., 2000 (21)]. The main findings in this study are provided in Table 7. After 24 hours, 58% of the applied dose remained on the skin surface and in the stratum corneum (33 and 25%, respectively) and 41.2% of the applied dose was recovered in the receptor fluid and in the epidermis and dermis (23 and 18.2%, respectively). Thus, it can be considered that 41.2% of the applied dose was absorbed percutaneously within 24 hours (penetration through the stratum corneum into deeper layers of the skin is considered to represent absorption). Triclosan was primarily absorbed through the skin as the parent compound, with some glucuronide and sulphate conjugates being detected in the receptor fluid. Glucuronidation was the primary route of metabolism of triclosan in rat skin.

Table 7: Findings from an *In Vitro* Percutaneous Absorption Study for Triclosan in Rat Skin

Method	Major Findings	Reference; GLP Status
Diffusion cell system using dorsal skin. Seven µL of 64.5 mM [^{3}H]-triclosan in an ethanol-water (9:1, v/v) solution (0.48 Mbq) was applied to the exposed skin surface (0.64 cm^2).	After 24 hours, approximately 23% of the applied dose appeared in the receptor fluid, and 33%, 25%, and 18.2% remained on the skin surface, stratum corneum, and epidermis and dermis, respectively. Of the radioactivity in the receptor fluid at 24 hours, 17.3% of the applied dose was present as triclosan, 4.1% as triclosan glucuronide, and 0.9% as triclosan sulfate. Of the radioactivity in the skin at 24 hours, 13% of the dose was recovered as triclosan, 1.4% as triclosan glucuronide, and 1.6% as triclosan sulfate.	Moss *et al.*, 2000 (21); GLP: not specified

In Vivo Studies of Dermal/Percutaneous Absorption

The main findings in the *in vivo* percutaneous absorption studies for triclosan are provided in Table 8.

Table 8: Findings from *In Vivo* Percutaneous Absorption Studies for Triclosan

Species	Method	Major Findings	Reference; GLP Status
Mouse	Single application of liquid soaps. Test site: 1 cm x 3 cm area on the back. 22.54 to 25.49 µg triclosan/cm^2 skin.	Triclosan deposition on hairless mouse skin was 0.98 ± 0.11 and 1.16 ± 0.13 µg/cm^2 skin, following application of approximately 22.54 and 25.49 µg triclosan/cm^2 skin. The percent of the applied dose deposited on the skin was 4.425 ± 0.617 and 4.809 ± 1.236, respectively.	Demetrulias, 1985 (22); GLP: not specified

Species	Method	Major Findings	Reference; GLP Status
Mouse	0.05 mL of [^{3}H]-Irgasan® DP300 (1.6 mg, 10 µCi) in olive oil was applied to a 5 cm^2 area of the shaved back.	Maximum tissue levels (at 12 or 18 hours) were highest in the gallbladder (402 ± 57 µg/g), followed by liver (13.4 ± 3.5 µg/g), fat (10.0 ± 3.5 µg/g), lung (7.5 ± 2.9 µg/g), blood (6.5 ± 1.9 µg/g), and kidney (4.3 ± 0.8 µg/g). Maximal levels in the testes, heart, and spleen were in the range of 1.1 to 1.8 µg/g. Low levels were detected in the brain (0.2 to 0.5 µg/g).	Kanetoshi *et al.*, 1992 (5); GLP: not specified
Mouse (CD-1)	0, 0.3, 0.6, 1.5, 3.0, or 6.0 mg/animal/d in acetone for 14 days, applied to 2x3 cm^2 area on the dorsal area once daily.	Toxicokinetic data from a dose range finding repeated dose dermal irritation study. Two pooled plasma samples per treatment group (5 mice/sex/pooled sample). Mean plasma triclosan levels in males: 32.5, 63.3, 98.8, 148.2, and 173.3 µg/mL at doses of 0.3, 0.6, 1.5, 3.0, and 6.0 mg/animal/d, respectively. Females: 63.2, 124.8, 144.0, 124.0, 295.2 µg/mL for same doses. Note that absorbed amount as percentage of applied dose was not calculated.	Burns, 1997a (8) GLP: compliant
Mouse (CD-1)	0, 0.3, 0.6, 1.5, 3.0, or 6.0 mg/animal/d in propylene glycol for 14 days, applied to 2x3 cm^2 area on the dorsal area once daily.	Toxicokinetic data from a dose range finding repeated dose dermal irritation study. Two pooled plasma samples per treatment group (5 mice/sex/pooled sample). Mean plasma triclosan levels in males: 7.4, 22.2, 38.6, 75.4, and 72.6 µg/mL at doses of 0.3, 0.6, 1.5, 3.0, and 6.0 mg/animal/d, respectively. Females: 9.2, 33.0, 47.0, 101.8, 112.1 µg/mL for same doses. Note that absorbed amount as percentage of applied dose was not calculated.	Burns, 1996 (9) GLP: compliant
Rat (Crl:CDBR)	0, 0.3, 0.6, 1.5, 3.0, or 6.0 mg/animal/d in acetone for 14 days, in a volume of 300 µL, applied to 2x3 cm^2 on the dorsal area once daily.	Toxicokinetic data from a dose range finding repeated dose dermal irritation study. Plasma samples were taken from 10 rats/sex/treatment group. Mean plasma triclosan levels in males: 1.0, 2.1, 6.6, 14.1, and 31.6 µg/mL at doses of 0.3, 0.6, 1.5, 3.0, and 6.0 mg/rat/day, respectively. Females: 1.2, 2.4, 5.2, 9.2, and 18.1 µg/mL. Note that absorbed amount as percentage of applied dose was not calculated.	Burns, 1997b (10) GLP: compliant
Rat	Triclosan in an ethanol solution, in shampoo, or in an aerosol deodorant was applied to 7.5 cm^2 clipped dorsal skin	Ethanol solution: 27.6% of the applied dose was absorbed within 48 hours. Blood levels were in the range of 0.07 to 0.30 µg/mL with T_{max} at 6 hours. Shampoo: Penetration of [H^3]-triclosan was dependent on concentration and independent of duration of contact (5, 10, or 20 minutes). Absorption after 48 hours was in the range of 2.8 to 4.1% of the applied dose. Deodorant: Report notes difficulty in administering a standard, accurate, known dose, so refers to data from the application using an ethanol solution as vehicle.	Black and Howes, 1975 (23); Predates GLP
Rat	Triclosan in an ethanol-water (9:1, v/v) solution (6.92 Mbq) was applied to a 9.6 cm^2 circular area on the shaved backs of the rats.	After 24 hours, 0.88% of radioactivity was in the urine, 11.84% in the faeces, 0.02% in the blood, 26.13% on the skin surface, 4.31% in the skin, 0.24% in the cage wash, 7.72% in the carcass, 36.33% in the stratum corneum, and 1.38% on the skin cover. Recovery of radioactivity was 90.46% of the dose.	Moss *et al.*, 2000 (21); GLP: not specified
Rat	Single application of triclosan (4 mg/cm^2, 400 mg/kg, 10 µCi/rat) to the shaven back	The applied dose remained primarily on the adhesive pad (~64%). Absorbed triclosan was primarily excreted in the faeces. 0.5%, 14.66%, 0.1%, 5.5%, 7.2% of the applied dose was recovered in the urine, faeces, blood, skin, and carcass/tissues, respectively.	Hong *et al.*, 1976 (24); Predates GLP

Species	Method	Major Findings	Reference; GLP Status
Rat	Single application of triclosan solution (ethanol, acetone), in cream, or in Vaseline to the neck	Solutions – within 96 hours, 2.8 to 3.7% and 69 to 89% of the applied dose were excreted in the urine and the faeces, respectively. Cream – within 48 hours, 1% and 22% of the applied dose were excreted in the urine and faeces, respectively. Vaseline – within 144 hours, 13% and 60% of the applied dose were excreted in the urine and faeces, respectively.	Ciba-Geigy, 1976b (25); Predates GLP
Rabbit	Single or repeated (5x) application of triclosan in solution (propylene glycol, DMSO, nickethamide, or hexane), in cream, or in soap solution (site not reported)	Solutions – 47 to 53% and 38% of the applied dose excreted in the urine and faeces, respectively, independent of dose. Creams – 29 to 48% of the applied dose was excreted in urine, inversely dependent on quantity of cream applied per unit area. ≤2% excreted in faeces. Soap solutions – 2 to 10% of the applied dose was absorbed (radioactivity measured in urine, faeces, skin, expired air, and organs and tissues).	Ciba-Geigy, 1976b (25); Predates GLP
Rabbit	Urine-soaked diapers containing 6.4 to 26.9 µg triclosan/g were applied to intact or abraded skin (dorsal and flank) and re-applied twice at 4-hour intervals, then left on overnight	Trace amounts of triclosan (*i.e.*, lower than the detection limit) were detected in some of the blood and faecal samples, but in most cases none was present. The amount of triclosan in the urine was in the range 0.1 to 3.8 µg (no difference between animals with intact or abraded skin).	Ciba-Geigy, 1977b (26); Predates GLP
Guinea pig	Single application or repeated application (twice daily for 5 days) of soap suspension or non-soap detergent suspension was applied to a 20 cm^2 area (40 cm^2 area in 1 experiment) on the clipped skin of the back for 2 minutes.	Triclosan remained primarily on the stratum corneum, and small amounts penetrated into the epidermis, dermis, hair follicles, and sebaceous glands. Repeated application did not increase localization in any area of the skin. Increasing concentrations of triclosan resulted in increasing triclosan deposition throughout the skin depth. Recovery of triclosan in rinse water accounted for 80-90% (single application) and 95% (repeated application mean) of the applied activity. Blood levels were in the range of 0.002 to 0.006 µg/mL and 0.014 to 0.027 µg/mL after a single application (40 cm^2 *vs.* 20 cm^2). The blood level was 0.019 µg/mL following repeated applications. Levels of triclosan in the tissues were extremely low (ng/g range). Triclosan was excreted mainly in the urine with relatively small amounts in the faeces.	Black *et al.*, 1975 (27); Predates GLP
Dog	1.3 to 5.0 mL mouthrinse, 15 minutes daily for 7 days	Peak blood levels (6-8 hrs post-dose) were 0.7 to 2.7% (mean = 1.4%) of the applied dose. Daily urinary excretion of free triclosan and conjugates was in the range of 1 to 4% (mean = 2%) of the applied dose.	Lin *et al.*, 1994 (28); GLP: not specified
Dog	Triclosan in water (200 mg/kg), once daily for 2 weeks, nuchal skin.	Blood levels were 130 and 165 ppb on Day 7 and 14, respectively.	Hong et al., 1976 (24); Predates GLP
Monkey	2 Rhesus monkeys, 3 days old, were washed once with a soap solution containing triclosan (1 mg/mL).	Blood levels reached a plateau by 8 to 12 hours and remained up to 24 hours after treatment. Levels of conjugated triclosan (glucuronide and sulfate) in the blood were in the range of 0.25 to 0.68 ppm. No free, unconjugated triclosan was detected. Triclosan sulfate levels in the blood increased, while triclosan glucuronide levels decreased with time post-treatment.	Parkes, 1978a (29); Predates GLP

Species	Method	Major Findings	Reference; GLP Status
Monkey	10 monkeys/group were washed daily for 90 days with 15 mL of a soap solution (0.1% triclosan) (size of application area not reported). 5/group were retained for a 30-day recovery period.	Levels of conjugated triclosan (glucuronide and sulfate) in the blood ranged from 0.17 to 0.97 ppm. No free, unconjugated triclosan was detected. Triclosan glucuronide predominated in the initial samples (Day 1 or 2). Triclosan sulfate predominated in all subsequent blood samples (80-90% of total present after 90 days). Urinary concentrations of triclosan were in the range of 0.3 to 4.8 ppm (primarily the glucuronide conjugate). Faecal concentrations of triclosan were in the range of <0.1 to 10.5 ppm (primarily the unconjugated form). Small amounts of triclosan (<0.1 to 1.9 ppm) were detected in tissues from treated animals. Following the 30-day recovery period, triclosan was detected only in skin samples.	Hazleton Laboratories, 1979b (30); Predates GLP

Deposition and absorption of triclosan were investigated in 4 mouse studies. In a skin deposition study, the triclosan residue on mouse skin was approximately 1 µg/cm^2 (4.4 to 4.8% of the applied dose) following application of liquid soap containing [^{14}C]-triclosan (22.5 to 25.5 µg/cm^2) for 10 minutes [Demetrulias, 1985 (22)]. In a percutaneous absorption study in the mouse, the maximal radioactivity in tissues (observed at 12 or 18 hours) following absorption of [^{3}H]-triclosan was in the range of 14 to 67% of the maxima obtained following oral administration (µg/g range) [Kanetoshi *et al.*, 1992 (5)]. The absorption of triclosan was assessed in two 14-day repeated dose dermal toxicity studies that showed dose-dependent increases in plasma triclosan levels following application of triclosan in propylene glycol and in acetone vehicles, respectively [Burns, 1996 (9), 1997a (8)]. Triclosan in plasma was detected at the lowest doses used in the studies (0.3 mg/mouse/ day, or 12 mg/kg bw/day in a 25 g mouse, giving plasma levels of 7.4 and 32.5 µg/mL using propylene glycol and acetone vehicles, respectively). These results indicate that triclosan was readily absorbed through the skin and distributed to tissues in the mouse.

Four percutaneous absorption studies were conducted in the rat. The results of these studies show that the extent of percutaneous absorption of triclosan is dependent on the vehicle used for application. The extent of triclosan absorption was in the range of 23 to 28% of the applied dose either in ethanol, ethanol/water, soap suspension, or a cream formulation. Greater absorption was observed with triclosan in an aqueous solution or in Vaseline, while lower absorption was observed with triclosan in shampoo. These data are shown in Table 9. In addition to the absorption data from these four studies, plasma data from rats that received 14 days of repeated dermal applications of triclosan in acetone showed dose-dependent increases in plasma triclosan levels starting at the lowest dose used in the study (0.3 mg/rat/day, or 1.2 mg/kg bw/day in a 250 g rat, giving plasma levels of 1.0 µg/mL) [Burns, 1997b (10)]. Taken together, these data indicate that triclosan is readily absorbed through the skin of rats.

Table 9: Percutaneous Absorption of Triclosan in the Rat

Vehicle	Time Point (hours)	% Dose Absorbed	Reference
Pure ethanol	48	28	Black and Howes, 1975 (23)
Shampoo	48	3 to 4	Black and Howes, 1975 (23)
Aerosol deodorant[1]	48	52	Black and Howes, 1975 (23)
Solution (ethanol, acetone)	96	93	Ciba-Geigy, 1976b (25)
Cream	48	23	Ciba-Geigy, 1976b (25)
Vaseline	144	73	Ciba-Geigy, 1976b (25)
Ethanol/water (9:1)	24	21	Moss *et al.*, 2000 (21)

Soap suspension	72	28	Hong *et al.*, 1976 (24)

[1] The determination of absorption following aerosol application was reported to be difficult by the authors of this study due to the lack of a standardized application of the spray. As such, the value of 52% should be interpreted cautiously.

The percutaneous absorption of triclosan was also investigated in the guinea pig [Black *et al*., 1975 (27)]. Autoradiography of skin after a single application of a soap suspension containing radiolabelled triclosan ([^{3}H] or [^{14}C]) showed that triclosan was absorbed with about 20% of the applied dose being absorbed percutaneously even after repeat applications of the soap solution.

The results of a percutaneous absorption study with diapers washed in a solution containing triclosan suggest that the absorption of triclosan through the skin was very low in rabbits [Ciba-Geigy, 1977b (26)]. It should be noted that in this study triclosan was not applied directly to the skin and that the amount of triclosan in the skin was not determined. In another percutaneous absorption study in rabbits, the extent of absorption of triclosan was dependent on the type of formulation (solutions: >85% of the applied dose was absorbed; creams: 29 to 48% was absorbed; soap solutions: 2 to 10% was absorbed), as was observed in studies with rats [Ciba-Geigy, 1976b (25)].

In a small percutaneous absorption study in dogs (n=3), triclosan in water (200 mg/kg) was applied to the shaven nuchal skin of dogs once daily for 2 weeks [Hong *et al.*, 1976 (24)]. Triclosan blood levels were 130 and 165 ppb (ng/mL) on Day 7 and 14, respectively. The total extent of absorption could not be determined, as levels in the urine, faeces, tissues, and expired air, were not reported. Another study in the dog investigated the absorption of triclosan through the oral mucosa [Lin *et al*., 1994 (28)]. In this study, peak blood levels, which occurred within 6 to 8 hours, represented 0.7 to 2.7% (mean = 1.4%) of the applied dose. Daily urinary excretion was in the range of 1 to 4% (mean = 2%) of the applied dose. There were no apparent sex differences in plasma concentrations or urinary excretion of triclosan in dogs. Again, the total extent of absorption was not determined.

The percutaneous absorption of unlabelled triclosan was investigated in a pilot study and a 90-day study with infant Rhesus monkeys [Parkes, 1978a (29); Hazleton Laboratories, 1979b (30)]. In the pilot study, triclosan was detected in all blood samples following a single dermal exposure to a soap solution containing triclosan (1 mg/mL, 0.1%), with blood levels detected up to 24 hours, and peak levels observed at 8 to 12 hours. In the 90-day study, only the glucuronide and sulfate conjugates were detected in blood samples, the glucuronide predominating in the early blood samples (Days 1 to 2), and triclosan sulfate predominating in all subsequent blood samples (samples were taken daily for the 90-day duration of the study). Triclosan was excreted in the urine primarily as the glucuronide conjugate, but was excreted in the faeces primarily in the free or unconjugated form. Low levels of triclosan were detected in tissues. The results of this monkey study indicate that triclosan was absorbed percutaneously following 90 days of daily washing with 15 mL of soap (1 mg triclosan/mL) and that the proportion of plasma glucuronide and sulphate conjugates altered following chronic administration.

Summary of Dermal/Percutaneous Absorption Data

In summary, data from the percutaneous absorption studies conducted with triclosan indicate that it was relatively well absorbed through the skin in all species tested. The extent of absorption was dependent on the formulation in which it was delivered (*e.g*., greater absorption was observed following application of triclosan in solution than in a cream or Vaseline formulation) and the duration of time that the applied dose remained on the skin (*e.g.*, lather/rinse off *vs.* apply/leave on). In the rat, the extent of percutaneous absorption was approximately 23 to 28% of the applied dose of triclosan in ethanol,

ethanol/ water, soap suspension, or a cream formulation (although higher absorption was observed in some studies).

3.3.5. Repeated dose toxicity

A number of repeated dose toxicity studies have been conducted for triclosan in mice, rats, hamsters, rabbits, dogs, and primates.

3.3.5.1. Repeated dose (14 days) dermal toxicity studies

Three short-term dermal toxicity studies were conducted in mice and rats for the purposes of establishing dose ranges for larger studies of longer durations. The major findings from these GLP dose range finding studies are presented in Table 10. Triclosan was tested in 2 mouse studies using different vehicles (acetone and propylene glycol). Using a similar study design, rats were administered triclosan in an acetone vehicle.
In the 2 mouse studies, triclosan was administered at dose levels of 0, 0.3, 0.6, 1.5, 3.0, or 6.0 mg/animal/day for 14 days in acetone [Ref.: 8] or in propylene glycol [Ref.: 9]. These dermally-applied doses correspond to systemic doses of approximately 12, 24, 60, 120, or 240 mg/kg body weight/day for a 25 g mouse (triclosan concentrations of 0.3, 0.6, 1.5, 3, and 6 % in 0.1 mL application volume). The triclosan was applied to a 2 x 3 cm^2 hairless area on the dorsal side of the animal. Appropriate untreated and vehicle controls were used. As these were dose range finding studies, although GLP-compliant, they were not entirely compliant with OECD guidelines for dermal studies (clinical chemistry, haematology, or urinalysis investigations were not conducted, and macroscopic and microscopic evaluations were limited). Both mouse studies showed similar dose-related dermal and liver findings such as dermal irritation, increased liver weight, coagulative necrosis and centrilobular hypertrophy. Dose-related dermal findings started at 1.5 mg / animal / day (60 mg/kg bw/d) for triclosan in propylene glycol and at 3.0 mg / animal / day for triclosan in acetone vehicle. Liver effects were observed in animals treated with dermal doses of ≥ 1.5 mg / animal / day (≥ 60 mg/kg bw/d). The NOAEL is 24 mg/kg bw/d.

Ref.: 8, 9

In rats, a similar dose range finding study was conducted using an acetone vehicle. Dermally-applied doses of 0.3, 0.6, 1.5, 3.0, or 6.0 mg/day in the rat study correspond to systemic doses of approximately 1.2, 2.4, 6, 12, or 24 mg/kg body weight/day for a 250 g rat (triclosan concentrations of 0.1, 0.2, 0.5, 1, and 2 % in 0.3 mL application volume). Skin irritation such as erythema was observed at 6.0 mg/day, with findings in one female animal at 1.5 mg/day considered to be incidental. Other related signs of skin irritation such as eschar, and oedema were also noted. Histopathology of the erythema, scaling, and eschar showed acanthosis and hyperkeratosis at the highest dose. Gross pathology revealed dark areas of the liver in a few treated animals (not dose-related); however, no change in organ weight was observed and no histopathology was associated with the gross pathology findings.

Ref.: 10

In summary, these 3 rodent dermal studies revealed a similar pattern of toxicity with respect to dose-related dermal irritation and hyperkeratosis at the site of application. In addition, liver-related effects such as increased organ weight associated with centrilobular hypertrophy were observed in both mouse studies but not in the rat, indicating a species difference in response to triclosan. The NOAEL was determined to be 0.6 mg/day (24 mg/kg body weight/day) in both mouse studies, based on liver effects observed at doses of ≥ 1.5 mg / animal / day. The NOAEL in the rat study was determined to be 3.0 mg / animal / day (12 mg/kg bw/day), based on dermal irritation at the highest dose of 6.0 mg / animal / day.

Table 10: Findings from GLP Short-Term Repeated Dose Dermal Toxicity Studies for Triclosan

Species (Strain)	Dosing Regimen and Duration	Major Findings	Reference; GLP and OECD Status
Mouse (CD-1)	0, 0.3, 0.6, 1.5, 3.0, or 6.0 mg/animal/d in acetone for 14 days, in a volume of 100 µL, applied to 2x3 cm^2 area on the dorsal area once daily (triclosan concentrations of 0, 0.3, 0.6, 1.5, 3, and 6%)	Dermal irritation such as erythema was observed at the site of application. Oedema, fissuring, eschar was observed at the 2 highest doses which correlated with hyperkeratosis. Acanthosis was also observed in males at 1.5 mg/d. The liver showed dose-related increase in absolute and relative liver weight in females at 1.5 and in both sexes at 3.0 and 6.0 mg/d. This increase associated with centrilobular hypertrophy. Mononuclear infiltrate was also observed but only at the high dose. The NOAEL is considered to be 0.6 mg/d. This corresponds to 24 mg/kg bw per day	Burns, 1997a (8) GLP: compliant OECD: comparable
Mouse (CD-1)	0, 0.3, 0.6, 1.5, 3.0, or 6.0 mg/animal/d in propylene glycol for 14 days, in a volume of 100 µL, applied to 2x3 cm^2 area on the dorsal area once daily (triclosan concentrations of 0, 0.3, 0.6, 1.5, 3, and 6%)	Dose-related dermal irritation was observed at the site of application starting at 1.5 mg/d. Dose-related increases in absolute and relative liver weights in both sexes at 6.0 mg/d and in males at 0.3, 1.5 and 3.0 mg/d. Centrilobular hypertrophy at doses ≥1.5 mg/d. The statistical increase in male liver weights at 0.3 mg/d was not correlated with any histopathology changes. The NOAEL is considered to be 0.6 mg/d. This corresponds to 24 mg/kg bw per day	Burns, 1996 (9); GLP: compliant OECD: comparable
Rat (Crl:CDBR)	0, 0.3, 0.6, 1.5, 3.0, or 6.0 mg/animal/d in acetone for 14 days, in a volume of 300 µL, applied to 2x3 cm^2 on the dorsal area once daily (triclosan concentrations of 0.1, 0.2, 0.5, 1, and 2%)	Dose-related skin irritation such as erythema was observed at 6.0 mg/d, with findings in 1 animal at 1.5 mg/d considered incidental. Other related signs of skin irritation such as eschar, oedema were also noted. Histopathology of the erythema, scaling and eschar showed acanthosis and hyperkeratosis at the highest dose. Gross pathology revealed dark areas of the liver noted in a few treated animals (not dose-related); however, no change in organ weight was observed and no histopathology accompanied this gross finding. The NOAEL was estimated by investigators to be 3.0 mg/animal/d equivalent to 12 mg/kg bw per day.	Burns, 1997b (10); GLP: compliant OECD: comparable

3.3.5.2. Repeated dose (21 days) inhalation toxicity

The inhalation toxicity of triclosan after 14 days of repeated dose administration was evaluated in the rat. This study was performed prior to GLP regulations and the establishment of OECD guidelines

In this study, groups of 10 male and 10 female rats were initially exposed to triclosan at concentrations ranging from 50 to 1,300 mg/m^3. Following the first day, due to the occurrence of deaths, dyspnoea and general clinical signs indicative of poor health in the treated animals, the test concentrations were reduced to 0, 50, 115, or 301 mg/m^3 for dosing on Days 2-15, *i.e.*, the remainder of the study. Although there were 12 unscheduled deaths related to high-dose level exposure, 11 of the 12 rats died during Day 1 as a result of the very high initial doses. Necropsy revealed congestion, inflammatory changes in mucous membranes and nasal cavity.
In the remaining animals, dose-related increases in leukocyte count and alterations in serum chemistry such as glutamic-pyruvic transaminase, alkaline phosphatase were

observed. Slight focal inflammation of the mucous membranes was observed in high-dose animals at the end of the study. The NOAEL for inhaled triclosan was determined in this study to be 50 mg/m^3, the lowest dose tested.

Ref.: 32

3.3.5.3. Repeated dose (28 days) oral toxicity study in mice

A GLP-compliant 28-day repeated dose oral toxicity study of triclosan was conducted in the mouse, using OECD Guideline 407 for the design of the study. In this mouse study, the significant findings were in the liver, which showed reversible hepatocellular hypertrophy and focal necrosis in mice that received the high dose (136 and 169 mg/kg body weight/day in males and females, respectively).

Ref.: 31

A brief summary of the major findings from the repeated dose oral toxicity study is presented in the table below.

Table 11: Findings from Short-Term Repeated Dose Oral Toxicity Studies for Triclosan

Species (Strain)	Dosing Regimen and Duration	Major Findings	Reference; GLP and OECD Status
Mouse (MAGf)	0, 50, or 1,000 ppm in the diet (0, 6.5, or 136 mg/kg bw/d in males and 0, 8.3, or 169 mg/kg bw/d in females) for 28 days with 14 days recovery	Slight, but significant reversible decreases in erythroid parameters. Biochemistry showed significant increases (2- to 3-fold) in liver function enzymes in plasma (high-dose animals); changes were almost completely reversed by the end of 14-d recovery. Slight changes in urea (high-dose animals) and creatinine (high-dose females) were not fully reversed. No macroscopic findings, but histopathological examination showed liver changes in high-dose animals, including hepatocyte hypertrophy, hepatocyte necrosis (focal) bordered by macrophages in some cases. Some Kupffer cells in the area contained a pigment that was assumed to be iron. These changes were reversed in 14-day recovery period. Low-dose animals showed no histological changes or changes in haematology or blood chemistry (except for a slight, not significant increase in liver function enzymes in males of the low-dose group). Electron microscopy of selected livers of high-dose animals showed reversible proliferation of smooth endoplasmic reticulum and increase in number and/or size of peroxisomes. No NOAEL was determined by investigators; however, it should be noted that no adverse effects were observed at the low dose (50 ppm).	Ciba-Geigy, 1987 (31); GLP: compliant OECD: No.407 consistent

Comment
A reversible decrease in phosphate was observed in females of both doses and liver enzymes were slightly increased but not significant in low dose males.

3.3.5.4. Sub-chronic (90 days) oral toxicity studies

The safety of triclosan has been evaluated in sub-chronic oral toxicity studies in mice, rats, hamsters, rabbits, dogs, and baboons, using either dietary administration or capsules. Test species were evaluated for clinical observations, body weight, body weight gain, food and water consumption, haematological, clinical chemistry, ophthalmological and urinalysis parameters, macroscopic observations, and microscopic findings. In addition, these investigations included a wide range of dose levels for triclosan. Pivotal studies conducted in mice, rats and hamsters were conducted pursuant to GLP and generally followed OECD guidelines. Findings from these studies are presented in Table 12 (GLP- and OECD-compliant studies). Rabbit, dog, and baboon studies were conducted prior to the

establishment of GLP standards and OECD guidelines. Findings from these studies are presented in Table 13 (non-GLP and/or non-OECD compliant studies).
In the pivotal studies conducted in compliance with GLP and following OECD guidelines for 90-day (sub-chronic) oral toxicology studies, the highest doses tested were 900 mg/kg body weight/day in the mouse, 600 mg/kg bw/day in the rat, and 900 mg/kg bw/day in the hamster.

Subchronic oral toxicity study in mice

In the GLP mouse study, CD-1 mice (15/sex/group) were administered triclosan *via* the diet at dose levels corresponding to 0, 25, 75, 200, 350, 750, or 900 mg/kg bw/day for 13 weeks. Additional satellite groups (10/sex/group), for interim sacrifice at 7 weeks, were administered 0, 25, 350, and 900 mg/kg body weight/day. This study contained a GLP and OECD compliance statement and was well designed and conducted. The results of this study indicated that the liver was the primary target organ in mice. Treatment-related increases in alkaline phosphatase were observed in females and males, at ≥25 mg/kg bw/day and at ≥200 mg/kg body weight/day, respectively. Decreased erythrocyte count and haemoglobin were noted at ≥25 mg/kg bw/d (male) and all animals at ≥75 mg/kg bw/d. Other changes noted included decreased total cholesterol in all animals at 25 mg/kg bw/day and higher and increased alanine aminotransferase (males at 350 mg/kg body weight/day and above, females at 750 mg/kg body weight/day and above). Increased gamma glutamyltransferase was observed in both males and females at 750 mg/kg body weight/day. Dose-related increases in absolute and relative liver weights were observed in both males and females starting at 75 mg/kg bw/day. Gross and histopathology findings in the liver included enlarged dark or thickened lobes, with histomorphologic centrilobular hepatocellular hypertrophy, vacuolization, pigment accumulations, necrosis, and/or inflammation. These hepatic changes were noted in males at 75 mg/kg bw/day and in both males and females at all higher doses. In the more severe cases of hepatocellular hypertrophy, hepatocytes were individualized, although the overall hepatic architecture was still intact. In addition to liver findings, increased extramedullary haematopoiesis was observed in the spleen of animals at 750 and 900 mg/kg bw/day and, at doses of 200 mg/kg body weight/day and higher, hyperplasia in male glandular stomachs and inflammation in female kidneys occurred. No histomorphologic alterations were observed in males at 25 mg/kg bw/day or in females at 25 or 75 mg/kg bw/day. A NOAEL was not established from this study since treatment-related changes in haematology parameters, increased alkaline phosphatase, and decreased cholesterol were observed at the low dose.

Ref.: 33

Subchronic oral toxicity study in rats

In a 90-day GLP toxicity study in rats that served as a dose range-finding study for a 2-year oral carcinogenicity study, triclosan (FAT 80'023) was administered to Sprague-Dawley rats (25/sex/group) *via* the diet at concentrations of 0, 1000, 3000, or 6000 ppm (approximately 0, 100, 300, or 600 mg/kg bw/day based on calculations of mean body weight, food consumption and target dose level. No test article-related mortality occurred. Decreased body weight along with gradually decreased food consumption was observed at the high-dose groups. Diet spillage at study initiation occurred at all treatment groups.
Treatment-related effects on erythrocyte parameters RBC, haemoglobin and haematocrit were observed in mid- and high-dose groups. Increased ketones were found in high-dose males and decreased triglycerides in high-dose males and females. Interim necropsy findings, conducted at 45 days, revealed increased liver weight changes in males (mid- and high-dose) and females (high-dose only). At terminal necropsy, absolute liver weight for high-dose males and relative liver weight for both male and female dose groups were increased. Kidneys weights were increased in males at the highest dose and spleen weights were decreased in mid-and high-dose males. Histopathologic examination revealed mild hepatic centrilobular cytomegaly and fatty methomorphosis in mid- and high-dose males. These changes were also common to female rats, but occurred at a lower frequency. No

histomorphologic changes were observed in the spleen. The low dose was not associated with any treatment-related findings; thus, the NOAEL was determined to be 1,000 ppm (~100 mg/kg body weight/day).

Ref.: 34

Subchronic oral toxicity study in hamsters

In the 13-week GLP hamster study, triclosan (FAT 80'023) was administered to Syrian Golden Hamsters (15/sex/treatment group; 20/sex/control, 10/sex/group for interim sacrifice at 7 weeks) *via* the diet at doses of 0, 75, 200, 350, 750, or 900 mg/kg body weight/day. Treatment was not associated with any mortality or clinical signs. Decreased body weight gain was observed at 750 and 900 mg/kg body weight/day. Food consumption was decreased in males at 900 mg/kg body weight/day and females at 350 mg/kg body weight/day and above. Water consumption was increased at all groups given 200 mg/kg body weight/day and higher. Increased coagulation times, changes in red cell morphology and red cell indices indicated microcytic type anaemia in high-dose animals (750 and 900 mg/kg body weight/day). Clinical chemistry disturbances of liver and kidney function were observed at doses of 750 mg/kg body weight/day and 900 mg/kg body weight/day. Biologically significant clinical chemistry changes noted in alkaline phosphatase and alanine aminotransferase indicated possible liver toxicity; however, organ weight determinations and macroscopic and microscopic examination revealed no corresponding findings. The main target organ toxicity in hamsters was dose-related nephrotoxicity (tubular casts, tubular basophilia, tubular dilation). Although the LOEL may be estimated to be 200 mg/kg body weight/day, study investigators determined the NOEL to be 75 mg/kg body weight/day due to increased water consumption and some changes in urinalysis parameters.

Ref.: 35

Comment of the SCCP
The NOAEL is set at 200 mg/kg bw/d based on nephrotoxicity indicated by microscopic findings and polyuria, haemoglobinuria and haematouria.

Table 12: Findings from GLP Sub-Chronic Oral Toxicity Studies for Triclosan

Species (Strain)	Dosing Regimen and Duration	Major Findings	Reference; GLP and OECD Status
Mouse (CD-1)	0, 25, 75, 200, 350, 750, 900 mg/kg bw/d for 13 weeks *via* dietary admixture. Interim sacrifice at 7 weeks.	Conducted pursuant to OECD Guideline No. 408. Clinical signs at highest dose. Decreased food consumption and decreased body weight at ≥750 mg/kg bw/d. Increased alkaline phosphatase in females at ≥25 mg/kg bw/d and males at ≥200 mg/kg bw/d. Decreased total cholesterol at ≥25 mg/kg bw/d, increased alanine aminotransferase (males at ≥350 mg/kg bw/d, females at ≥750 mg/kg bw/d). GGT increased ≥750 mg/kg; however, not statistically significant. Decreased erythrocyte count and haemoglobin at ≥25 mg/kg bw/d (male) and all animals at ≥75 mg/kg bw/d. Increased liver weight and liver to body weight ratio at doses of ≥75 mg/kg bw/d in the main study and at doses of ≥350 mg/kg bw/d at interim sacrifice. Decreased kidney weights were observed in males at ≥350 mg/kg bw/d and females at 900 mg/kg bw/d. Enlarged dark/thickened lobes correlated with histomorphologic centrilobular hepatocellular hypertrophy, vacuolization, pigment accumulations, necrosis and/or inflammation noted in males at 75 mg/kg bw/d and all animals at 200, 350, 750 mg/kg bw/d. Extramedullary haematopoiesis was observed in the spleen of higher dose (≥750 mg/kg) animals. No histomorphologic alterations were observed in males at 25 mg/kg bw/d and in females at 75 mg/kg bw/d. A NOAEL could not be determined.	Trutter, 1993 (33); GLP: compliant OECD: No.408 consistent
Rat (Sprague-Dawley)	0, 1,000, 3,000, or 6,000 ppm *via* diet (~ 0, 100, 300, or 600 mg/kg bw/d) for 90 days *via* dietary admixture. Interim sacrifice at Day 45.	Conducted pursuant to OECD Guideline No. 408. High doses were accompanied by decreased body weight with gradual decreased food consumption and diet spillage at initiation for all treatments. Treatment-related effects on erythrocyte parameters were observed. Interim necropsy revealed increased liver weight changes in males (mid- and high-dose) and females (high dose only). At terminal necropsy, liver weight for high-dose males and relative liver weight for both male and female dose groups were increased. Histopathologic examination revealed mild hepatic centrilobular cytomegaly and fatty methomorphosis in high and mid-dose males. These changes were also common to female rats; however, at a lower frequency. The NOAEL was considered to be 1,000 ppm (~100 mg/kg bw/d).	Litton Bionetics, 1983 (34); GLP: compliant OECD: No.408 consistent

Species (Strain)	Dosing Regimen and Duration	Major Findings	Reference; GLP and OECD Status
Hamster (Syrian Golden)	0, 75, 200, 350, 750 or 900 mg/kg bw/d for 13 weeks *via* dietary admixture. Interim sacrifice at Week 7.	Conducted pursuant to OECD Guideline No. 408. Decreased body weight gains were observed at ≥750 mg/kg bw/d. Food consumption was decreased in males at 900 mg/kg bw/d and females at ≥350 mg/kg bw/d. Water consumption was increased at all groups given 200 mg/kg bw/d and higher. Increased coagulation times, changes in red cell morphology and red cell indices in high-dose animals (≥750 mg/kg bw/d). Slightly increased alanine aminotransferase levels in females at the highest dose, and in both sexes increased cholesterol at the highest dose. At ≥750 mg/kg bw/d, decreased levels of alkaline phosphatase were observed. At 900 mg/kg bw/d, decreased gamma glutamyltransferase was observed. Urinalysis revealed polyuria, haemoglobinuria and haematouria in all animals given ≥350 mg/kg bw/d. Interim and terminal necropsy revealed no change in any major organ weight in females. In males, increased absolute and relative kidney weights at 750 mg/kg bw/d after 13 weeks and 900 mg/kg bw/d at the interim and terminal necropsy. Associated with these findings was tan discolour and/or granulated kidneys. Histopathology showed nephrotoxicity (*e.g.*, tubular casts, tubular basophilia, tubular dilation) which was dose-related with respect to incidence and severity; microscopic changes were noted starting at the dose of 350 mg/kg bw/d. Stomach inflammation (gastritis, glandular erosions) was also observed at ≥750 mg/kg bw/d. Based on changes noted in water consumption, which were not considered adverse, the LOEL was determined to be 200 mg/kg bw/d; study investigators determined the NOEL to be 75 mg/kg bw/d. At 750 and 900 mg/kg/day, the kidneys were identified as a target organ based on macroscopic, histopathologic and clinical findings. Clinical chemistry changes also indicated liver effects; however, no microscopic findings were observed. The red blood cell also showed treatment-related effects. At 350 mg/kg/day, microscopic findings of nephrotoxicity were observed. The NOEL was determined by study investigators to be 75 mg/kg bw/day.	Schmid *et al.*, 1994 (35); GLP: compliant OECD: No.408 consistent

Subchronic oral toxicity study in rabbits

The results of 2 studies in rabbits were inconsistent; in 1 study, triclosan was reported to be well-tolerated, with no treatment-related effects up to the dose level of 125 mg/kg bw/day, whereas in a second study, the NOAEL was determined to be 3 mg/kg bw/day, as animals given doses of 30 or 150 mg/kg bw/day showed pulmonary infections. However, it should be noted that study investigators stated that the relationship of the lung findings to triclosan was unclear.

Ref.: 36, 37

Subchronic oral toxicity study in dogs, study 1

Inconsistency also was found in a comparison of 2 sub-chronic dog studies, the first of which showed haematology, clinical chemistry, macroscopic and microscopic findings at all doses tested, including some effects at the lowest dose tested of 25 mg/kg bw/day. As a result, no NOAEL was determined for the study.

Ref.: 38

Subchronic oral toxicity study in dogs, study 2

In a subsequent study, where doses were lowered to 0, 5, 12.5, or 25 mg/kg body weight/day, there were no treatment-related findings at any dose, even though the highest dose tested in the second study was the same as the lowest dose tested in the first study.
Ref.: 39

Subacute & subchronic oral toxicity study in baboons

In baboon studies 2 – 20 animals per dose level were administered 0, 1, 3, 10, 30 and 100 mg/kg via oral gelatine capsules for 4 or 13 weeks (6 animals 3 mg/kg bw/d), respectively. Symptoms such as agitation, anger and aggression were observed in the one female receiving 100 mg/kg. No other clinical signs were observed in other treatment groups. No treatment-related changes were found in ophthalmoscopic examinations and in water consumption, haematology and body weight. Findings were similar among terminal histopathological examination of treated groups from 4 weeks and 13 weeks of treatment. Evidence of chronic interstitial pneumonitis was seen throughout all animals. Lymphocytic infiltration was seen in the liver and large intestines. These findings were not different among control and treated animals.
Ref.: 40

Long term (1 year) oral toxicity study in baboons

A total of 56 animals (3/sex/dose (main study) plus 2/sex/dose (interim, 6 months) plus 2/sex/dose (recovery group, 4 weeks after dosing) were used. Animals were dosed orally, once daily, with prefilled capsules with 0, 30, 100, or 300 mg/kg bw/d triclosan. The controls received 1 capsule containing 600 mg lactose + 0.5% magnesium stearate per day. Vomiting was observed in some mid and high dose animals accompanied by an increased incidence of low food intake. Deterioration of condition and abdominal pain was observed in High Dose animals. Incidence of diarrhoea was increased in Mid Dose animals and greatly increased in High Dose animals. No significant effects in Males were observed except at 39 and 52 weeks, when decreased WBC noted in Mid Dose and High Dose groups. Females showed slight decreases in erythroid parameters at all time points for High Dose group, but only significant up to 26 weeks. Also decreased WBC was observed in High Dose Females at 52 weeks (not significant). Decrease in absolute brain weight and increases in kidney and liver weights (relative to BW) in High Dose animals were significant when Male and Female data combined (data at termination). NOEL is estimated to be 30 mg/kg bw/d based on the absence of any effect, including diarrhoea, in baboons at the low dose level in this study.
Ref.: 41

Comment
In baboon studies conducted at doses up to 300 mg/kg bw/d, both 4/13-week and 1-year investigations showed time-dependent haematology findings. Incidental clinical chemistry changes were observed; however, there was no evidence of hepatic or renal injury accompanying these findings [Ref. 40; 41]. Clinical signs observed in the longer-term study were not observed in the 4/13-week study. Significant differences in study design as well as quality of investigation likely contributed to discrepancies between these studies.

Table 13: Findings from Non-GLP, Non-OECD Sub-Chronic Oral Toxicity Studies for Triclosan

Species (Strain)	Dosing Regimen and Duration	Major Findings	Reference; GLP and OECD Status
Rabbit (New Zealand White)	0, 12.5, 25, 62.5 or 125 mg/kg bw/d for 90 days *via* dietary admixture.	Conducted prior to OECD guidelines, but considered similar in design. Terminal examination revealed no organ weight changes or gross pathology findings. Histopathology examination found no differences in high-dose animals compared to control animals. Incidental microscopic changes noted in high-dose animals included granulomatous infiltrations in lungs in 1 female and 2 males and nephrosis in 1 high-dose female. Overall, triclosan was well tolerated at all doses. No NOAEL was determined in this study, in which the highest dose used did not produce treatment-related effects.	Leuschner *et al*., 1970a (36); Predates GLP and OECD OECD: comparable
Rabbit (Albino)	0, 3, 30, 150 mg/kg bw/d for 13 weeks *via* oral gavage.	Conducted prior to OECD guidelines, but considered similar in design. Dose-related mortality was observed at 30 and 150 mg/kg bw/d. At doses of 30 and 150 mg/kg bw/d, neutrophilia and lymphopenia were observed on various observation days but were not consistent throughout the study. The lung (which was associated with macroscopic and microscopic changes) appeared to be the target organ. Pulmonary infection was observed in 3/6 rabbits at 30 mg/kg bw/d and 3/6 rabbits from 150 mg/kg bw/d. Perirenal abscess in 1 rabbit was observed at 30 mg/kg bw/d. Limited organ weight determinations showed no treatment-related findings. Gross macroscopic findings in the lung corresponded with histopathologic lung lesions, oedema in 30 and 150 mg/kg bw/d treated animals and lung necrosis in 2 high-dose animals. No such histomorphology changes were noted in control or 3 mg/kg bw/d animals. Based on findings of neutrophilia and lymphopenia at 30 mg/kg bw/d and absence of histomorphologic alterations, 3 mg/kg bw/d was determined to be the NOAEL. However, the study authors stated that the relationship of the lung lesions, infection, oedema and sometimes necrosis observed at the 2 highest doses to test article administration is unclear. Dosing accidents or regurgitation with resultant pulmonary infection was suggested.	Paterson, 1969 (37); Predates GLP and OECD OECD: comparable
Dog (Beagle)	0, 25, 50, 100 or 200 mg/kg bw/d *via* gelatine capsules for 91 days.	Conducted prior to OECD guidelines, but considered similar in design, with the exception that clinical pathology evaluations were limited. Seven unscheduled deaths (1-25 mg/kg bw/d, 2-100 mg/kg bw/d and 4-200 mg/kg bw/d). Severity of diarrhoea was dose-related. Haematology evaluations revealed decreased haemoglobin, PCV and red blood cells and increased ESR and reticulocytes in dogs at ≥50 mg/kg bw/d as well as during interim evaluation in moribund dogs. Increased alkaline phosphatase in all animals at doses of ≥50 mg/kg bw/d. Additionally, high SGOT and SGPT were noted in these animals. Bile salts, polymorphonuclear leukocytes were observed in dogs at ≥25 mg/kg bw/d. Terminal organ weight determinations revealed increased liver, pancreas and adrenal weights for 100 and 200 mg/kg bw/d animals. Bile retention, necrosis, pathological fat and unusual Kupffer cell activation were observed histopathologically in the liver. In animals which showed severe liver damage, the bone marrow was hyperplastic. In 2 dogs given 200 mg/kg bw/d and one dog at 100 mg/kg bw/d, focal interstitial nephritis was observed. Convoluted epithelium of the kidney was observed in 2 dogs given 25 mg/kg bw/d but not observed at any other dose. Based on the findings in this study, no NOAEL was determined.	Paterson, 1967 (38); Predates GLP and OECD OECD: comparable

Species (Strain)	Dosing Regimen and Duration	Major Findings	Reference; GLP and OECD Status
Dog (Beagle)	0, 5, 12.5 or 25 mg/kg bw/d *via* diet for 90 days.	Conducted prior to OECD guidelines, but considered similar in design. Occasional pasty to thin faeces were noted in all groups. Haematology, clinical chemistry and urinalysis evaluations were limited to high-dose group animals. Macroscopic evaluations found no gross pathology for any organ. Organ weight measurements showed no changes in relative or absolute weights. Histopathologic evaluation of all major organs from all animals showed no difference among treated and control animals. No NOAEL was determined in this study, in which the highest dose used did not produce treatment-related effects.	Leuschner *et al*., 1970b (39); Predates GLP and OECD OECD: comparable
Baboons (papio cynocephalus and anubis)	4-week dosing: 0, 1, 10, 30, or 100 mg/kg bw/d *via* gelatine capsules. 13-week dosing: 0 or 3 mg/kg bw/d *via* gelatine capsules.	Conducted prior to OECD guidelines, but considered comparable in design (but limited in number of parameters evaluated). Blood chemistry revealed no haematology changes. Urinalysis showed no changes. Clinical chemistry changes included high plasma urea levels in 2 animals at 30 mg/kg bw/d, 2 animals at 3 mg/kg bw/d, 1 control baboon and increased SGPT in individual male baboons at 1 mg/kg bw/d and 3 mg/kg bw/d. Terminal necropsy after 4 weeks and after 13 weeks showed no organ weight differences among treated and controls. No gross macroscopic findings were observed. After 4 weeks of treatment, minor changes such as dark nodules in the large intestine wall at ≥10 mg/kg bw/d. Adhesion in lung surface and rib cage, slight thickening of capsule of the liver in 10 mg/kg bw/d female and 1 mg/kg bw/d male, and control male and female were observed. Limited conclusions can be derived from this study due to insufficient number of animals evaluated. A NOAEL was not determined for this study.	Noel *et al*., 1969 (40); Predates GLP and OECD OECD: comparable
Baboons (papio)	0, 30, 100, or 300 mg/kg bw/d *via* capsules daily for 1 year. Necropsies were scheduled at 6 months, 12 months, and 13 months (28 days post-treatment).	Conducted prior to OECD guidelines, but considered similar in design (but limited in number of parameters evaluated). Vomiting and diarrhoea occurred at mid and high-doses. Haematology parameters showed decreased white blood cells in males at Week 29 and 52 in mid and high-dose groups. In females, slight decreases in erythroid parameters at all time points were observed for the high-dose group (statistically significant only up to Week 26). Incidental changes in potassium and sodium were observed. Slight changes, both increases and decreases in SGOT and AP were observed; however, not considered treatment-related. No differences were found in urinalysis. At necropsy, decreased absolute brain and increases in kidney and liver weights were observed in high-dose animals. Histopathologic examination found similar histology in both treated and control animals. The NOEL was determined to be 30 mg/kg bw/d.	Ciba-Geigy, 1975a (41); Predates GLP and OECD OECD: comparable

3.3.5.5. Sub-Chronic Dermal Toxicity

The sub-chronic dermal toxicity of triclosan has been investigated in rats, dogs, and monkeys. The studies conducted in weanling dogs and newborn Rhesus monkeys were not GLP-compliant and not conducted pursuant to OECD guidelines. The pertinent details from these studies are summarized in Tables 14 (GLP rat study) and 15 (non-GLP, non-OECD studies).

Sub-Chronic Dermal Toxicity (90 d) in rats

The rat study was conducted according to both GLP and OECD guidelines (Crl:CDBR (VAF/Plus) strain). IRGASAN DP300 (0, 10, 40, 80 mg/kg bw/d) diluted in propylene glycol was applied to clipped, unabraded dorsal surface of each animal (approx. 10% of total body

surface) at dose volume of 2.0 mL/kg daily (approximately 0.5 to 4.0% triclosan). The appropriate dose was applied under a gauze pad and covered. Daily contact exposure was for at least 6 hours. Dermal observations of erythema and/or showed oedema in all treated groups. No treatment-related changes were found in ophthalmoscopic examinations and in water consumption and body weight. Occult blood was observed in the urine of high-dose and satellite male rats and to a lesser extent in mid-dose males and females. Isolated changes were observed in erythrocyte parameters in high dose animals. Small but statistically significant changes were observed in some serum chemistry parameters. NOAEL = 80 mg/kg/day (excluding dermal irritation)

Ref.: 11

Sub-Chronic Dermal Toxicity (90 d) in dogs

Weanling dogs exposed to triclosan through dermal application for 90 days of doses ranging from 2 to 200 mg/kg bw/day in a non-GLP study showed no toxicity except for dermal irritation at the highest dose tested.

Ref.: 12

Sub-Chronic Dermal Toxicity (90 d) in monkeys

The major findings from a 90-day bathing study conducted in newborn Rhesus monkeys showed that repeated exposure to triclosan (0.1% in a soap solution, 5 min exposure) was well-tolerated. No treatment-related toxicities were observed.

Ref.: 13

Table 14: Findings from GLP Sub-Chronic Dermal Toxicity Studies for Triclosan

Species (Strain)	Dosing Regimen and Duration	Major Findings	Reference; GLP and OECD Status
Rat (Crl: CDBR VAF/ Plus strain)	0, 10, 40, or 80 mg/kg bw/d in propylene glycol applied under gauze for at least 6 hours (90 days). Triclosan was applied to approx. 10% of total body surface in a volume of 2 mL/kg bw. Approximate triclosan test concentrations were ~0, 0.5, 2, and 4% for a 0.25 kg animal. A recovery group was included at the high dose.	Conducted pursuant to OECD Guideline No. 411. Dermal erythema and/or oedema was observed in all treatment groups. Haematology parameters showed isolated changes in erythrocyte parameters in high-dose animals; however, these findings were within expected range. Similarly, clinical chemistry findings showed small but statistically significant changes in serum chemistry. Occult blood in urine was observed in the high dose and satellite male rats and to a lesser extent in mid-dose females and males. Histopathology examinations observed eschar and desquamation, hyperplasia/hyperkeratosis of epidermis, dermal inflammation and focal necrosis observed at all doses. Reversal of the dermal effects was seen during the 28-day recovery period. Microscopic changes in the urinary bladder of 3 males were observed. Coagulative necrosis of hepatocytes was also observed. Both findings lacked a dose-response. With respect to general toxicity, the NOAEL was determined to be 80 mg/kg bw/d (the highest dose tested). No NOAEL for dermal toxicity was determined.	Trimmer, 1994 (11); GLP: compliant OECD: compliant

Table 15: Findings from Non-GLP, Non-OECD Sub-Chronic Dermal Toxicity Studies for Triclosan

Species (Strain)	Dosing Regimen and Duration	Major Findings	Reference; GLP and OECD Status
Dog (Beagle weanling)	0, 2, 20, or 200 mg/kg bw/d applied to a shaved area of the neck for 90 days (12.5% triclosan added to 50% corn-starch vehicle w/vol; size of application area not reported.)	Conducted prior to OECD guidelines and missing a number of study parameters. Upper respiratory disease during the study up to Day 25. Diarrhoea in all animals was not attributed to the test substance. Dermal irritation (dermatitis) was observed at the high dose and was dose-dependent. There were no treatment-related biochemical or haematological changes. Microscopic examination revealed no histomorphology changes in the skin. Test substance was reported to show "little toxic effect" aside from dermal irritation (pathology data not reported).	Dorner, 1973 (12); Predates GLP and OECD
Monkey (Rhesus, newborn)	0.1% soap solution, lathered for 5 minutes and then washed for 90 days (size of application area not reported). Recovery group: 30 days without bathing.	Conducted prior to OECD guidelines and not comparable to current OECD guidelines (*e.g.*, exposure method). Mild anaemia, red blood cell and haemoglobin values showed variations between animals, but was not considered treatment-related. These changes were attributed to frequent sampling. Limited clinical chemistry parameters were evaluated due to limitations in blood sampling; however, of the parameters measured, no consistent changes were noted. Termination examination showed no organ weight changes. Histopathologic evaluation revealed similar findings of focal adrenal mineralisation, pulmonary infiltration, and low-grade pneumonia in both treated and control animals. Hepatocellular vacuolar changes and hepatic extramedullary haematopoiesis were observed in both treatment and control animals. No histological changes were observed in skin sections taken for examination. No differences were noted between animals sacrificed after 90 days of treatment and recovery animals. This 0.1% triclosan soap solution was well-tolerated under the conditions of this study.	Hazleton Labs, 1979a (13); Predates GLP and OECD OECD: no applicable guidelines

3.3.5.6. Chronic (> 1 year) Toxicity

Chronic toxicity was assessed in the carcinogenicity studies with rats, mice and hamsters (See also 3.3.7).

Long Term Toxicity/Carcinogenicity study – 18 Months (Mouse)

Animals were dosed daily *via* the diet for 544-552 days in total. Dietary admixtures were mixed weekly for 13 weeks, then every 4 weeks; amounts of triclosan were adjusted using most recent weekly body weight and feed consumption data. 0, 10, 30, 100, or 200 mg/kg bw/d in the diet (control, low dose, low mid dose, high mid dose, or high dose).
A significant decrease in survival was observed in High Mid Dose males and high dose females whereas high dose males the decrease was slight but not significant.
No adverse effects on Body weight, food consumption and urine parameters attributable to the test substance were observed. In biochemistry significant changes at 18 months were increased (230-560%) liver function enzymes in high Mid Dose and High Dose Males and Females; decreased (75-90%) cholesterol in all treated Males and Females; decreased bilirubin (up to 67%) in Females (all doses except Low Dose). In haematology: small (<15%) but significant dose-related changes in erythroid parameters (Males and Females). Increased % reticulocytes (males) and platelets (males and females; 25-37%, dose-related) significant at 18 months. Increased WBC, neutrophils, & lymphocytes at higher doses in

Males (6 months) were not considered indicative of test substance toxicity by the study authors. No changes were recorded at low Dose.
Dose-related, significant increases in liver weights were observed in males and females at low Mid Dose, high Mid dose and High Dose. Increase in incidence of hepatic nodules/masses and/or discolorations in Males and Females of high Mid Dose and High Dose groups compared to control. Slight increase in testicular germinal epithelium degeneration/atrophy was observed at High Dose (only controls and High Dose examined). No treatment-related histopathological changes other than in liver. Hepatocyte hypertrophy was present in all animals except Low Dose Females. Brown pigment in hepatocytes in higher dose rats was found to be lipofuscin and iron. The LOAEL was 10 mg/kg bw/d based on liver changes. This dose level was considered as NOAEL based on haematotoxicity when excepting the target organ liver.

Ref.: 66

Long Term Toxicity/Carcinogenicity study – 104-week (Rat)

Animals were dosed daily *via* the diet at concentrations of 0, 300 (Low Dose), 1,000 (Mid Dose), 3,000 (High Dose) ppm in 104-week study. An extra group of rats was given a "toxic" dose of 6,000 ppm and killed at 52 weeks. Doses were calculated weekly based on food intake and weekly mean BW. At 52 weeks, calculated doses were 0, 12, 40, and 127 mg/kg bw/d (Males) and 0, 17, 56, and 190 mg/kg bw/d (Females), respectively. The dose of 6,000 ppm gave doses of 247 (Males) or 422 (Females) mg/kg bw/d.
No treatment-related effects on mortality were observed. Body weight was significantly decreased in High Dose Females up to week 52. Food Consumption was generally significantly increased in High Dose Males. In biochemistry transient changes in protein, BUN, glucose, bilirubin, triglyceride and liver enzymes were found.
In haematology slight (4-10%) but significant changes in erythroid parameters (red blood cell count in Males and Females), including in Low Dose Males rats at week 104 were recorded. Also increases in mean corpuscular haemoglobin concentration was observed in females at mid and high dose and in males in addition at low dose. Slightly increased altered red cell morphology was observed in High Dose Males. Increased clotting time (High Dose Males), decreased WBC (33%) in Females was measured at 104 weeks. Decreases in % monocytes were transient (High Dose Females) but significant at 104 weeks (High Dose Males).
At 52 weeks slight relative weight decreases at High Dose were observed: liver, brain, kidneys (Males) and heart, ovaries and spleen (Females). At 104 weeks increased absolute adrenal weight (Mid Dose Males) and decreased brain weight (High Dose Males); increased absolute and relative ovary weight (High Dose Females), decreased relative spleen weight (High Dose Females), and decreased absolute and relative spleen weights (Mid Dose Females).
Morphology revealed hepatocyte hypertrophy and hepatocytic inclusions (hyaline-staining, round-shaped) in Male rats: significant – High Dose (13 weeks), 6,000 ppm (52 weeks). No other histological lesions were considered to be treatment-related. The study authors considered the NOAEL as ~48 mg/kg/d for both sexes, combined (~56 mg/kg bw/d in Females and ~40 mg/kg bw/d in Males).

Ref.: 67

Comment
SCCP considers the NOAEL as 12 mg/kg bw/d due to haematoxicity and decreased absolute and relative spleen weights (Mid Dose Females).

Long Term Toxicity/Carcinogenicity study – 95 Weeks (Hamster)

The animals were administered 0, 12, 75, or 250 mg/kg bw/d FAT 80'023/S. in the diet for 90 weeks. Survival was significantly decreased in males and was generally poor (38-58%) in females at 90 weeks. Body weight gain was significantly decreased in all high-dose hamsters and was accompanied by a slight (3%), but significant decrease in food

consumption in high-dose females. Biochemical changes observed at termination included increases (<50%) in blood urea nitrogen in high-dose hamsters and in triglycerides in mid- and high-dose males. Haematological changes observed included slight (<15%), but significant decreases (but not dose-related) in erythroid parameters in mid- and high-dose animals, increased white blood cells in high-dose animals, and increased lymphocytes in high-dose females at termination. At termination, renal nephropathy was observed in animals of all dose groups, with increased incidence and severity at the high-dose level. In addition, males showed atypical hyperplasia in the stomachs (fundic region), along with spermatozoa and germ cell effects at the high dose. One high-dose female showed atypical hyperplasia in the fundic region that was considered to be treatment-related. High-dose females showed distended gastric glands and a few treated females of all doses showed benign papillomas of the non-glandular region of the stomach (not discussed in report). Hepatic effects were few, with only rarified hepatocytes reported in a few male animals (6/60 in high dose *vs.* 3/121 in controls).
The NOAEL was set as 75 mg/kg bw/d.

Ref.: 68

Comment on setting a NOAEL

In the Table below the derived NOAELs from subchronic and chronic studies in different species were compiled.

EPA in its recent evaluation selected the NOAEL of the baboon study (30 mg/kg bw/d) for risk assessment based on clinical signs of toxicity which are presumably due to oral treatment. This might not be relevant for cosmetic uses.

The applicant in its safety evaluation used the NOAEL of the 95-week study in hamsters as this species was judged to be the most relevant to humans based on pharmacokinetics (75 mg/kg bw/d). Alternatively as a more conservative value, the NOEL of the 104-week rat study (≈ 48 mg/kg bw/d for both sexes) was used.

SCCP considers the NOAEL of this long term toxicity study in rats as 12 - 17 mg/kg bw/d (≈ 14.5 mg/kg bw/d) due to haematoxicity and decreased absolute and relative spleen weights. Haematoxicity was also detected in the 13-week subchronic oral toxicity studies in mice and rats, in hamsters only at higher doses and in the 1-year toxicity study in baboons. This was further confirmed by changes in haematology parameters in the long term studies in mice and hamsters. Interestingly, also in the 13-week subchronic dermal toxicity study in rats changes in erythrocytes parameters were observed.
The SCCP will use the NOAEL of 12 mg/kg bw/d of the long term toxicity study in rats for risk assessment.

Subchronic oral toxicity study in mice	A NOAEL was not established from this study since treatment-related changes in haematology parameters, increased alkaline phosphatase, and decreased cholesterol were observed at the low dose 25 mg/kg body weight/day.
Subchronic oral toxicity study in rats	The low dose was not associated with any treatment-related findings; thus, the NOAEL was determined to be 1,000 ppm (~100 mg/kg body weight/day).
Subchronic oral toxicity study in hamsters	The NOAEL is set at 200 mg/kg bw/d based on nephrotoxicity indicated by microscopic findings and polyuria, haemoglobinuria and haematouria.
Long term (1 year) oral toxicity study in baboons	NOEL is estimated to be 30 mg/kg bw/d based on the absence of any effect, including diarrhoea, in baboons at the low dose level in this study.
Long Term Toxicity / Carcinogenicity study – 18 Months (Mouse)	The LOAEL was 10 mg/kg bw/d based on liver changes. This dose level was considered as NOAEL based on haematotoxicity when excepting the target organ liver.
Long Term Toxicity / Carcinogenicity study – 104-week (Rat)	The study authors considered the NOAEL as ≈ 48 mg/kg/d for both sexes, combined (≈ 56 mg/kg bw/d in Females and ≈ 40 mg/kg bw/d in Males). SCCP considers the NOAEL as 12 mg/kg bw/d due to haematoxicity and decreased absolute and relative spleen weights (Mid Dose Females).
Long Term Toxicity / Carcinogenicity study – 95 Weeks (Hamster)	The NOAEL was set as 75 mg/kg bw/d.

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

Study 1

Guidelines:	/
Species/Strain:	*Salmonella typhimurium* TA92, TA98, TA100, TA1535, TA1537
Replicates:	triplicates in a single test
Test substance:	FAT 80 023/A
Solvent:	DMSO
Batch:	/
Purity:	/
Concentrations:	0.1, 0.3, 0.9, 2.7 and 8.1 µg/ml without metabolic activation Additionally 24.3 and 72.9 µg/ml without metabolic activation for TA92 0.1, 0.3, 0.9, 2.7, 8.1, 24.3 and 72.9 µg/ml with metabolic activation
Treatment:	Plate incorporation method with 48 h incubation time
GLP:	/
Date:	May 1978

The Ames-test was performed with the bacterial tester strains *Salmonella typhimurium* TA92, TA98, TA100, TA1535 and TA1537 with and without S9-mix. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Toxicity was evaluated on the basis of a reduction in the number of spontaneous revertant colonies. The experiment was performed with the direct plate incorporation method. Justified negative and positive controls were concurrently tested.

Results
The test compound did not induce an increase in the number of revertant colonies in any strain at any concentration tested both in the presence or absence of metabolic activation.

Conclusion
Under the experimental conditions used FAT 80 023/A was not mutagenic in the gene mutation tests in bacteria both in the absence and the presence of S9 metabolic activation.
Ref.: 42

Comment
The test is performed before the implementations of OECD guidelines. Batch number and purity were not reported. The test has only limited value.

Study 2

Guideline:	OECD 471
Strain:	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537
Replicates:	3 replicates in 2 individual experiments both in the presence and absence of S9-mix.
Test substance:	Triclosan [Irgasan DP 300; 2,4,4'-trichloro-2'-hydroxy diphenyl]
Solvent:	DMSO
Batch:	S 15155 TO1
Purity:	> 99%
Concentrations:	0.015, 0.05, 0.15, 0.5 and 1.5 µg/plate both without and with S9-mix
Metabolic activation:	Experiment 1: 3% and 10% Experiment 2: 10% and 30%

Treatment:	pre-incubation method with 60 minutes incubation and a selection period of 72 h.
GLP:	In compliance
Date:	March – September 1988

Triclosan was investigated for the induction of gene mutations in *Salmonella typhimurium* (Ames test). Test concentrations were based on the results of a preliminary toxicity study with concentrations up to the (nowadays) prescribed maximum concentration of 5000 µg/plate. Toxicity was evaluated on the basis of a substantial reduction in the number of micro-colonies in the background bacterial lawn. The experiments were performed with the pre-incubation method. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Negative and positive controls were in accordance with the OECD guideline.

Results
In the preliminary toxicity study triclosan was toxic towards the tester strains at the higher doses and consequently 1.5 µg/plate was chosen as the top dose level. In the main experiments toxicity was reported at the top dose level. No substantial increases in revertant colony numbers in any of the tester strains were observed at any of the doses tested either in the absence or presence of metabolic activation.

Conclusion
Under the experimental conditions used triclosan was not genotoxic (mutagenic) in the gene mutation tests in bacteria both in the absence and the presence of metabolic activation.

Ref.: 43

Study 3

Guideline:	OECD 471	
Strain:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	
Replicates:	triplicates both in the presence and absence of S9-mix TA100: triplicates in 2 retests in the absence of S9-mix	
Test substance:	39316	
Solvent:	DMSO	
Batch:	CC# 14663-09	
Purity:	/	
Concentrations:	Experiment 1:	0.00167, 0.005, 0.0167, 0.05, 0.1 and 0.167 µg/plate without S9-mix 0.05, 0.167, 0.5, 1.67, 2.5 and 5 µg/plate with S9-mix
	Experiment 2 and 3:	0.000167, 0.0005, 0.00167, 0.005, 0.01, 0.0167, 0.0333, 0.05, 0.1 and 0.167 µg/plate without S9-mix for TA100 only.
Treatment:	direct plate incorporation with 48 incubation without and with S9-mix	
GLP:	In compliance	
Date:	March 1993	

Test compound 39316 was investigated for the induction of gene mutations in *Salmonella typhimurium* (Ames test). Test concentrations were based on the results of a preliminary toxicity pre-screen with concentrations up to the prescribed maximum concentration of 5000 µg/plate evaluating the growth of the background lawn and/or frequency of spontaneous revertants. The experiments were performed with the direct plate incorporation method. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Negative and positive controls were in accordance with the OECD guideline.

Results
Toxicity was reported at the higher doses tested. However, due to excessive toxicity for strain TA100 without S9-mix only three acceptable dose levels remained. In a retest a statistical significant but not dose dependent increase in revertant colonies was observed. This finding was not confirmed in a second retest. Biologically relevant, dose dependent increases in the number of revertants were also not observed in the other strains at any of the doses tested both in the absence or presence of metabolic activation.

Conclusion
Under the experimental conditions used test compound 39316 was not genotoxic (mutagenic) in the gene mutation tests in bacteria both in the absence and the presence of S9 metabolic activation.

Ref.: 44

Comment
The test was not repeated. Purity of test substance 39316 (triclosan) was not reported.

Mutagenicity test with *Saccharomyces cerevisiae*

Study 1

Guideline:	/
Strain:	*Saccharomyces cerevisiae* MP-1
Replicates:	triplicates
Test substance:	FAT 80 023/A
Solvent:	DMSO
Batch:	/
Purity:	/
Concentrations:	10, 20, 30, 40, 50, 60 and 200 mg/l
Treatment:	3.5 h treatment, followed by an expression period of 4-6 days for inter- and intragenic recombinants or 8 days for cycloheximide resistance.
GLP:	/
Date:	November 1978

FAT 80 023/A was investigated for the induction of gene mutations in a mutagenicity test with *Saccharomyces cerevisiae*. Test concentrations were based on the results of a preliminary toxicity study. 4-nitroquinoline-N-oxide served as positive control. For the determination of inter- and intragenic recombinants yeast cells were cultured on normal and tryptophan free agar, for the determination of cycloheximide resistance, an indication of forward mutations, on cycloheximide agar.

Results
In the main experiment no colonies were found at the highest dose as a result of the inhibitory effect of FAT 80 023/A on the growth of the yeast cells. Treatment with FAT 80 023/A did not result in a significant increase in the incidence of intergenic recombinants nor in an increase in cycloheximide resistance. The occasional increases in intragenic recombinants in the mid doses of 30 and 40 mg/l were not dose dependent and can be attributed to variation inherent in the test system

Conclusion
Under the experimental conditions used FAT 80 023/A was not mutagenic in this mutagenicity test in yeast.

Ref.: 45

Comment

The test is performed before the implementations of OECD guidelines. Batch number and purity of FAT 80 023/A (triclosan) were not reported. The test has only limited value.

Study 2

Guideline:	/
Strain:	*Saccharomyces cerevisiae* MP-1
Replicates:	20 replicates in 3 experiments
Test substance:	Irgasan DP 300 [5-chloro-2-(2,4-dichlorophenoxy)-phenol]
Solvent:	DMSO
Batch:	/
Purity:	99.7% isomer pure
Concentration:	0.2 mg/ml
Treatment:	3.5 h treatment, followed by an expression period of 4 days for inter- and intragenic recombinants or 8 days for actidione resistance.
GLP:	/
Date:	June 1978

Irgasan DP 300 was investigated for the induction of gene mutations in a mutagenicity test with *Saccharomyces cerevisiae*. Only one test concentration was used. For the determination of inter- and intragenic recombinants yeast cells were cultured on normal and tryptophan free agar, for the determination of actidione resistance, an indication of forward mutations, on actidione agar. A positive control was not included.

Results
The results demonstrated that Irgasan DP 300 had an effect in the mutation and intergenic recombination system but not on the intragenic recombination system.

Conclusion
Under the experimental conditions used Irgasan DP 300 was mutagenic in this mutagenicity test in yeast.

Ref.: 46

Comment
The test is performed before the implementations of OECD guidelines. Batch number was not reported. Only one dose was tested. The test has only very limited value.

***In vitro* gene mutation assay with Mouse Lymphoma cells**

Study 1

Guideline:	/
Species/strain:	Mouse lymphoma cell line L5178Y/$tk^{+/-}$
Replicates:	Duplicates, two independent tests
Test substance:	FAT 80 023/A
Solvent:	0.05 N NaOH
Batch:	/
Purity:	/
Concentrations:	15.8 µg/ml (18h treatment) and 28.9 µg/ml (4 h treatment) without metabolic activation
Treatment:	4 or 18 h treatment and an expression period of 3 days. The selection period was not mentioned (probably 12 days)
GLP:	/
Date:	May 1978

FAT 80 023/A was assayed for gene mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of S9 metabolic activation. Test concentrations were based on the results of a toxicity test on cell survival. The concentration required to produce 80% cell-kill was calculated. In the main test, cells were treated for 4 or 18 followed by an expression period of 3 days to fix the DNA damage into mutations. The incidence of mutants was determined with methothrexate, cytosine arabinoside and thymidine as antimetabolites. Negative and positive controls were not included.

Results
Following treatment with FAT 80 023/A an increase in the mutant frequency was not observed at both dose levels tested in the absence of S9-mix.

Conclusion
Under the experimental conditions used FAT 80 023/A was not mutagenic in mouse lymphoma cells *in vitro*.

Ref.: 49

Comment
The test is performed before the implementations of OECD guidelines. Toxicity was not measured in the main experiment. Batch number and purity of FAT 80 023/A (triclosan) were not reported. The test has only limited value.

Study 2

Guideline:	/
Cells:	L5178Y mouse lymphoma cells
Replicates:	duplicate cultures in 2 independent experiments
Test substance:	Triclosan
Solvent:	DMSO
Batch:	S15155 TO1
Purity:	> 99%
Concentrations:	Experiment 1: 5.0, 7.5, 10.0, 15.0, and 20.0 µg/ml without S9-mix 3.5, 7.5, 10.0 and 15.0 µg/ml with S9-mix Experiment 2: 2.5, 5.0, 7.5, 10.0 and 15.0 µg/ml without S9-mix 1.0, 5.0, 7.5, 10.0 and 15.0 µg/ml with S9-mix
Treatment	3 h treatment without and with S9-mix; expression period 48 h and selection period of 12 days
GLP:	In compliance
Date:	September 1988

Triclosan was assayed for gene mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of S9 metabolic activation. Test concentrations were based on the results of a preliminary toxicity test considering suspension growth. In the main test, cells were treated for 3 h in the absence or presence of S9-mix followed by an expression period of 48 h to fix the DNA damage into a stable *tk* mutation. Liver S9-mix fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Toxicity was measured in the main experiments as mean % survival relative to the solvent control cultures. Negative and positive controls were included.

Results
In experiment 2 without S9-mix and in experiment 1 with S9-mix the highest dose tested showed the appropriate level of toxicity (10-20% adjusted relative total growth after the highest dose). In the experiment 1 without S9-mix and in experiment 2 with S9-mix excessive toxicity was seen at the highest dose but the first analysable doses did show the appropriate level of toxicity.

A more or less dose dependent increase in the mutant frequency was found in all experiments. However, the increase in mutant frequency was either less than 2-fold the background mutant frequency or for concentrations with a mutant frequency higher than the 2-fold back ground level the relative survival exceeded the 10%.

Conclusion
Under the experimental conditions used, triclosan was considered not mutagenic in the mouse lymphoma assay at the *tk* locus.

Ref.: 50

Comments
The test was not performed according the OECD guideline.

In vitro unscheduled DNA synthesis test

Study 1

Guideline:	/	
Species/strain:	primary rat hepatocytes	
Replicates:	triplicate subcultures	
Test substance:	Triclosan	
Solvent:	DMSO	
Batch:	S15155 TO1	
Purity:	/	
Concentrations:	Experiment 1:	0.6, 1.3, 2.5, 5, 10, 20, 40 and 80 µg/ml
	Experiment 2:	0.16, 0.3, 0.6, 1.3, 2.5, 5, 10 and 20 µg/ml
Treatment:	18 - 20 h treatment	
GLP:	In compliance	
Date:	May - September 1988	

Triclosan was investigated for the induction of unscheduled DNA synthesis (UDS) in primary hepatocytes of rats. Hepatocytes were isolated from male Fischer F344 rats. Hepatocytes were exposed simultaneously to triclosan and 10 µCi/ml 6^3H-thymidine for 18 - 20 h. After treatment cultures were divided in 3 subcultures for quantification of UDS whereas a fourth subculture was used to determine the toxicity of each treatment. Evaluation of autoradiography was done 7 days after exposure.
UDS was measured by counting nuclear grains and subtracting the average number of grains in 3 nuclear-sized areas adjacent to each nucleus; this value is referred to as net nuclear grain count. Unscheduled synthesis was determined in 50 randomly selected hepatocytes. Negative and positive controls were included.

Results
In experiment 1 at concentrations above 10 µg/ml no viable cells were seen at the end of the exposure period. In experiment 2 at 10 µg/ml only 14% survival was observed at the end of exposure whereas at 20 µg/ml no viable cells were seen at all. Although autoradiography was performed on all treated cultures, the autoradiographs of 20, 40 and 80 µg/ml of experiment 1 and of 20 µg/ml of experiment 2 were not scored due to excessive toxicity.
At none of the remaining dose levels in both experiments neither an increased net nuclear grain count, nor a notable increase in cells with 6 or more nuclear grains per cells, nor a notable increase in cells with 20 or more nuclear grains per cells (cells in repair) was observed.

Conclusion
Under the experimental conditions used triclosan did not induce unscheduled DNA synthesis in primary rat hepatocytes and, consequently, is not genotoxic in the *in vitro* UDS test.

Ref.: 52

Comment
The test was not performed according the OECD guideline. Purity was not reported. The test has only limited value.

Study 2

Guideline:	/
Species/strain:	primary rat hepatocytes
Replicates:	triplicate cultures
Test substance:	39317
Solvent:	DMSO
Batch:	/
Purity:	/
Concentrations:	0.25, 0.5, 1 and 2.5 µg/ml
Treatment:	18 - 20 h treatment
GLP:	In compliance
Date:	March - April 1993

39317 was investigated for the induction of unscheduled DNA synthesis (UDS) in primary hepatocytes of rats. Hepatocytes were isolated from male Fischer F344 rats. Hepatocytes, grown on cover slips, were exposed simultaneously to 39317 and 10 µCi/ml ^{3}H-thymidine (specific activity 50 - 80 Ci/mM) for 18 - 20 h. Test concentrations were based on the results of a preliminary screen on precipitation of 39317. Evaluation of autoradiography was done 7 days after exposure. Coverslips from each dose were pre-screened for toxicity by visual inspection under a microscope.
UDS evidenced as a net increase of grains over the nucleus was quantified by determining the nuclear and cytoplasmic grain counts. Cytoplasmic grain count was performed in 3 nuclear-sized areas adjacent to each nucleus. Unscheduled synthesis was determined in 150 nuclei per dose point. Negative and positive controls were included.

Results
Analytical results of the test article performed by the sponsor indicated that the actual concentrations were 0.3, 0.6, 1.18 and 3 µg/ml.
At none of the dose levels an increased net nuclear grain count nor an increase in cells with 5 or more nuclear grains per cells (cells in repair) was observed.

Conclusion
Under the experimental conditions used 39317 did not induce unscheduled DNA synthesis in primary rat hepatocytes and, consequently, is not genotoxic in the *in vitro* UDS test.

Ref.: 53

Comment
The test was not performed according the OECD guideline. The test was not repeated. Batch number and purity were not reported. However, stability and purity were reported as "the responsibility of the sponsor".

***In vitro* chromosome aberration test**

Study 1

Guideline:	OECD 473 (1983)
Species/strain:	Chinese hamster ovary (CHO-K_1 –BH_4) cells
Replicates:	duplicates in a single test

Test substance:	Triclosan
Solvent:	DMSO
Batch:	S15155 TO1
Purity:	> 99%
Concentrations:	0.1, 0.3, 0.5 and 1.0 µg/ml without metabolic activation 4.8, 9.5, 19 and 30 µg/ml with metabolic activation
Treatment:	24 h treatment without S9-mix. 6 h treatment with harvest time 24 h after start of treatment with S9-mix
GLP:	in compliance
Date:	April – August 1988

Triclosan has been investigated in the absence and presence of metabolic activation for the induction of chromosomal aberrations in CHO cells. Test concentrations were chosen on the basis of the results of a preliminary toxicity test measuring cell death and decline in mitotic index. In the absence of S9 cells were treated for 24 h and immediately harvested; in the presence of S9 cells were treated for 6 h and harvested 24 h after the start of treatment. Two hours before harvest, each culture was treated with colchicine solution (final concentration 0.25 µg/ml) to block cells at metaphase of mitosis. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Chromosome (metaphase) preparations were stained with 10% Giemsa and examined microscopically for chromosomal aberrations. Negative and positive controls were in accordance with the OECD guideline.

Results
In both the absence and the presence of a metabolic activation system, triclosan did not cause a statistical increase in cells with chromosome aberrations.

Conclusion
Under the experimental conditions used triclosan did not show evidence for a genotoxic (clastogenic) activity in CHO cells *in vitro*.

Ref: 47

Comment
It is not known whether in the main test the cells were sufficiently exposed since the mitotic index was not determined. Yet, in the experiment with metabolic activation a dose dependent increase was seen with a 4-fold increase in the highest dose compared to the untreated control. Therefore the SCCP consider this test as equivocal. The test was not repeated. The results of this test can only be used as supportive evidence.

Study 2

Guideline:	OECD 473, 1983
Species/strain:	Chinese hamster V79 cells Duplicates in a single experiment
Test substance:	FAT 80'023/Q
Solvent:	ethanol
Batch:	EN 91390.76
Purity:	/
Concentrations:	0.1, 1.0 and 3.0 µg/ml without and with S9-mix
Treatment:	4 hours with harvest times 7 (high dose only), 18 and 28 h (high dose only) after the start of treatment.
GLP:	in compliance
Date:	February – December 1990

FAT 80'023/Q was investigated in the absence and presence of metabolic activation for the induction of chromosomal aberrations in V79 cells. Test concentrations were chosen on the basis of the results of a pre-experiment for toxicity test measuring reduction in plating efficiency. Cells were treated for 4 h. Harvest times were 7 h (high dose only), 18 h or 28 h (high dose only) after the beginning of treatment. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Two hours (7 h harvest time) or 2.5 h (18 and 28 h harvest times) before harvest, each culture was treated with colchicine solution (final concentration 0.2 µg/ml) to block cells at metaphase of mitosis. Chromosome (metaphase) preparations were stained with Giemsa and examined microscopically for chromosomal aberrations. Negative and positive controls were in accordance with the OECD guideline.

Results
In the cytogenetic experiment, the mitotic index was reduced after treatment with the highest concentration at each fixation interval, except at interval 18 and 28 h in the presence of S9-mix. At the harvest time 7 h (both without and with S9) and 28 h (with S9) no increase in cells with chromosomal aberrations was observed.
A dose dependent and biologically relevant increase in cells with chromosomal aberrations was found at the harvest times of 18 h (both without and with S9-mix) and 28 h (without S9-mix).

Conclusion
Under the experimental conditions used FAT 80'023/Q induced an increase in the number of aberrant cells and, consequently, is mutagenic (clastogenic) in V79 cells *in vitro*.

Ref: 48

Comment
Exposure of the cells (measured as a decrease in the mitotic index) was not determined in the main test. In the protocol, it is mentioned that at harvest time 28 h only the high dose (3.0 µg/ml) is tested. However, in the table with the result at harvest time 28 h without S9-mix, (table 22 of the study report) the test article concentration is reported as 1.0 µg/ml. The test was not repeated. Purity of FAT 80 023/A (triclosan) was reported as "cf. Analytical Certificate in sponsor's file".

Summary: genotoxicity/mutagenicity of triclosan *in vitro*

strain/cell type	test compound	concentration	S9	result	quality	ref
Bacterial gene mutation assay						
TA92, TA98, TA100, TA1535, TA1537	FAT 80 023/A	0.1-8.1-(72.9) µg/plate 0.1-72.9 µg/plate	- +	negative negative	limited value	42
TA98, TA100, TA1535, TA1537	triclosan	0.015-1.5 µg/plate	-/+	negative	appropriate	43
TA98, TA100, TA1535, TA1537, TA1538	39316	0.000167-0.167 µg/plate 0.05-5 µg/plate	- +	negative negative	sufficient	44
Yeast gene mutation assay						
S. cerevisiae MP-1	FAT 80 023/A	10-200 mg/l	-	negative	limited value	45
S. cerevisiae MP-1	Irgasan DP 300	0.2 mg/ml	-	mutagenic	limited value	46
Gene mutation assay in mammalian cells						
L5178Y/$tk^{+/-}$ mouse lymphoma cells	FAT 80 023/A	15.8-28.9 µg/ml	-	negative	limited value	49

strain/cell type	test compound	concentration	S9	result	quality	ref
L5178Y/$tk^{+/-}$ mouse lymphoma cells	triclosan	2.5-20 µg/ml 1-15 µg/ml	- +	negative negative	appropriate	50
Unscheduled DNA Synthesis test						
primary rat hepatocytes	triclosan	0.16-80 µg/ml	-	negative	limited value	52
primary rat hepatocytes	38317	0.25-2.5 µg/ml	-	negative	sufficient	53
Chromosome aberration test						
CHO cells	triclosan	0.1-1 µg/ml 4.8-30 µg/ml	- +	negative negative	supportive evidence	47
V79 cells	FAT 80'023/Q	0.1-3 µg/ml	-/+	mutagenic	appropriate	48

3.3.6.2. Mutagenicity / Genotoxicity *in vivo*

Host mediated assay with *S. typhimurium* in mice

Guideline: /
Species: albino NMRI mice
Bacteria: *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537
Group sizes: 6 mice/group
Test substance: FAT 80 023/A
Solvent: 2% CMC
Batch: /
Purity: /
Dose levels: 50, 100, 200 and 400 mg/kg bw
Route: oral gavage
Cell removal: 1 h after the injection of the bacteria
GLP: /
Date: March 1979

FAT 80 023/A was investigated for the induction of gene mutations in the host mediated assay with *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537. After being fasted for 16 h the mice were treated orally by gavage 2 h, 1 h and immediately before bacteria were injected. The bacteria were injected in the lateral vein of the tail. One h after the injection of the bacteria the mice were killed, the livers removed and homogenised. The homogenate was centrifuged and the centrifugate was resuspended in saline. Five plates with 0.2 ml of the undiluted samples were used for the determination of the mutant count. The total bacterial count present in the animals was determined for each dosage or control group as a whole. Dilutions of 10^{-5} and 10^{-6} of the pooled samples were spread on 5 and 4 NB (nutrient brooth) plates respectively. The mutant rate is calculated from the mutant count and the total bacterial count. The mutation factor is the ratio of the mean mutation rate for each dosage group to the mean mutation rate of the control group. Negative and positive controls were not included.

Results
At the tested doses 50, 100, 200 and 400 mg/kg the mutation rates in comparison with those of the control were not significantly increased in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537

Conclusion
Under the experimental conditions used FAT 80 023/A was not mutagenic in this host mediated assay with *S. typhimurium* in mice.

Ref.: 54

Comment
The test is performed before the implementations of OECD guidelines. Batch number and purity of FAT 80 023/A (triclosan) were not reported. The test has only limited value.

Host mediated assay with mouse lymphoma cells in mice

Guideline:	/
Species:	DBA/2f/Bom mice
Cell type:	Mouse lymphoma cell line L5178Y/$tk^{+/-}$
Group sizes:	6 mice/group
Test substance:	FAT 80 023/A
Solvent:	0.05 N NaOH
Batch no:	/
Purity:	/
Dose levels:	1313 mg/kg bw
Route:	oral gavage
Cell removal:	6 days after cell inoculation and 3 days after compound administration
GLP:	/
Date:	May 1978

FAT 80 023/A was investigated for the induction of gene mutations in the host mediated assay. Test concentrations were based on the results of a toxicity test on cell survival. The mice were inoculated intraperitoneally with 10^6 cells per animal. FAT 80 023/A was administered orally. Six days after cell inoculation and 3 days after FAT 80 023/A administration, the cells were removed from the peritoneal fluid under a-septic precautions. The cells were seeded and cultured to fix the DNA damage into mutations. The incidence of mutants was determined with methothrexate, cytosine arabinoside and thymidine as antimetabolites. Negative and positive controls were not included.

Results
At 1313 mg/kg bw the target cell count was reduced with 50%. There was no increase in the number of mutant colonies in comparison with the control.

Conclusion
Under the experimental conditions used FAT 80 023/A was not mutagenic the host mediated assay with mouse lymphoma cells.

Ref.: 49

Comment
The test is performed before the implementations of OECD guidelines. Batch number and purity of FAT 80 023/A (triclosan) were not reported. The test has only limited value.

Mouse spot test

Study 1

Guideline:	/
Species/strain:	T stock males and C57BL/6JHan females
Group size:	/
Test substance:	Irgasan DP 300
Batch:	/
Purity:	/
Dose level:	50 mg/kg bw

Route: intraperitoneal injection
Day of administration: day 10 of pregnancy
Vehicle: Hanks balanced salt solution
GLP: /
Date: August 1978

Irgasan DP 300 has been investigated for the induction of mutations in the mouse spot test. Female C57BL/6JHan mice were exposed by ip injection to a single dose of 50 mg/kg bw Irgasan DP 300 at day 10 of pregnancy. Between 2 and 5 weeks of age the F_1 animals were analysed for the occurrence of spots.

Results
The examination was rendered difficult because with the exception of some midventral white spots all spots consisted of a more or less large mixture of mutant and non-mutant hairs.
The frequency of colour spots in mice in the test and control groups clearly show that Irgasan DP 300 in a dose of 50 mg/kg is active in the spot test. Compared to other compounds the authors consider Irgasan DP 300 as a mutagen of only medium effectiveness.

Conclusion
Under the experimental conditions used Irgasan DP 300 was mutagenic in the mouse spot test.

Ref.: 63

Comment
The test is performed before the implementations of OECD guidelines. Group size, batch number and purity were not reported. The test has only limited value.

Study 2

Guideline: /
Species/strain: male T stock and female C57BL/E mice
Group size: /
Test substance: Irgasan ®DP 300 (triclosan)
Batch: /
Purity: 99.7%
Dose level: 0, 1, 2, 4, 8 and 25 mg/kg bw
Day of administration: day 9.25 or 10.25 of pregnancy
Vehicle: 60% methanol
Scoring for mutations: day 12 after birth
GLP: /
Date: March - October 1979

Triclosan has been investigated for the induction of somatic mutations in the mouse spot test. Female C57B1/E mice were mated with T stock males. On days 9.25 or 10.25 post fertilisation triclosan was administered by ip injection. At 12 days after birth the offspring was scored for coat colour spots. A positive control was not included.

Results
Triclosan when injected in 60% methanol killed 12 out of 41 treated females of the 25 mg/kg group. In the other treatment groups all animals survived. Prenatal survival was clearly reduced by 25 mg/kg triclosan in both the 9.25 and 10.25-day treated groups. Level of exposure below 25 mg/kg produced no obvious effects on prenatal survival. Postnatal survival was severely reduced after 25 mg/kg and slightly but significantly reduced after 8

mg/kg in both the 9.25 and 10.25-day treated groups and significantly reduced after 4 mg/kg (average exposure 3.2 mg/kg) only in the 10.25-day treated group.
Within the 9.25 day group the incidence of recessive spots and midventral white spots is not increased by triclosan treatment. Within the 10.25 day set triclosan has a clear effect on the incidence of midventral white spots at 25 mg/kg but no effect on the incidence of recessive spots in comparison to the control group. However, 25 mg/kg was markedly toxic both to the mothers and the animals exposed *in utero*.

Conclusion
Under the conditions of this test, triclosan did not induce somatic mutations at non/sub toxic concentrations and, consequently, triclosan is not mutagenic in this mouse spot test.
Ref.: 64

Comment
The experiment is conducted before the development of the OECD guidelines. The batch nr was not reported. The test can be used as supportive evidence.

Bone marrow chromosome aberration test in Chinese hamsters (*Cricetulus griseus*)

Study 1

Guideline:	/
Species:	Female Chinese hamsters (*Cricetulus griseus*)
Group sizes:	4 females/group
Test substance:	GP 41 353 (triclosan)
Batch:	3
Purity:	/
Dose levels:	150, 300 and 600 mg/kg bw
Vehicle:	0.5 % CMC
Treatment:	oral by gavage, daily application on 2 consecutive days, 24 apart.
Sacrifice times:	6 h after the last treatment
GLP:	/
Date:	1973

Triclosan has been investigated for the induction of chromosome aberrations in bone marrow cells of Chinese hamsters. Chinese hamsters were exposed twice by oral gavage 24 h apart on two consecutive days. The highest dose 600 mg/kg bw is approximately 1/3 of the LD_{50}. Two h after the second treatment the animals were injected intraperitoneally with 10 mg colcemid/kg. Bone marrow cells were collected 6 h after the last treatment. Bone marrow preparations were stained with acetic-orcein and examined microscopically for chromosome aberrations. Cyclophosphamide was used as positive control.

Results
In comparison to the vehicle control there was no biologically relevant or statistically significant enhancement in the number of cells with chromosome aberrations with any dose level tested.

Conclusion
Under the experimental conditions used triclosan is not genotoxic (clastogenic) in bone marrow cells of Chinese hamsters.
Ref.: 55

Comment

The test is performed before the implementations of OECD guidelines. Purity was not reported. There were no indications of toxicity and thus sufficient exposure is not proven. The test has only limited value.

Study 2

Guideline:	/
Species:	Female Chinese hamsters (*Cricetulus griseus*)
Group sizes:	6 male and 6 females/group
Test substance:	FAT 80 023/A
Batch:	652
Purity:	/
Dose levels:	75, 150, 300 and 600 mg/kg bw
Vehicle:	0.7 % CMC
Treatment:	oral by gavage, thrice weekly for twelve weeks.
Sacrifice times:	6 h after the last treatment
GLP:	/
Date:	February 1979

FAT 80 023/A has been investigated for the induction of chromosome aberrations in bone marrow cells of Chinese hamsters after long term treatment. Chinese hamsters were exposed thrice weekly by oral gavage for 12 weeks. The highest dose 600 mg/kg bw is approximately 1/3 of the LD_{50}. Two h after the last treatment the animals were injected intraperitoneally with 10 mg colcemid/kg. Bone marrow cells were collected 6 h after the last treatment. Bone marrow preparations were stained with acetic-orcein and examined microscopically for chromosome aberrations. The solvent was used as negative control; a positive control was not included.

Results
Of the 12 animals treated with 600 mg/kg bw 5 died within the first week, additionally 3 by the 3rd, 4th and 9th week. One of the animals in the low dose group died at the end of the experiment.
In comparison to the vehicle control animals, long term treatment with FAT 80 023/A did not result in a biologically relevant or statistically significant enhancement in the number of cells with chromosome aberrations with any dose level tested.

Conclusion
Under the experimental conditions used FAT 80 023/A is not genotoxic (clastogenic) in bone marrow cells of Chinese hamsters.

Ref.: 56

Comment
The test is performed before the implementations of OECD guidelines. Long term treatment is not a routinely performed protocol. Purity of test substance FAT 80 023/A (triclosan) was not reported. There were no data on toxicity and thus sufficient exposure is not proven. The test has only limited value.

Bone marrow chromosome aberration test in rats

Guideline:	OECD 475 (1984)
Species/strain:	Wistar rats
Group size:	5 rats/sex/group
Test substance:	FAT 80'023/Q
Batch:	EN 91390.76
Purity:	/

Dose level:	4000 mg/kg bw
Route:	oral, once
Vehicle:	1% carboxymethylcellulose-suspension
Sacrifice times:	6 h, 24 h and 48 h.
GLP:	In compliance
Date:	December 1990 – April 1991

FAT 80'023/Q has been investigated for the induction of chromosome aberrations in bone marrow cells of rats. The test concentration was based on a pre-experiment for toxicity measuring acute toxicity. In the main experiment rats were exposed orally to 4000 mg/kg bw, the maximum tolerated dose. From approximately 18 h before treatment with the test compound the animals were fasted. Bone marrow cells were collected 6 h, 24 h or 48 h after dosing. Two and a half h before sacrifice animals were injected intraperitoneally with the spindle inhibitor colcemid (2.0 mg/kg bw) to arrest cell in metaphase. To describe a cytotoxic effect, and thus exposure of the target cells, the mitotic index (percentage cells in mitosis) was determined. Bone marrow preparations were stained with Giemsa and examined microscopically for the mitotic index and chromosomal aberrations. Negative and positive controls (24 h sacrifice time only) were in accordance with the OECD guideline.

Results
FAT 80'023/Q did not induce a reduction in the mitotic index and is considered not cytotoxic for bone marrow cells and thus exposure of the target cells is not proven. Exposure to 4000 mg/kg bw FAT 80'023/Q did not induce a biological relevant increase in cells with chromosomal aberrations in bone marrow cells of the rat up to a sacrifice time of 48 h after treatment.

Conclusion
Under the experimental conditions used FAT 80'023/Q was not genotoxic (clastogenic) in bone marrow cells of rats.

Ref.: 57

Comment
Purity of FAT 80'023/Q (triclosan) was reported as "see Analytical Certificate in sponsor's file". Since the test substance did not induce a decrease in the mitotic index as compared to the concurrent negative control data, evidence of exposure of the target cells is lacking and thus the test has only limited value.

Bone marrow micronucleus test in mice

Guideline:	OECD 474 (1982)
Species/strain:	CD-1 mice
Group size:	5 mice/sex/group
Test substance:	Triclosan
Batch:	S 15155 TO1
Purity:	≥ 99%
Dose level:	5000 mg/kg bw
Route:	oral by gavage, once
Vehicle:	1% methylcellulose
Sacrifice times:	24 h, 48 h and 72 h after dosing.
GLP:	In compliance
Date:	March - August 1988

Triclosan has been investigated for the induction of micronuclei in bone marrow cells of mice. The test concentration was based on a preliminary toxicity test with concentrations up to the maximal prescribed dose of 5000 mg/kg bw recording cell death and signs of malreaction. In the main experiment rats were exposed orally to 5000 mg/kg bw. Following

dosing the animals were examined regularly and any mortalities or clinical signs of reaction to the test compounds were recorded. Bone marrow cells were collected 24 h, 48 h and 72 h after dosing. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and normochromatic erythrocytes (PCE/NCE ratio). Bone marrow preparations were stained with 10% Giemsa and examined microscopically for the PCE/NCE ratio and micronuclei. Negative and positive controls were in accordance with the OECD guideline.

Results
In the preliminary toxicity study mice treated with the highest dose (5000 mg/kg bw) showed slight piloerection, hunched posture, waddling and ptosis. One male mouse treated with the highest dose (5000 mg/kg bw) died. In the main test the mice only showed the first 6 h after exposure piloerection and hunched posture.
At the sacrifice time 24 h and 48 h but not at 72 h triclosan induced a reduction in the PCE/NCE ratio confirming exposure of the target cells.
Exposure to 5000 mg/kg bw triclosan did not induce a biological relevant increase in cells with micronuclei in bone marrow cells of mice up to a sacrifice time of 72 h after treatment.

Conclusion
Under the experimental conditions used triclosan was not genotoxic (cacogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 60

Nucleus anomaly test on somatic interphase nuclei of Chinese hamsters

Study 1

Guideline:	/
Species:	Chinese hamsters (*Cricetulus griseus*)
Group size:	3 rats/sex/group
Test substance:	GP 41 353 (triclosan)
Batch:	Mg. 3
Purity:	/
Dose level:	150, 300 and 600 mg/kg bw
Route:	oral, twice 24 h apart
Vehicle:	0.5 % carboxymethylcellulose solution
Sacrifice times:	24 h after the last dose.
GLP:	/
Date:	May 1974

Triclosan has been investigated for the occurrence of nucleus anomalies in interphase nuclei of bone marrow cells of Chinese hamsters (*Cricetulus griseus*). Chinese hamsters were exposed twice by oral gavage 24 h apart on two consecutive days. The highest dose 600 mg/kg bw is approximately 1/3 of the LD_{50}. Bone marrow cells were collected 24 h after the last treatment. Bone marrow preparations were stained with undiluted May-Grünwald and subsequently with 5% Giemsa and examined microscopically for nuclear anomalies. As anomalies were registered: single Jolly bodies, fragments of nuclei in erythrocytes, micronuclei in erythroblasts, micronuclei in leucopoietic cells, bizarre forms of nuclei, polyploid cells and necrobiotic cells. Cyclophosphamide was used as positive control.

Results
In all triclosan exposed groups the percentage of cells displaying anomalies of nuclei did not differ significantly from the negative control.

Conclusion

Under the experimental conditions used triclosan was not genotoxic in bone marrow cells of Chinese hamsters.

Ref.: 58

Comment
The test is performed before the implementations of OECD guidelines. The nuclear anomaly test is not a routinely performed test. Purity was not reported. The test has only limited value

Study 2

Guideline:	/
Species:	Chinese hamsters (*Cricetulus griseus*)
Group size:	6 rats/sex/group
Test substance:	FAT 80 023/A
Batch:	652
Purity:	/
Dose level:	75, 150, 300 and 600 mg/kg bw
Route:	oral, thrice weekly for 12 weeks
Vehicle:	0.7 % carboxymethylcellulose solution
Sacrifice times:	6 h after the last dose.
GLP:	/
Date:	August 1978

FAT 80023/A has been investigated for the occurrence of nucleus anomalies in interphase nuclei of bone marrow cells of Chinese hamsters (*Cricetulus griseus*) after long term treatment. Chinese hamsters were exposed thrice weekly by oral gavage for 12 weeks. The highest dose 600 mg/kg bw is approximately 1/3 of the LD_{50}. Bone marrow cells were collected 6 h after the last treatment. Bone marrow preparations were stained with undiluted May-Grünwald and subsequently with 5% Giemsa and examined microscopically for nuclear anomalies. As anomalies were registered: single Jolly bodies, fragments of nuclei in erythrocytes, micronuclei in erythroblasts, micronuclei in leucopoietic cells and polyploid cells. The solvent was used as negative control; a positive control was not included.

Results
The bone marrow of only 3 female and 3 male hamsters (for the highest dose only 2 male hamsters) was evaluated. Of the animals treated with 600 mg/kg bw 1 died within the 2^{nd} week, additionally 3 by the 7^{th}, 8^{th} and 9^{th} week and further 3 in the 12^{th} week. One of the animals treated with 300 mg/kg bw died within the 5^{th} week. In all FAT 80023/A exposed groups the percentage of cells displaying anomalies of nuclei did not differ significantly from the negative control.

Conclusion
Under the experimental conditions used FAT 80 023/A was not genotoxic in bone marrow cells of Chinese hamsters.

Ref.: 59

Comment
The test is performed before the implementations of OECD guidelines. The nuclear anomaly test is not a routinely performed test. Purity of test substance FAT 80 023/A (triclosan) was not reported. The test has only limited value

Sex linked recessive lethal test in *Drosophila melanogaster*

Guideline:	/	
Species:	Male *Drosophilas melanogaster* of wild type strain Karsnäs 60	
Test substance:	Irgasan	
Batch no:	/	
Purity:	/	
Dose levels:	Experiment 1:	1000 ppm Irgasan in 1% sucrose
	Experiment 2:	1000 ppm Irgasan in Ringer solution
	Experiment 3:	1000 ppm in corn agar substrate
Treatment:	Experiment 1:	In glass tubes to which 0.5 ml of the sucrose solution was added for 24 h
	Experiment 2:	Injection of 1000 ppm Irgasan
	Experiment 3:	In ordinary vials with corn agar containing 1000 ppm Irgasan for 7 days.
GLP:	not in compliance	
Date:	1979	

Irgasan was tested in a sex linked recessive lethal assay in *Drosophila melanogaster*. Dose selection was based on the results of a toxicity test with adult male flies with and without pre-treatment with the inducer of the metabolic detoxication enzymes. Males were either treated with 1000 ppm Irgasan in 1% sucrose in glass tubes for 24 h, or injected with 1000 ppm Irgasan in Ringer solution or kept on vials with corn agar containing 1000 ppm Irgasan for 7 days. The flies which were treatment with Irgasan in sucrose were before treatment put for 4 h in empty tubes in order to increase the liquid consumption. Three broods of flies were studies: 0-3, 4-6, 7-10 days after treatment. New virgin females were given to the male for each brood.
Flies treated with Irgasan in sucrose solution and flies injected with Irgasan in Ringer solution were analysed chemically by GLC for the determination of the Irgasan content immediately after treatment and between 1 and 4 days after.

Results
Both in flies treated with Irgasan in sucrose solution or injected with Irgasan in Ringer solution the concentration of Irgasan reached control values in less than less 72 h or 48 h, respectively. Apparently Irgasan is excreted very fast and will thus not accumulate in the flies.
No effect of Irgasan in any of the broods within the experiments was recorded. The frequency of the lethals recorded after treatment with Irgasan is in accordance both with the parallel and the historic controls with this particular wild type strain.

Conclusion
Under the experimental conditions described, Irgasan did not induce an increase in sex linked recessive lethals and, consequently, Irgasan was not genotoxic in the sex linked recessive lethal test in *Drosophila melanogaster.*

Ref.: 51

Comment
The test was not performed according the OECD guideline. Batch number and purity were not reported. The test has only limited value.

Dominant lethal test

Guideline:	/
Species:	NMRI mice
Group sizes:	12 male mice/group

Test substance:	GP 41 353
Solvent:	2% sodium carboxymethylcellulose
Batch:	3
Purity:	/
Dose levels:	750 and 1500 mg/kg bw
Route:	oral gavage
Sacrifice times:	females were autopsied at day 14 of pregnancy
GLP:	/
Date:	October 1971

GP 41 353 has been investigated for the induction of dominant lethals in mice. Mice were exposed by oral gavage with 750 and 1500 mg/kg bw which are equal to approximately 1/6 and 1/3 of the LD_{50} respectively. Each male mouse from each group was mated with 4 untreated females immediately after treatment. After 1 week the females were removed from the cages and replaced by another group of 3 untreated females. This procedure was continued for 8 consecutive weeks. This time of 8 "mating periods" comprises all stages of the maturation of the male germ cell. The mated females were sacrificed and autopsied on the 14^{th} day of pregnancy. The number of alive and dead foetuses as well as early embryonic resorptions was listed. A positive control was not included.

Results
Until 3 days after treatment the males of the high dose group were found to display signs of intolerance as indicated by slight convulsions. The symptoms did not exert any influence on the mating performance. An increase in the number of implementations, live embryos, death embryos and early embryonic resorptions due to treatment with GP 41 353 was not observed.

Conclusion:
Under the conditions tested GP 41 353 did not induce an increase in dominant lethals and consequently is not genotoxic in this dominant lethal test in mice.

Ref.: 65

Comment
The test is performed before the implementations of OECD guidelines. Purity was not reported. The test has only limited value.

Chromosome aberration test in male germinal epithelium of the mouse

Study 1

Guideline:	/
Species/strain:	Male NMRI mice
Group size:	6 mice /group
Test substance:	FAT 80 023/A
Batch:	652
Purity:	/
Dose level:	189, 378, 756 and 1512 mg/kg bw
Route:	oral by gavage, on 5 consecutive days
Vehicle:	2% CMC
Sacrifice times:	1 day after the last dose
GLP:	/
Date:	December 1978

FAT 80 023/A has been investigated for the induction of chromosome aberrations in male germinal epithelium cells, in particular on spermatogonia, of mice. Mice were exposed on 5 consecutive days by oral gavage. Germinal epithelium cells were collected 1 day after the

last dose. Three h before section the mice were injected intraperitoneally with 10 mg colcemid/kg. Germinal epithelium cell preparations were stained and examined microscopically for chromosome aberrations. The solvent was used as negative control; a positive control was not included.

Results
In the 1512 mg/kg bw group 7 out 8 animals died in the course of the second to last treatment. The remaining animal was not included in the evaluation. In the other treatment groups all animals survived.
In comparison to the vehicle control animals, FAT 80 023/A did not induce a biologically relevant or statistically significant enhancement in the number of cells with chromosome aberrations at the three remaining dose levels tested.

Conclusion
Under the experimental conditions used FAT 80 023/A is not genotoxic (clastogenic) in germinal epithelium cells (spermatogonia) of mice.

Ref.: 61

Comment
The test is performed before the implementations of OECD guidelines. Purity of test substance FAT 80 023/A (triclosan) was not reported. Raw nor summarized data on the occurrence of chromosomal aberrations were not available in the report. The test has only limited value.

Study 2

Guideline:	/
Species/strain:	Male NMRI mice
Group size:	6 mice /group
Test substance:	FAT 80 023/A
Batch:	652
Purity:	/
Dose level:	189, 378, 756 and 1512 mg/kg bw
Route:	oral by gavage, 5 intermittently doses on days 0, 2, 3, 5 and 9
Vehicle:	2% CMC
Sacrifice times:	3 days after the last dose
GLP:	/
Date:	February 1979

FAT 80 023/A has been investigated for the induction of chromosome aberrations in male germinal epithelium cells, in particular on spermatogonia, of mice. Mice were exposed intermittently on days 0, 2, 3, 5 and 9 by oral gavage. Germinal epithelium cells were collected 3 days after the last dose. Three h before section the mice were injected intraperitoneally with 10 mg colcemid/kg. Germinal epithelium cell preparations were stained and examined microscopically for chromosome aberrations. The solvent was used as negative control; a positive control was not included.

Results
In the 1512 mg/kg bw group only 4 animals survived. These animals were not included in the evaluation. In the other treatment groups all animals survived.
In comparison to the vehicle control animals, FAT 80 023/A did not induce a biologically relevant or statistically significant increase in the number of cells with chromosome aberrations at the three remaining dose levels tested.

Conclusion

Under the experimental conditions used FAT 80 023/A is not genotoxic (clastogenic) in germinal epithelium cells of mice.

Ref.: 62

Comment
The test is performed before the implementations of OECD guidelines. Purity of test substance FAT 80 023/A (triclosan) was not reported. The test has only limited value.

Summary: genotoxicity/mutagenicity of triclosan *in vivo*

strain/cell type	test compound	concentration		result	quality	ref
Host mediated assay with S. typhimurium in mice						
albino NMRI mice	FAT 80 023/A*	50-400 mg/kg bw		negative	limited value	54
Host mediated assay with Mouse lymphoma cells in mice						
DBA/2f/Bom mice	FAT 80 023/A*	1313 mg/kg bw		negative	limited value	49
Mouse spot test						
T stock x C57BL/6JHan	Irgasan DP 300	50 mg/kg bw		mutagenic	limited value	63
T stock x C57BL/E	Irgasan DP 300	1-25 mg/kg bw		negative	supportive evidence	64
Bone marrow chromosome aberration test						
Chinese hamsters (female)	GP 41 353	150-600 mg/kg bw		negative	limited value	55
Chinese hamsters (female)	FAT 80 023/A*	75-600 mg/kg bw long term exposure		negative	limited value	56
Wistar rats	FAT 80'023/Q*	4000 mg/kg bw		negative	limited value	57
Bone marrow micronucleus test						
CD-1 mice	triclosan	5000 mg/kg bw		negative	appropriate	60
Nucleus anomaly test on somatic interphase nuclei						
Chinese hamsters	GP 41 353	150-600 mg/kg bw		negative	limited value	58
Chinese hamsters	FAT 80 023/A*	75-600 mg/kg bw long term exposure		negative	limited value	59
Sex-linked recessive lethal test						
Drosophila melanogaster, strain Karsnäs 60	Irgasan	1000 ppm		negative	limited value	51
Dominant lethal test						
NMRI mice	GP 41 353	750-1500 mg/kg bw		negative	limited value	65
Chromosome aberration test in male germinal epithelium cells						
male NMRI mice	FAT 80 023/A*	189-1512 mg/kg bw		negative	limited value	61
male NMRI mice	FAT 80 023/A*	189-1512 mg/kg bw		negative	limited value	62

3.3.7. Carcinogenicity

Carcinogenicity studies of 18 months, 104 weeks, and 90 to 95 weeks in duration have been conducted in the rat, mouse, and hamster to assess chronic exposure effects of triclosan (Pharmaco LSR, 1995 (66); Ciba-Geigy, 1986 (67); Huntingdon Life Sciences, 1999 (68)]. The three life-time bioassays were designed according to OECD guidelines Nos. 451 and/or 453, and GLP compliant.

Triclosan showed no tumourigenic effects in the rat and the hamster studies. The mouse oncogenicity study showed both hepatic adenomas and hepatocellular carcinomas following an 18-month exposure to triclosan. Descriptions of the rat, mouse, and hamster data are found in the table below.

Table 16: Findings from GLP Carcinogenicity Studies for Triclosan

Species (Strain)	Dosing Regimen	Duration of Treatment	Major Findings[1]	Reference; GLP and OECD Status
Mouse (CD-1)	Oral (diet) doses of 0, 10, 30, 100, or 200 mg/kg bw/d 50/sex/ dose + 20/sex/ dose (6-month interim)	18 months	Decreased survival occurred in males (100 mg/kg bw/d) and high-dose females (a smaller decrease in high-dose males was not significant); therefore, only the survival in females was considered to be affected by triclosan. Increased plasma levels of liver function enzymes were observed in higher dose mice, and decreased cholesterol was measured at all doses. There were no biochemical or pathological indications of kidney effects. Increased incidences of hepatic nodules or masses or discolorations were observed in higher dose animals. No histopathological changes other than in liver (hepatocellular hypertrophy in all animals except low-dose females) were attributed to triclosan treatment. Data show dose-dependent increases in liver adenomas and carcinomas, with increased incidence in males *vs.* females. Incidences of mice bearing at least 1 hepatic tumour (*i.e.*, combined adenoma and carcinoma data) were: 6, 10, 17, 32, 42 in males and 0, 1, 3, 6, 20 in females at doses of 0, 10, 30, 100, and 200 mg/kg bw/d, respectively. In summary, effects in the liver, serum cholesterol decreases (males and females) and hepatocellular hypertrophy (males only) were observed at the lowest dose of 10 mg/kg bw/d; investigators concluded that there was no NOEL based on liver changes at all dose levels.	Pharmaco LSR, 1995 (66); GLP-compliant OECD: No. 451 consistent
Rat (Sprague-Dawley	Oral (diet) doses of 0, 12, 40, or 127 mg/kg bw/d in males and 0, 17, 56, or 190 mg/kg bw/d in females 60/sex/ dose + 10/sex/ dose or 20/sex/ control group (52-week interim) 20/sex in additional high-dose	104 weeks	Increased food intake was found in high-dose males. Mean body weight decreases were observed to be transient during the study except for consistent significant decreases of up to 10% in high-dose females. Sporadic changes such as slight changes in protein, glucose, bilirubin, triglyceride, and blood urea nitrogen found in the first 52 weeks of the study had disappeared by 78-104 weeks. Slight changes in erythroid parameters were sporadic. Decreases in monocytes and white blood cells in F and increased clotting time in males was observed at 104 weeks. Absolute adrenal weights were increased in mid-dose males; absolute brain weights were decreased in high-dose males; absolute and relative ovary weights were increased in high-dose females; absolute and relative spleen weights were decreased in mid-dose females, and relative spleen weights were decreased in high-dose females at 104 weeks. Hepatocyte hypertrophy and hepatocytic inclusions (hyaline-staining) were occasionally noted in male rats at early time points but were not seen at 104	Ciba-Geigy, 1986 (67); GLP-compliant OECD: No. 453 consistent

Species (Strain)	Dosing Regimen	Duration of Treatment	Major Findings[1]	Reference; GLP and OECD Status
	group (247 or 422 mg/kg bw/d in males and females) at 52 weeks		weeks. No other histological lesions, either neoplastic or non-neoplastic, were observed that were considered to be treatment-related. Specifically, there were no treatment-related tumours, including hepatic tumours, in any of the treated rats examined histologically at 52 or 104 weeks. Animals in the additional high-dose group killed at 52 weeks (247 or 422 mg/kg bw/d in males and females, respectively) showed similar types of toxicity as the main study animals, excepting that the severity or incidences of events were increased. No tumours were observed in these animals. In summary, the results show that triclosan is not tumourigenic in a 2-year study in rats at doses of up to 127 mg/kg bw/d in males and 190 mg/kg bw/d in females. A NOEL of 1,000 ppm was determined (48 mg/kg bw/d for females and males combined).	
Hamster (Syrian)	Oral (diet) doses of 0, 12, 75, or 250 mg/kg bw/d 60/sex/ dose + 10/sex/ dose (52-week interim)	Females: 90 weeks Males: 95 weeks	Survival was significantly decreased in males and was generally poor (38-58%) in females at 90 weeks. Body weight gain was significantly decreased in all high-dose hamsters and was accompanied by a slight (3%), but significant decrease in food consumption in high-dose females. Biochemical changes observed at termination included increases (<50%) in blood urea nitrogen in high-dose hamsters and in triglycerides in mid- and high-dose males. Haematological changes observed included slight (<15%), but significant decreases in erythroid parameters in mid- and high-dose animals, increased white blood cells in high-dose animals, and increased lymphocytes in high-dose females at termination. Significant observations at 52 weeks (interim) were mainly renal changes in high-dose animals. At termination, renal nephropathy was observed in animals of all dose groups, with increased incidence and severity at the high-dose level. In addition, males showed atypical hyperplasia in the stomachs (fundic region), along with spermatozoa and germ cell effects at the high dose. One high-dose female showed atypical hyperplasia in the fundic region that was considered to be treatment-related. High-dose females showed distended gastric glands and a few treated females of all doses showed benign papillomas of the non-glandular region of the stomach (not discussed in report). Hepatic effects were few, with only rarified hepatocytes reported in a few male animals (6/60 in high dose *vs.* 3/121 in controls). There were no tumours considered to be treatment-related. In summary, triclosan had little to no effect in hamsters at 12 and 75 mg/kg bw/d, and toxic effects at 250 mg/kg bw/d that resulted in the general deterioration of high-dose males after Week 80. Overall results show that triclosan is not tumourigenic in hamsters at doses of up to 250 mg/kg bw/d. However, survival in females at 90 weeks was poor. NOAEL=75 mg/kg bw/d.	Huntingdon Life Sciences, 1999 (68); GLP-compliant OECD: No. 451 consistent

[1] Findings reported in table are significant compared to controls unless otherwise noted in text.

Doses tested in the 3 carcinogenicity assays were comparable, ranging from 10 to 200 mg/kg body weight/day in mice, 12 to 127 mg/kg body weight/day (for males) and 17 to 190 mg/kg body weight/day (for females) in rats, and 12 to 250 mg/kg body weight/day in hamsters. Survival was not altered by triclosan treatment in the rat study, whereas decreased survival was observed in both the hamster and the mouse studies. In general, neither biochemistry/clinical chemistry nor haematology analyses showed any serious

effects that could be attributed to triclosan treatment, except in the case of liver-related changes in the mouse study.

Rats showed no changes in liver function enzyme activity in plasma, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which would be indicative of hepatic damage. Liver effects in rats in the carcinogenicity study were limited to increased incidences of hepatocyte hypertrophy and hyaline-staining inclusions in high-dose males at early time points in the study. These findings were not observed in animals at the termination of the study.

As with rats, hamsters showed no alterations in liver function enzymes. The effects of triclosan in hamster liver, as observed in the carcinogenicity study, were limited to a slight decrease in organ weight in high-dose females. This finding was attributed to the decreased body weight gain at this dose level. In addition to the organ weight effects, slight, but statistically significant, decreases in erythroid parameters and in triglycerides were measured in mid-dose females. The only notable histopathological finding was an increased incidence of rarified hepatocytes in a few high-dose males.

In contrast to observations in rats and hamsters, liver effects in the mouse included increased liver function enzymes in plasma; decreased blood cholesterol levels; significant increases in liver weights; increased incidences of hepatic nodules, discolorations, or masses; hepatocellular hypertrophy; and, increased incidences of both hepatocellular adenomas and hepatocellular carcinomas, depending on dose level. Signs of liver effects, increases in cholesterol (both sexes) and hypertrophy (males only) were seen at the lowest dose of 10 mg/kg body weight/day. Hypertrophy was seen in mice of both sexes at 30 mg/kg body weight/day, and increases in liver function enzyme levels were seen in mice of both sexes at doses of 100 and 200 mg/kg body weight/day. Increases in numbers of hepatic tumours were observed at doses of 30 mg/kg body weight/day and higher.

Summary and NOAEL Values from the Rodent Carcinogenicity Studies

Three rodent lifetime bioassays have been conducted to evaluate the carcinogenic potential of triclosan. Triclosan produced hepatic effects and hepatic tumours in mice, but little evidence of toxicity and no tumours in rats. Hamsters showed increased liver toxicity relative to the rat, but no tumours.

No NOAEL could be determined for the mouse, based on findings of liver effects at all doses (effects of hepatocyte hypertrophy and decreased plasma cholesterol). Increased incidences of liver tumours were observed at doses of 30 mg/kg body weight/day and higher in mice.

It should be noted that triclosan is a peroxisome proliferator in mouse liver.

The NOEL for the rat study was determined by study investigators to be the mid-dose of 1,000 ppm, or ~48 mg/kg body weight/day for males and females combined, based on the finding of hepatocyte changes in the high-dose group of 3,000 ppm (~127 mg/kg body weight/day in males and 190 mg/kg body weight/day in females). Although absolute adrenal weights were elevated at the 1,000 ppm dose in rats, this change was not considered to be adverse for a number of reasons, including a lack of change in relative adrenal weights, a lack of change in adrenal weights in the high-dose group, and the absence of accompanying histopathological changes in the adrenal glands.

The NOAEL for the hamster study was determined to be the mid-dose of 75 mg/kg body weight/day. Although the values for certain haematologic parameters were significantly altered at doses of 12 and 75 mg/kg body weight/day, the erythroid changes were slight (on the order of 5 to 10%), and not considered to be adverse effects *per se*. An increase in triglycerides (40 to 50%) was not considered to be an adverse effect, as the increase was not accompanied by any changes in liver weight or histopathology.
The NOAEL values for the three rodent lifetime cancer bioassays are summarized in Table 17.

Table 17: Summary of NOAEL Values from GLP Carcinogenicity Studies for Triclosan in Rodents

Species	NOAEL (mg/kg bw/day)	Comment
Mouse	10 (this value is a LOEL for tumour formation only)	No overall NOAEL due to hepatotoxic effects observed at the lowest dose tested. There were no tumours observed in other tissues.
Rat	~48 (NOAEL)[1]	NOAEL based on systemic toxicity. There was no evidence of tumour formation, including in liver. There was no evidence of hepatotoxicity.
Hamster	75	NOAEL based on systemic toxicity. There was no evidence of tumour formation, including in liver.

[1] 1,000 ppm ~48 mg/kg body weight/day for males and females, combined.

Comment
According to the EU classification system, triclosan is not considered classifiable as a carcinogen. It should be noted that triclosan is a peroxisome proliferator in mouse liver.

3.3.8. Reproductive toxicity

The reproductive and developmental toxicology of triclosan has been investigated in teratology studies in the mouse, rat, and rabbit, and a two-generation reproductive toxicity study in the rat. Based on international guidelines (ICH, 2005), effects on fertility and reproduction and perinatal development should be evaluated in rodents (typically, the rat), and teratology studies be conducted in both rodent and non-rodent species (typically, the rat and the rabbit). The available studies for triclosan meet these recommendations.

3.3.8.1. Two generation reproduction toxicity

One GLP-compliant two-generation study was conducted in rats, providing both fertility and reproduction and post-natal data [Morseth, 1988 (69)]. The study was consistent with OECD guidelines (Two-Generation Reproductive Toxicity Study), with the exception that the coagulating gland was not preserved, and with ICH (Fertility and Early Embryonic Development, Stages A and B; Pre- and Post-Natal Development, ICH Stages D, E, and F) guidelines, with the exception that sperm counts and viability assessments, and specific tests for physical, sensory, reflexes and behaviour, were not conducted. In addition to the post-natal data provided in the two-generation rat study, the GLP-quality study in Colworth Wistar rats provided post-natal data [Denning *et al*., 1992 (74)]. This study was generally consistent with international guidelines, with the major exceptions being those listed above [*i.e*., as for the study by Morseth, 1988 (69)], that maturation and fertility were not assessed, and that relatively few animals (5 per treatment group) were allowed to deliver and rear offspring. The fertility, reproduction, and post-natal data from these studies are summarized in the table below.

Table 18: Findings from the Reproductive and Developmental Toxicity Studies that Examined Fertility and Reproduction and/or Post-Natal Parameters

Species (Strain)	Dosing Regimen (mg/kg bw/d)	Major Findings[1]	Reference, GLP and OECD Status
Charles River CD® (SD)Br rats	0, 300, 1,000, or 3,000 ppm (Doses of ~17, 56, and 176 mg/kg bw/d in males and ~23,	**Fertility and Reproduction:** F_0 Generation: There were no remarkable effects on F_0 treated rats at any dose, including no effects on mating, pregnancy, duration of gestation, and parturition. F_1 Generation: There were no treatment-related effects on reproduction indices.	Morseth, 1988 (69) GLP-compliant

Species (Strain)	Dosing Regimen (mg/kg bw/d)	Major Findings[1]	Reference, GLP and OECD Status
	73, and 229 mg/kg bw/d in females, based on food consumption and body weight data after 10 weeks of dosing. Both males and females were dosed starting 10 weeks prior to mating. The rats received the appropriate control or test diet throughout the growth, mating, gestation, and lactation phases, or until terminal sacrifice.	**Post-natal Parameters:** F_1 Generation: Post-natal survival from Day 0 to Day 4 (*i.e.*, viability index) was slightly reduced in the high-dose group (90, 94, 96, and 82% in the control, 300, 1,000, and 3,000 ppm groups, respectively). Adjusted mean body weights of pups in the high-dose group were generally lower than controls throughout the Day 0 to 21 lactation period. With regard to development, mean body weights of high-dose pups were lower than controls during Weeks 0 to 12. Mean body weights were slightly decreased in low-dose group females *vs*. controls during Weeks 4-8 and 11. Overall, the most common findings were dilated pelvises of the kidney that showed a slight increase in incidence at the high dose in pups, but no differences between treatment groups in adult animals. F_2 Generation: The mean number of live F_2 pups on Day 0, viability index (survival to Day 4), and survival to weaning were slightly lower in high-dose animals *vs.* controls. Adjusted mean body weights of high-dose pups were slightly, but significantly lower than controls on Day 0. There were no other remarkable treatment-related findings in the F_2 pups, or of mature F_2 offspring. Although study investigators considered the data to be somewhat equivocal, they determined the **Overall NOAEL** for the study to be 1,000 ppm (~65 mg/kg bw/d for males and females, combined), based on evidence of slightly reduced survival and pup body weights at the high dose. However, the data in the study indicate that a **Fertility and Reproduction NOEL** of 3,000 ppm (~203 mg/kg bw/day for males and females combined) would be appropriate based on the absence of treatment-related effect in both the F_0 and F_1 generations. The **Foetal and Post-Natal NOAEL** would be 65 mg/kg bw/day (based on pup body weights).	OECD: No. 416 consistent
Rat (Colworth Wistar)	Dams received 0, 30, 100, 300 mg/kg bw/day) *via* oral gavage, in corn oil on Days 6-15 of gestation	[Maternal and Foetal Data are presented in Table 19] **Post-natal Parameters:** Survival and development of pups from birth to weaning was comparable to controls. There was no significant increase in the number of pups with anomalies. Study investigators did not determine NOEL values for this study. However, the data indicate that a **Foetal and Post-Natal NOEL** of 300 mg/kg bw/d would be appropriate, based on the lack of foetal and pup effects at this dose.	Denning *et al*., 1992 (70); GLP-compliant[2] OECD: comparable

[1] Statistically and biologically significant findings have been outlined.
[2] Based on the presence of a statement of Quality Assurance, the study by Denning *et al*., [1992 (74)] in Colworth Wistar rats was assumed to contain GLP-quality data.

3.3.8.1.1 Fertility and Reproduction

Fertility and reproduction parameters of mating, pregnancy, duration of gestation, and parturition were assessed in the two-generation rat study [Morseth, 1988 (69)]. Based on the absence of effects in F_0 and F_1 generation rats, the NOAEL (NOEL) for fertility and reproduction was determined to be 3,000 ppm (~203 mg/kg body weight/day using male and female doses, combined), the highest dose tested.

3.3.8.1.2 Post-Natal Parameters

In the two-generation study that examined post-natal development in rats, the primary findings were a slight decrease in mean foetal body weights and a slight decrease in the Day 0 to Day 4 survival in the F_1 and F_2 generations at the high dose of 3,000 ppm (~203 mg/kg body weight/day) [Morseth, 1988 (69)]. Thus, the foetal and post-natal NOEL for this study was determined to be 1,000 ppm (~65 mg/kg body weight/day using

combined male and female doses) for triclosan administered in the diet. Results from the study in Colworth Wistar rats showed no effect of triclosan on the survival and development of pups from birth to weaning [Denning *et al*., 1992 (70)]. In this study, the post-natal NOEL was determined to be 300 mg/kg body weight/day for triclosan administered *via* oral gavage.

In summary, taking into account both of the studies containing post-natal data, the lowest post-natal NOAEL is considered to be 65 mg/kg body weight/day, the post-natal NOEL from the two-generation study using dietary administration of triclosan.

3.3.8.2. Teratogenicity

One-Generation Reproduction Toxicology: Developmental Toxicity Studies

3.3.8.2.1 GLP Studies

GLP-compliant teratology studies were conducted in mice, rats, and in rabbits using the oral route of administration. All 3 of the main studies were conducted along OECD guidelines. One preliminary range-finding study was conducted for each species, in addition to the definitive investigations. NOEL or NOAEL values were determined for each of the definitive studies in each species. Brief summaries of the pertinent findings from the GLP teratology studies are presented in table 19. Where appropriate, findings of foetal effects were classified either as *foetal variations* (an alteration that may occur at a relatively high frequency and/or represent a (reversible) retardation or acceleration in development, a transitory alteration, or a permanent alteration not considered to adversely affect survival, growth, development, or functional competence in a given species or strain) or *foetal malformations* (permanent change/anomalies in which there is a morphologic defect of an organ, resulting from an abnormal developmental process that occur at low incidences in a given species or strain of animal) (U.S. FDA, 2001).

Table 19: Findings from GLP Teratology/Developmental Toxicity (Segment 2, ICH Stage C) Studies for Triclosan

Species (Strain)	**Dosing Regimen (mg/kg bw/d)**	**Major Findings[1]**	**Reference, GLP and OECD Status**
Mouse (Crl:CD®-1(ICR)BR)	0, 5, 10, 20, 40, 80, or 160 mg/kg bw/d *via* the diet (Days 6-15 of gestation)	Dose range-finding study. **Maternal Parameters:** Maternal body weight gain and food consumption were reduced in the 160 mg/kg bw/d group. At the doses of 80 and 160 mg/kg bw/d, absolute liver weights and relative liver weights (relative to terminal body weights and to brain weights) were increased. **Foetal Parameters:** Foetal body weight data were lower for the 40, 80, and 160 mg/kg bw/d groups than for the vehicle control group. Litter averages for resorptions (early and late resorptions, percentage of resorbed conceptuses and the number of dams with resorptions) were increased at 160 mg/kg bw/day. There were no other remarkable findings. Study investigators did not determine NOEL values for this study.	Argus Research Laboratories, 1992a (70) GLP-compliant OECD: not applicable for a dose range finding study
Mouse (Crl:CD®-1(ICR)BR)	0, 10, 25, 75, or 350 mg/kg bw/d in the diet (Days 6-15 of gestation)	**Maternal Parameters:** Body weights and body weight gains were increased in the 350 mg/kg bw/d group. Absolute liver weights and relative liver weights (relative to terminal body weights and to brain weights) were significantly increased in the 75 and 350 mg/kg bw/d dose groups. The 350 mg/kg bw/d dose caused increases in the numbers of mice with tan-coloured livers, along with one	Argus Research Laboratories, 1992b (71) GLP-compliant

Species (Strain)	Dosing Regimen (mg/kg bw/d)	Major Findings[1]	Reference, GLP and OECD Status
		mouse in the 75 mg/kg bw/d dose group. **Foetal Parameters:** Foetal body weight data were slightly lower for the 75 and 350 mg/kg bw/d groups compared to the vehicle control group. There were reversible delays in ossification caused by the test article in the 75 and 350 mg/kg bw/d dosage groups, including skull ossification and reductions in the average numbers of ossified forepaw phalanges and hind paw phalanges. There were no other remarkable findings. **Maternal NOEL** = 25 mg/kg bw/d (based on tan-coloured livers and increased liver weights) **Foetal NOEL** = 25 mg/kg bw/d (based on decreased foetal body weights and delayed ossification)	OECD: consistent with "Teratogenicity" guideline
Rat (Charles River CD® Sprague-Dawley derived)	0, 5, 10, 25, 50, or 75 mg/kg bw/d *via* oral gavage, in 1% carboxymethyl-cellulose in a 20% glycerine in water suspension (Days 6-15 of gestation)	Dose range finding study. **Maternal Parameters:** The lower maternal body weights that occurred at the high dose were due to the weight loss in a single dam. There were no other remarkable findings. **Foetal Parameters:** Foetal body weight data were lower for all treatment groups *vs*. controls; however, only the 75 mg/kg bw/d group had foetal body weights outside the low range of historical control data. There were no other remarkable findings. Study investigators did not determine NOEL values for this study.	Bio/dynamics, 1992a (72) GLP-compliant OECD: not applicable for a dose range finding study
Rat (Charles River CD® Sprague-Dawley derived)	0, 15, 50, or 150 mg/kg bw/d *via* oral gavage, in 1% carboxymethyl-cellulose in a 20% glycerine in water suspension (Days 6-15 of gestation)	**Maternal Parameters:** There was a slight but significant decrease in food consumption from Days 6 through 11 of gestation at the high dose (70±7 *vs*. 76±5 g/kg bw/d in controls). There were no other remarkable findings. **Foetal Parameters:** Foetal development showed retarded ossification at the high dose (cranium, vertebrae, sternebrae, metacarpals, and pelvic girdle). There were no other remarkable findings. **Maternal NOEL** = 50 mg/kg bw/d (based on decreased food consumption at the high dose) **Foetal NOEL** = 50 mg/kg bw/d (based on delayed ossification at the high dose)	Bio/dynamics, 1992b (73) GLP-compliant OECD: consistent with "Teratogenicity" guideline
Rat (Colworth Wistar)	0, 30, 100, 300 mg/kg bw/d *via* oral gavage in corn oil (Days 6-15 of gestation)	**Maternal Parameters:** At the 300 mg/kg bw/d dose level, slight maternal toxicity was manifested as transient diarrhoea, retarded body weight gain (*e.g*., 9.4 *vs*. 13.0 g for controls during the period of Days 6 to 10), and reduced food consumption (5-15% decrease *vs*. controls), and increased water intake (<10% increase *vs*. controls). There were no other remarkable findings. **Foetal Parameters:** There were no remarkable findings. Study investigators did not determine NOEL values for this study. However, the data indicate that a **Maternal NOEL** of 100 mg/kg bw/d would be appropriate, based on decreased body weights and slight diarrhoea, with a **Foetal NOEL** of 300 mg/kg bw/d (based on a lack of foetal effects).	Denning *et al*., 1992 (74); GLP-compliant[2] OECD: comparable
Rabbit (New Zealand White)	0, 5, 10, 25, 50, or 75 mg/kg bw/d *via* oral gavage in 1% carboxymethyl-cellulose in a 20% glycerine in water	Dose range-finding study. **Maternal Parameters:** There were slight mean maternal body weight losses and lower terminal body weights of the does treated with 75 mg/kg bw/d, as well as decreased food consumption as measured on Days 6, 8-11, 13, and 16, but not on Days 7 and 18, for this dose group. There were no other remarkable findings. **Foetal Parameters:** There were no remarkable findings	Bio/dynamics, 1992c (77) GLP-compliant OECD: not applicable for a dose range

Species (Strain)	Dosing Regimen (mg/kg bw/d)	Major Findings[1]	Reference, GLP and OECD Status
	suspension (Days 6 to 18 of gestation)	Study investigators did not determine NOEL values for this study.	finding study
Rabbit (New Zealand White)	0, 15, 50, or 150 mg/kg bw/d *via* oral gavage in 1% carboxymethyl-cellulose in a 20% glycerine in water suspension (Days 6-18 of gestation)	**Maternal Parameters:** There were slight decreases in mean maternal body weight (-5.1% at Day 10 to -7.9% at Day 16, with significant decreases on Days 14 and 16) and occasional decreased body weight gains during the dosing period at the high dose, as well as decreased food consumption for this dose group (from -7.05 at Day 11 to -41.4% at Day 14). There were no other remarkable findings. **Foetal Parameters:** There were no remarkable findings at any of the doses tested. **Maternal NOEL** = 50 mg/kg bw/d (based on decreased maternal body weights and food consumption) **Foetal NOEL** = 150 mg/kg bw/d (based on the absence of effects at the highest dose tested)	Bio/dynamics, 1992d (78) GLP-compliant OECD: consistent with "Teratogenicity" guideline

[1] Being consistent with industry standards, statistical analyses were conducted for definitive, but not range-finding, studies. Statistically and biologically significant findings have been outlined.
[2] Based on the presence of a statement of Quality Assurance, the study by Denning *et al*. [1992 (74)] in Colworth Wistar rats was assumed to contain GLP-quality data.

GLP Studies in the Mouse

The potential for triclosan to induce developmental toxicity effects has been investigated in 1 range-finding and 1 definitive teratology study in mice [Argus Research Laboratories, 1992a (71); Argus Research Laboratories, 1992b (72)]. Based on the study report, the definitive study was consistent with OECD (Teratogenicity) and ICH (Embryo-foetal development, Stage C) guidelines, with the exception that the placenta was not grossly examined. This deviation would not have affected the interpretation of the results. The test article was administered *via* the diet; as triclosan did not affect food consumption, the calculated average dosages were comparable to the targeted dose levels in the definitive study.

Doses in the definitive mouse study were 0, 10, 25, 75, or 350 mg/kg body weight/day administered in the diet on Days 6 to 15 of gestation. Maternal toxicity was observed at doses greater than 25 mg/kg body weight/day, including liver effects (*i.e.*, increased liver weights and tan-coloured livers). Triclosan was not teratogenic in either the dose range finding or the definitive study. Foetal effects (classified as foetal variations) included slightly decreased body weights at the two higher doses that also caused maternal toxicity, as well as reversible delays in ossification at the same doses. A foetal NOEL of 25 mg/kg body weight/day was determined based upon decreases in foetal body weights and delayed ossification at higher dose levels in the definitive study.

GLP Studies in the Rat

In rats, triclosan has been investigated in 1 range-finding and 2 definitive teratology studies [Bio/dynamics, 1992a (73); Bio/dynamics, 1992b (74); Denning *et al*., 1992 (70)]. The study in Colworth Wistar rats [Denning *et al*., 1992 (70)], although lacking a formal statement of GLP compliance, included a statement of Quality Assurance and was, therefore, considered to contain GLP-quality data. Based on the reports, the studies were generally consistent with the OECD (Teratogenicity) and ICH (Embryo-foetal development, Stage C) guidelines, with the major exceptions being that the placenta was not grossly examined in the Bio/dynamics studies, and there were relatively few numbers of animals in the post-natal survival and development portion of the study by Denning *et al*. (see Section 3.8.1). Triclosan was orally administered by gavage in all of the studies, although different vehicles were used (1% carboxymethylcellulose in 20% glycerine in water suspension in the

Bio/dynamics studies, and corn oil in the Colworth Wistar rat study). Rats were treated on Days 6 to 15 in each of the studies.

Doses of 0, 15, 50, or 150 mg/kg body weight/day were administered in the definitive oral gavage study using triclosan in 1% carboxymethylcellulose [Bio/dynamics, 1992b (73)]. The dams showed evidence of maternal toxicity in the form of slight, but significant, decreases in food consumption from Days 6 to 11 of gestation in high-dose animals. There was no evidence of any teratogenic effect of triclosan. Foetal effects (foetal variations) were manifest as delayed ossification at the high dose of 150 mg/kg body weight/day, and with maternal toxicity occurring at the same dose. Based on observations of delayed ossification at the high dose, the foetal NOEL in this study was determined to be 50 mg/kg body weight/day. The maternal NOEL was also 50 mg/kg body weight/day.

The 1992 study by Denning *et al*. was conducted in Colworth Wistar rats at doses of 0, 30, 100, or 300 mg triclosan/kg body weight/day administered *via* oral gavage in corn oil, and examined both embryo/foetal and post-natal parameters. The results from this study demonstrated no foetal effects in terms of anomalies, body weights, numbers of live foetuses, *etc*. Maternal toxicity was in the form of delayed body weight gain, and reduced food consumption, as well as transient diarrhoea, at the highest dose, resulting in a maternal NOEL of 100 mg/kg body weight/day. Based on the absence of foetal effects at any of the doses tested, the foetal NOEL for this study was determined to be 300 mg/kg body weight/day.

In summary, triclosan showed no teratogenic effects at any of the doses in either of the GLP studies in rats. The only embryo or foetal toxic effect observed was classified as a foetal variation (delayed ossification) in the 1% carboxymethylcellulose vehicle study at the dose of 150 mg/kg body weight/day, a dose that was associated with evidence of maternal toxicity (significantly decreased food consumption). The findings of maternal toxicity at the high dose in each of the studies indicated that dose levels were adequately high in each case. (It should be noted that the observed decreases in food consumption followed the administration of triclosan by oral gavage, and therefore would not likely have been due to any decreased palatability of the diet.) Hepatotoxic effects were not noted in the dams in any of the studies. No embryo or foetal toxic effects were seen at any of the doses in the corn oil study. None of the studies showed evidence of any teratogenic effects of triclosan. Taking into account both of the GLP rat teratology studies, the foetal NOEL is considered to be 50 mg/kg body weight/day in rats given triclosan *via* oral gavage in a 1% carboxymethylcellulose vehicle, with the maternal NOEL being also 50 mg/kg body weight/day.

GLP Studies in the Rabbit

In rabbits, triclosan has been investigated in one range-finding and one definitive teratology study [Bio/dynamics, 1992c (75), Bio/dynamics, 1992d (76)]. Based on the reports, the studies were generally consistent with the OECD (Teratogenicity) and ICH (Embryo-foetal development, Stage C) guidelines, with the major exception being that the placenta was not grossly examined. Triclosan was orally administered by gavage in a vehicle of 1% carboxymethylcellulose in 20% glycerine in water suspension. The does were treated from Days 6 to 18 of gestation.

Doses of 0, 15, 50, or 150 mg/kg body weight/day were administered in the definitive oral gavage study in rabbits [Bio/dynamics, 1992d (76)]. The does showed evidence of significant maternal toxicity in the form of decreased body weights, body weight gains, and food consumption at the high dose. There was no evidence of embryo or foetal toxicity or of teratogenic effect of triclosan. Based on the lack of foetal effects in this study, the foetal NOEL was determined to be 150 mg/kg body weight/day administered by gavage, with the maternal NOEL being 50 mg/kg body weight/day.

In summary, triclosan showed no teratogenic and no embryotoxic effects at any of the doses in the definitive GLP study conducted in rabbits. Findings of maternal toxicity (decreases in maternal body weight gains) at the high dose indicated that dose levels were adequately high in the study. Hepatotoxic effects were not observed in any of the dams. The foetal NOEL is considered to be 150 mg/kg body weight/day in rabbits given triclosan *via* oral gavage, with the maternal NOEL being 50 mg/kg body weight/day.

Non-GLP Studies

Table 20 presents summaries of the non-GLP developmental toxicity studies for triclosan, including findings from a genotoxicity study in mice that examined embryo/foetal toxicity following triclosan administered *via* the intraperitoneal route of administration.

Table 20: Findings from non-GLP Teratology/Developmental Toxicity (Segment 2, ICH Stage C) Studies for Triclosan

Species (Strain)	Dosing Regimen (mg/kg bw/d)	Major Findings[1]	Reference, GLP and OECD Status
Mouse (C57Bl/E cross, specific for the genotoxicity assay conducted)	Single intraperitoneal injections of 0, 1, 2, 4, 8, or 25 mg/kg bw on either Day 9 or Day 10 or gestation	Note that this was primarily a genotoxicity study (not a reproductive/developmental toxicity study). **Maternal Parameters:** Only mortality was assessed. There were deaths in the high-dose group (12/41 dams), but no deaths in the 267 animals treated at the 1, 2, 4, and 8 mg/kg doses. **Foetal Parameters:** The principle endpoints assessed were embryo and post-natal survival. Embryo survival (reductions in litter size) was reduced at the high dose in animals dosed on either Day 9 or Day 10. Post-natal survival was markedly reduced at the high dose. Post-natal survival was slightly decreased at the dose of 8 mg/kg bw (Day 9 or Day 10 treatment), and at the doses of 2 and 4 combined. Study investigators did not determine NOEL values for this study. It should be noted that, due to the design of the study (*e.g.*, lack of evaluation of parameters typically assessed in reproductive toxicity studies), it was not possible to reach any clear interpretation and conclusion of the impact of these data.	Russell and Montgomery, 1980 (64) GLP: not specified OECD: not specified
Rat (Wistar)	0, 100, 200, or 400 mg/kg bw/d *via* oral gavage in olive oil (Days 7-17 of gestation)	**Maternal Parameters:** Clinical signs of piloerection, incontinence, and diarrhoea were observed at the high dose, together with a decrease in food consumption during the dosing period. **Foetal Parameters:** There was a significant increase in numbers of foetal deaths at the high dose of 400 mg/kg bw/d (7.05% mortality *vs*. 1.59% in the controls). Study investigators did not determine NOEL values for this study. However, the data indicate that **Maternal** and **Foetal NOEL**s of 200 mg/kg bw/d would be appropriate, based on the absence of any significant maternal and foetal effects at this dose level.	Kawashima *et al*., 1987 (77) GLP: not specified OECD: not specified

Species (Strain)	Dosing Regimen (mg/kg bw/d)	Major Findings[1]	Reference, GLP and OECD Status
Rat (Wistar) and Hamster (Syrian)	0, or 1/1000, 1/500, 1/250, 1/100, or 1/50 of the LD_{50} dose in a pilot study (approximately 4, 8, 16, 40, and 80 mg/kg bw/d based on the LD_{50} of 4 g/kg bw in rats – doses in hamsters were similarly 1/1000, 1/500, 1/250, 1/100, or 1/50 of the LD_{50} dose). Animals were treated during gestational Days 6-15 (rats) or Days 6-10 (hamsters).	Limited numbers and types of parameters were assessed (dams: body weight, clinical signs, mortality; foetuses: body weights, placental weights, foetal length, foetal tail length, number of foetuses, number of males, numbers of resorptions, gross abnormalities). **Maternal Parameters (Rat):** There were no remarkable findings. **Foetal Parameters (Rat):** A significant decrease in number of live foetuses and number of males was reported in the 1/1000 (lowest dose) group *vs*. controls. However, this was not considered to be treatment-related based on a lack of dose relationship. Also, the decrease in number of foetuses did not appear to be due to an increase in resorptions but could have reflected a decrease in ovulation or implantation rate (could not be confirmed due to lack of corpora lutea data). **Maternal Parameters (Hamster):** Mortality was reported at the highest dose tested (no details provided). **Foetal Parameters (Hamster):** Body weights were significantly decreased in the high-dose group. The significant decrease in number of live foetuses in the low dose group is not considered to be treatment-related due to the lack of a dose relationship. Also, there was no increase in number of resorptions, suggesting that there might have been a decrease in ovulation or implantation rate (could not be confirmed due to lack of corpora lutea data). The decrease in number of live foetuses in the high-dose group was accompanied by maternal mortality. Study investigators did not determine NOEL values for this study. It should be noted that, due to the design of the study (*e.g.*, lack of evaluation of parameters typically assessed in reproductive toxicity studies), it was not possible to reach any clear conclusion of the impact of these data.	Piekacz, 1978 (78) Predates GLP and OECD

[1] Statistically and biologically significant findings have been outlined.

Non-GLP Studies in the Mouse

In addition to the GLP studies in mice, limited reproductive toxicity data were found in a published, non-GLP mouse "spot test" genotoxicity study [Russell and Montgomery, 1980 (64)]. Since this was a genotoxicity study, and not a reproductive toxicity study, there were a large number of deviations from international guidelines for the conduct of reproductive toxicity studies: the lack of evaluation of typical parameters for evaluation in reproductive and developmental toxicity studies (*e.g.*, external and internal examination of the foetuses, litter parameters); the dosing regimen (a single injection on gestational Days 9 or 10 *vs*. dosing throughout the period of organogenesis); the route of administration (the intraperitoneal route is not appropriate for reproductive toxicity testing); the choice of vehicle solution (although vehicle controls were included and no toxic vehicle effects were reported, 60% methanol is not considered to be an appropriate, non-toxic vehicle for use in a reproductive toxicity study), and the choice of statistical analytical methods (it is presumed, based on the use of the Fisher's Exact test for the genotoxicity data, that the same test was used for the reproductive effects data). As such, the significance of the study's stated statistically-significant findings are questionable. As maternal deaths were reported to have occurred in the high-dose group, developmental toxicity findings at this dose level could be attributed to maternal toxicity, and not to the direct toxicity of triclosan to foetuses *per se*. Without data from the evaluation of appropriate reproductive toxicity study parameters, it was not possible to reach a conclusion regarding the significance, if any, of the post-natal survival data. Overall, the choice of methods used indicates that the data in this non-GLP mouse study are of limited value.

Non-GLP Studies in the Rat and Hamster

Additional reproductive and developmental toxicity data in rats were provided in two non-GLP studies [Kawashima *et al*., 1987 (77); Piekacz, 1978 (78)].

Although no statement of GLP compliance was included in the published report, the study by Kawashima *et al*. [1987 (77)] appears to have been well-conducted using methodology comparable to published guidelines for the conduct of reproductive toxicity studies. Effects of significantly increased foetal mortality were reported at the high dose of 400 mg/kg body weight/day (7.05 *vs*. 1.59% mortality in controls, with the accompanying finding of increased numbers of resorptions at the high dose)[1]. These effects could not conclusively be attributed to the direct action of triclosan, as this dose also caused maternal toxicity (diarrhoea, incontinence, piloerection, decreased food consumption). There was no evidence of teratogenic effects caused by triclosan at any of the doses tested. Although study investigators did not determine a NOEL for this study, a NOEL value of 200 mg/kg body weight/day may be considered appropriate based on the absence of any significant maternal or foetal effects at this dose.

The early study by Piekacz [1978 (78)] included data from experiments conducted using both rats and hamsters. This study was not consistent with current guidelines for the conduct of reproductive toxicity studies, especially with regard to the parameters evaluated, the species used (*i.e.*, hamsters are not conventionally used as they are considered to be a highly sensitive species for reproductive studies), the number of animals (*i.e.*, only 10 per group), and the statistical methods used (no evidence that a necessary correction (adjustment) for multiple comparisons was included). As maternal toxicity was not evaluated, excepting mortality and body weight changes, the doses selected for the study could not be evaluated for appropriateness and, moreover, any reproductive or developmental toxicity effects observed could not be evaluated with reference to maternal effects. In this study, decreases in numbers of live foetuses were reported to occur at the low dose in rats and in both the low and high doses in hamsters. These effects in the low-dose groups were not considered to be directly treatment-related, as, with regard to both rats and hamsters, there was no dose-relationship. There was also no high-dose effect in the rats. For the low dose groups (rat and hamster), there were no increases in resorptions, indicating that the decreased number of live foetuses may have reflected decreases in implantation or ovulation rates (could not be confirmed due to lack of corpora lutea data). Foetal effects in hamsters of decreased foetal numbers at the high dose of 80 mg/kg body weight/day were accompanied by maternal toxicity effects consisting of deaths, and thus were not clearly a direct effect of triclosan. Overall, regardless of the contention that the study may have been inadequately conducted compared to current standards, the study provides little to no evidence of any treatment-related effect of triclosan on the development of rats or hamsters. As with the study by Kawashima *et al*. [1987 (77)], there were reported to be no teratogenic effects of triclosan at any of the doses tested. No NOEL level was determined for this study.

3.3.8.3 Summary and NOAEL Values from Reproductive and Developmental Toxicology Studies

Both GLP and non-GLP reproductive and developmental toxicology studies have investigated the effects of triclosan on fertility, development, parturition and lactation. The pivotal studies were conducted pursuant to GLP regulations and generally followed the relevant ICH and OECD guidelines. Appropriate test species (*i*.e., rats and rabbits) and a clinically relevant route of administration (*i.e*., oral) were used in the studies. Although toxicokinetic parameters were not measured in these studies, the doses achieved in these test systems reached as high as 300 mg/kg body weight/day in rats and 350 mg/kg body weight/day in

[1] It should be noted that the mortality rates were 1.51 and 8.19% in the control and high-dose groups, respectively, as presented in the published paper by Kawashima *et al*. (1987). However, a re-calculation of the data (number of dead implants / total number of implants x 100) yielded mortality rates of 1.59 and 7.05% in the control and high-dose groups, respectively. Following appropriate chi-square statistical analysis, the high dose group data were found to be significantly different from control ($p<0.05$).

mice (not a species typically used in reproductive toxicity studies, due to the high sensitivity of the species) and are considered adequate to assess the potential for triclosan to cause human reproductive toxicity. In addition, evidence of maternal toxicity was observed in all of the studies, indicating that adequately high doses of triclosan have been tested. An overall, integrated view of the foetal data together with maternal data is necessary when assessing the relevance of the findings in these reproductive toxicity studies (U.S. FDA, 2001).

Taking the studies all together, there was no evidence of teratogenic effects of triclosan in any of the studies, both GLP and non-GLP, and at any of the dose levels tested in rats and rabbits. Similarly, there was no evidence of teratogenic effects in studies conducted in mice and hamsters. Furthermore, in the definitive GLP studies, the effects on developing foetuses were limited to foetal variation effects of reversible, delayed ossification in rats, there being no effects observed in rabbits.

It is important to note that foetal variations were observed at doses that also produced significant maternal toxicity. In the GLP-compliant mouse study, consistent with the findings from the rat studies, there were clear indications that the foetal variations of significantly decreased body weight and delayed ossification observed at the two higher doses in the mouse study were likely to be secondary to maternal toxicity consisting of hepatic effects of tan-coloured livers and increased absolute and relative liver weights (there were no foetal effects at doses that were not maternally toxic in mice). No liver effects were observed in any of the studies in rat or rabbits using doses up to 300 mg/kg body weight/day. While there were reports of decreases in numbers of live foetuses in the non-GLP studies [Kawashima *et al*., 1987 (77); Russell and Montgomery, 1980 (64); Piekacz, 1978 (78)], these effects also were observed at doses of triclosan that produced significant maternal toxicity (*e.g*., deaths). Thus, the reported effects were unlikely to have been a direct effect of triclosan but, rather, secondary to maternal toxicity. It is considered that only findings potentially indicative of reproductive toxicity at doses that do not produce maternal toxicity are of increased concern for human reproductive or developmental toxicity. Thus, taken altogether, triclosan was not teratogenic and provided no clear evidence of direct effects on reproduction, embryo/foetal toxicity, or post-natal developmental toxicity in any of the reproductive and developmental toxicology studies.

Triclosan also has been investigated for potential actions as an oestrogen disruptor, as its chemical structure resembles that of known non-steroidal estrogens (*e.g.*, DES, bis-phenol A). In a published, non-GLP study [Foran *et al*., 2000 (79)], Japanese medaka fry were exposed to concentrations of up to 100 µg triclosan/µL for 14 days, but there was no evidence of any effect of triclosan on sex ratios in developing fish. The results of this experiment were consistent with the findings from GLP studies in mammals indicating that triclosan has no effect on sex ratios or on reproductive maturity.

NOAEL (NOEL) values from the definitive GLP studies are summarized in Table 21. The developmental toxicity effects of decreased foetal body weights and delayed ossification in all of the studies were observed at doses that also caused maternal toxicity. Based on the data in Table 21, the mouse NOAEL (both maternal and foetal) could represent an overall NOAEL for reproductive and developmental toxicity effects of triclosan. However, as observed in the repeated dose studies, the mouse is uniquely sensitive, showing liver effects at low doses of triclosan, including in the dams in the teratology study. Thus the low NOAEL value for foetal effects that was determined based on the mouse study may be attributed to the sensitivity of the maternal mice to liver effects, and is not due to any direct effect of triclosan on foetuses *per se*. The next lowest value for an overall NOAEL for reproductive and developmental toxicity is 50 mg/kg body weight/day from the study in rats. Of note, a lack of liver effects at doses up to 300 mg/kg body weight/day was seen in studies in both rats and rabbits, indicating a consistency between these two species.

Table 21: Summary of NOAEL[1] Values from GLP Reproductive and Developmental Toxicity Studies for Triclosan

Species	Route of Administration	Maternal NOAEL (NOEL)[1] (mg/kg bw/d)	Foetal NOAEL (NOEL)[1] (mg/kg bw/d)	Comment
Fertility and Reproduction Effects				
Rat	Diet	203[2]		No adverse effects on fertility and reproduction, based on lack of remarkable findings in F_0 and F_1 pups.
Foetal Effects				
Mouse	Diet	25	25	Maternal liver effects noted in 2 higher dose groups. Foetal effects limited to decreased foetal body weights and delayed ossification at doses that caused maternal toxicity.
Rat	Oral (gavage; carboxymethyl-cellulose)	50	50	Decreases in food consumption in high-dose dams. Reversible, delayed ossification in foetuses at same dose.
Rat	Oral (gavage; corn oil)	100	300	Decreases in body weight and diarrhoea in high-dose dams. No remarkable foetal effects.
Rabbit	Oral (gavage)	50	150	Decreases in body weight and food consumption in high-dose dams. No remarkable foetal effects.
Pre- and Post-Natal Effects				
Rat	Diet	65[2]		Slight decreases in foetal body weights and in mean number of live pups at the highest dose.

[1] In all cases, the NOEL values were taken to be the NOAEL values for the study as only NOEL values were determined.
[2] Male and female doses combined

3.3.9. Toxicokinetics

More than 30 non-clinical pharmacokinetics and/or toxicokinetics studies investigating absorption, distribution, metabolism, and excretion of triclosan have been reviewed for this dossier. The kinetics of triclosan and its metabolites have been studied in mice, rats, hamsters, guinea pigs, rabbits, dogs, and monkeys. In these studies, the oral, intravenous, dermal, intraperitoneal, intravaginal, and intraduodenal routes of administration have been examined. In mice, rats, and hamsters, single, stand-alone studies were conducted that investigated all pharmacokinetic (PK) parameters (*i.e.*, absorption, distribution, metabolism, excretion, ADME).

Unlabelled triclosan, ^{14}C-labelled triclosan, and ^{3}H-labelled triclosan have been used to study the pharmacokinetics of triclosan. The pharmacokinetic data assessed included the maximum concentrations in blood and tissues (C_{max}), the corresponding time required to reach the peak concentrations (T_{max}), the area under the curve (AUC), the elimination half-life ($t_{1/2}$), and the pattern of excretion. In general, the pharmacokinetic studies used similar methodologies to monitor and quantitate the pharmacokinetic data. Following a single or series of extraction and/or partitioning step(s), the blood and tissue samples were analysed by thin layer chromatography (TLC), gas chromatography (GC) with electron capture, high performance liquid chromatography (HPLC), and liquid scintillation counting. Whole body autoradiography was used to determine the relative location of the ^{14}C-labelled triclosan in distribution studies.

Three stand-alone PK/ADME studies for triclosan were conducted in mice, rats, and hamsters [van Dijk, 1994 (80); van Dijk, 1995 (81); van Dijk, 1996 (82)]. These studies were similar in design, with the exception that the rat study did not investigate the metabolism of triclosan. The remainder of the data available were generated from studies where toxicokinetics was not the primary focus. These kinetic data were derived from toxicokinetic studies conducted in conjunction with toxicology studies [Caudal *et al*., 1975 (83); Parkes, 1986 (84); Hohensee and Berke, 1991 (85)]. Overall, however, the three PK/ADME studies in mice, rats, and hamsters provide definitive single-dose data, with supporting evidence from the remaining studies.
The pharmacokinetic data for triclosan in all species investigated, including the route of administration and the pharmacokinetic parameters assessed, are summarised in Table 22.

Table 22: Kinetic Parameters Measured in Non-clinical Studies

Species	Route[1]	Parameter Measured	Reference
Mouse	oral	C_{max}, T_{max}, $T_{1/2}$, AUC	van Dijk, 1995 (81)
Rat	oral	C_{max}, T_{max}, $T_{1/2}$, AUC	Lin and Smith, 1990 (86), Black *et al*., 1975 (27), van Dijk, 1996 (82)
Rat	i.v.	$T_{1/2}$	Siddiqui and Buttar, 1979 (87)
Rat	i.v.g.	C_{max}, T_{max}	Siddiqui and Buttar, 1979 (87)
Guinea Pig	oral	$T_{1/2}$	Black *et al*., 1975 (27)
Hamster	oral	C_{max}, T_{max}, $T_{1/2}$, AUC	van Dijk, 1994 (80)
Rabbit	oral	C_{max}, T_{max}	Hong *et al*., 1976 (24)
Dog	oral	C_{max}, T_{max}	Ciba-Geigy, 1976a (88)
Dog	i.v.	C_{max}, T_{max}	Stierlin, 1972a (89)
Monkey	oral	C_{max}, T_{max}	Parkes, 1978b (90), Ciba-Geigy, 1976a (88), Ciba-Geigy, 1977a (91)
Monkey	dermal	C_{max}, T_{max}	Hazleton Laboratories, 1979b (30)

[1] i.v.=intravenous; i.v.g.=intravaginal

3.3.9.1.1 Single Dose Data

PK/ADME parameters have been examined following single oral doses of ^{14}C-labelled triclosan to mice, rats, and hamsters [van Dijk, 1994 (80); van Dijk, 1995 (81); van Dijk, 1996 (82)]. The single-dose data are summarized in Table 23. Other details from these studies are included under the appropriate heading (*i.e.*, absorption, distribution, metabolism, excretion).

Table 23: Summary of Plasma Toxicokinetics of ^{14}C-Triclosan after Single Oral Administration at Two Dose Levels to Rodents

				Plasma Levels[1]				
Species	Route	Target Dose (mg/kg bw)	Sex	C_{max} (µg/g)	T_{max} (hr)	$t_{1/2}$ (hr)	AUC[2] (mg•hr/L)	C_{96} (mg/L)
Mouse[3]	oral	2	M	19.48	4	9.1	166.1	0.02
		200	M	212.8	4	11.8	4,505	1.1716
		2	F	8.79	1	8.9	119.3	0.007
				7.67	4			
		200	F	267.2	2	9.9	6,322	0.742
				263.3	4			
Rat[3]	oral	2	M	4.772	1	12.6	63.9	0.086

				Plasma Levels[1]				
Species	**Route**	**Target Dose (mg/kg bw)**	**Sex**	**C_{max} (µg/g)**	**T_{max} (hr)**	**$t_{1/2}$ (hr)**	**AUC[2] (mg•hr/L)**	**C_{96} (mg/L)**
				4.458	4			
		200	M	153.4	1	10.0	3,237	1.25
				183.3	4			
Hamster[3]	oral	2	M	7.684	1	29.1	178.0	0.181
		200	M	359.171	1	32	6,010	7.784
		2	F	7.357	1	24.5	79.8	0.071
		200	F	384.920	1	27.0	4,298	4.556

[1] C_{max} =maximal concentrations in blood and tissues, T_{max} =the time required to reach the peak concentration, AUC= the plasma area under the concentration-time curve, $t_{1/2}$= the elimination half-life of the radioactivity, C_{96}=plasma concentration at 96 hr
[2] AUC values representative of time of animal sacrifice: 96 h (mice, rats); 168 h (hamsters) (T_{max})
[3] Data from: van Dijk, 1994 (80); van Dijk, 1995 (81); van Dijk, 1996 (82)
[4] Animals sacrificed at 96 h (mice, rats) and 168 h (hamsters).

Triclosan is rapidly absorbed as indicated by the time to reach C_{max}. Two peaks (2 x T_{max}) in plasma triclosan levels were detected in mice and rats, with peak plasma concentrations occurring after 1 and 4 hours in these 2 species. C_{max} values obtained following 2 *versus* 200 mg/kg body weight/day did not reflect the 100-fold increase in dose of triclosan. A comparison of the C_{max} data for the low and high doses indicates that the process of absorption may be at least partially saturated at the high dose level and elimination may be enhanced. Triclosan appeared to have high systemic exposure following oral administration, based on urinary excretion data (*i.e.*, high levels of radioactivity were excreted in the urine following dosing, indicating good absorption).

Single Dermal Dose

With respect to dermal applications of triclosan, a single-application study in newborn Rhesus monkeys using a triclosan-containing soap solution (1 mg/mL, 0.1%) resulted in a T_{max} of 12 hours and a C_{max} of 0.5 to 0.7 ppm (500 to 700 ng/mL) [Parkes, 1978a (29)]. In comparison to the single-exposure monkey data, rats displayed 2 peaks in plasma concentration 2 hours (0.278 ppm or 278 ng/mL) and 6 hours (0.264 ppm or 264 ng/mL) following the dermal application of triclosan in an ethanol solution to a 7.5 cm^2 section of rat skin [Black and Howes, 1975 (23)].

3.3.9.1.2 Repeated Dose Data

Repeated Dose Data from Pharmacokinetic/ADME Studies

In the PK/ADME studies in mice, rats, and hamsters considered to be definitive, plasma concentrations and, in the case of rats, AUC, were determined following 13 days of triclosan administration in the diet. PK analyses were conducted on blood and tissue samples taken following a dose of ^{14}C-labelled triclosan on Day 14 of repeated dosing. Plasma concentrations at C_{max} were comparable following the bolus radiolabelled dose on Day 14 compared to the single dose in both rats and hamsters. In contrast, the C_{max} level for a single oral dose of triclosan in plasma following repeated daily exposure was decreased in mice, compared to plasma levels following a single dose. These findings are summarized in Table 24.

Table 24: Summary of C_{max} Values Following Single and Repeated Doses of 2 mg/kg bw/day of Triclosan[1]

Species	Time (h)	Single Dose C_{max} (µg/peq/g)[2]	Repeated Dose C_{max} (µg/peq/g)[1]
Mouse	4	14.326	4.977
Rat	1-2	4.772	4.491
Hamster	1-2	4.883	5.003

[1] Data from: van Dijk, 1994 (80); van Dijk, 1995 (81); van Dijk, 1996 (82)
[2] µg parent equivalents per gram plasma

Data from the study in rats that examined AUC following both single and repeated doses of triclosan indicate AUC levels in plasma did not change after repeated dosing for 14 days, as shown in the table below.

Table 25: Comparison of Calculated AUC Values Following Single and Repeated Doses of Triclosan in Rats

Dose (mg/kg bw/day)	Single Dose AUC (ng*hr/mL)	Repeated Dose AUC (ng*hr/mL)
2	64,000	77,400
200	3,237,000	3,581,000

Repeated Dose Toxicokinetic Data from Oral Toxicology Studies

Plasma levels of triclosan were determined following 12 to 14 days of oral (dietary) administration to mice of daily doses of 10 mg/kg body weight were 22,500, 22,000, and 23,600 ng/mL on Days 12, 13, and 14 of dosing, respectively [Huntingdon Life Sciences, 1997 (92)]. The AUC value calculated for these data was 489,000 ng*hr/mL.

Plasma levels of triclosan were also determined in the chronic carcinogenicity bioassays conducted in mice, rats, and hamsters [Pharmaco LSR, 1995 (66); Ciba-Geigy, 1986 (67); Huntingdon Life Sciences, 1999 (68)]. Tables 26 through 28 inclusive present the blood levels of triclosan from these 3 carcinogenicity studies. For purposes of comparison, Table 29 presents triclosan levels following at least 6 months of daily oral (dietary) doses in these studies, including dose-normalized data. These data indicate that in chronic dosing studies, plasma levels in mice were slightly higher or comparable to plasma levels in rats (based on dose-normalized data) and much higher than plasma levels in hamsters (greater than 4- to 5-fold).

Table 26: Plasma Concentrations (ng/mL) of Triclosan in Mice Following Chronic Dietary Administration

Dose (mg/kg/d)	Interim (6 months)				Termination (18 months)			
	Males		Females		Males		Females	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10	16,800	4,260	21,900	8,220	20,600	11,100	21,100	7,300
30	58,900	15,300	75,700	11,500	62,400	26,100	98,900	21,300
100	134,600	25,000	172,100	27,200	150,200	37,400	169,000	69,400
200	177,600	49,300	191,500	37,700	197,200	43,100	236,500	59,900

Source: Pharmaco LSR, 1994 (66).

Table 27: Plasma Concentrations (ng/mL) of Triclosan (FAT 80'023/S) in Rats Following Chronic Dietary Administration[1]

Males					Females				
Dose (mg/kg/d)[2]	Interim (52 weeks)		Termination (104 weeks)		Dose (mg/kg/d)[3]	Interim (52 weeks)		Termination (104 weeks)	
	Mean	SD	Mean	SD		Mean	SD	Mean	SD
12-9	21,808	9,008	10,576	3,392	17-11	28,160	12,928	26,496	18,032
40-34	55,024	12,656	43,296	15,760	56-34	70,624	22,736	42,048	31,888
127-107	120,368	44,464	86,784	26,416	190-114	170,656	32,928	138,560	43,648

Source: Ciba-Geigy, 1986 (67).
[1] Note that the original total values for triclosan (free + conjugates) were in units of ng/mL in blood, not plasma. Conversions to values in ng/mL plasma (presented in this table) were based on an average blood volume of 64 mL/kg (range: 58-70 mL/kg) and an average plasma volume of 40 mL/kg (range: 36-45 mL/kg). Source for value for rat plasma volume: McGuill and Rowan, 1989.
[2] Average daily dose calculated at approximately 52 weeks (first number) and 104 weeks (second number) for males consuming dietary doses of 300, 1,000, and 3,000 ppm. Daily doses (mg/kg/d) were decreased by15-20% at 104 weeks compared to 52 weeks.
[3] Average daily dose calculated at approximately 52 weeks (first number) and 104 weeks (second number) for males consuming dietary doses of 300, 1000, and 3,000 ppm. Daily doses (mg/kg/d) were decreased by 35-40% at 104 weeks compared to 52 weeks.

Table 28: Plasma Concentrations (ng/mL) of Triclosan (FAT 80'023/S) in Hamsters Following Chronic Dietary Administration

Dose (mg/kg/d)	Interim (52 weeks)				Termination (95 weeks for Males; 90 weeks for Females)			
	Males		Females		Males		Females	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12.5	1,411	467	578	188	1,322	232	655	148
75	5,310	972	2,578	847	8,162	3,404	3,683	664
250	19,390	3,465	9,942	3,055	50,985	34,197	43,624	56,279

Source: Chasseaud *et al.*, 1994 (93), located in Huntingdon Life Sciences, 1999 (68)

Table 29: Steady State Concentrations of Triclosan in Mice, Rats, and Hamsters Following Chronic Dosing

Species	Dose (mg/kg bw/d)	Sex	Interim[1] (ng/mL)		Termination[2] (ng/mL)	
			Mean	SD	Mean	SD
Mouse[3]	100	M	134,600	25,000	150,200	37,400
		F	172,100	27,200	169,000	69,400
	200	M	177,600	49,300	197,200	43,100
		F	191,500	3,700	236,500	59,900
	Dose-Normalized	M	888-1,346	--	986-1,502	--
		F	958-1,721	--	1,183-1,972	--
Rat[4]	127-107[5]	M	120,368	44,464	86,784	26,416
	190-114[6]	F	170,656	32,928	138,560	43,648
	Dose-Normalized	M	947	--	811	--
		F	898	--	1,216	--
Hamster[3]	250	M	19,390	3,565	50,985	34,197
		F	9,942	3,055	43,624	56,279
	Dose-Normalized	M	78	--	204	--

		F	40	--	174	--

[1] Mice=6 months, Rats=52 weeks, Hamsters=52 weeks
[2] Mice=18 months, Rats=104 weeks, Hamsters=95 weeks (M); 90 weeks (F)
Abbreviations: M=Male, F=Female
[3] Plasma concentration
[4] Conversions from blood concentration to plasma concentration were based on an average blood volume of 64 mL/kg (range: 58-70 mL/kg) and an average plasma volume of 40 mL/kg (range: 36-45 mL/kg). Source for rat plasma volume: McGuill and Rowan, 1989.
[5] Average daily dose calculated at approximately 52 weeks (first number) and 104 weeks (second number) for males consuming dietary doses of 3,000 ppm. Daily doses (mg/kg/d) were decreased by 15-20% at 104 weeks compared to 52 weeks
[6] Average daily dose calculated at approximately 52 weeks (first number) and 104 weeks (second number) for males consuming dietary doses of 3,000 ppm. Daily doses (mg/kg/d) were decreased by 35-40% at 104 weeks compared to 52 weeks.

In summary, in the 3 rodent studies presented in Tables 23 and 24, target dose levels of 2 and 200 mg/kg body weight were used in each study, and thus relevant comparisons of exposure between species can be made following single doses of triclosan. The AUC data (measured over 96 hours in mice and rats and 168 hours in hamsters) show that the mouse and hamster have much higher levels of exposure (almost 3-fold) compared to the rat. The levels of exposure in the mouse and hamster are shown to be similar. Plasma levels at 96 hours were much higher in hamsters than in mice and rats. Based on data from the rat PK/ADME study, there was no change in AUC after 14 days of repeated daily dosing. Data from chronic dosing studies showed that plasma levels in mice were slightly higher or comparable to plasma levels in rats (based on dose-normalized data) and much higher than plasma levels in hamsters (greater than 4- to 5-fold). Plasma levels in the chronic mouse study were comparable to those in the 14-day study (16,800 and 21,900 ng/mL in males and females, respectively).

Repeated Dermal Doses

In the 90-day study in newborn Rhesus monkeys that used a 0.1% triclosan soap solution, plasma levels ranged from 0.17 to 0.97 ppm (170 to 970 ng/mL) [Hazleton Laboratories, 1979b (30)]. The data from this study showed that plateau levels of triclosan in plasma were reached within 15 days of daily treatment. Other pharmacokinetic data (*e.g.*, AUCs or $t_{1/2}$) were not available for triclosan administered *via* the dermal route.

3.3.9.1.3 Bioaccumulation/Bioretention

The accumulation or retention of triclosan in organs and tissues was investigated in the definitive PK/ADME studies in rodents [van Dijk, 1994 (80); van Dijk, 1995 (81); van Dijk, 1996 (82)]. Tissue distribution data from the hamster study showed persistently higher plasma levels of triclosan compared to levels in liver, kidney, and lung following administration of either the low or high dose of ^{14}C-labelled triclosan (2 or 200 mg/kg body weight/day) [van Dijk, 1994 (80)]. Liver, kidney, and lung contained the highest levels of triclosan following oral administration, followed by the gastrointestinal tract. Levels of triclosan in plasma, liver, kidney, and lung following single *versus* repeated oral doses of ^{14}C-labelled triclosan are shown in Table 30. A comparison of levels following single dose *versus* repeated shows no evidence of accumulation or retention of triclosan in liver, kidney, or lung. Taken altogether, the data indicate that triclosan is efficiently eliminated from organs/tissues of the hamster and that there is no accumulation in these tissues after repeated exposure to triclosan.

Table 30: Levels of Triclosan in Plasma, Liver, Kidney, and Lung Following Single or Repeated Doses of ^{14}C-Labelled Triclosan in Hamsters[1]

Organ/ Tissue	2 mg/kg bw/day		200 mg/kg bw/day	
	Male	Female	Male	Female

	Single Dose	Repeated Dose	Single Dose	Repeated Dose	Single Dose	Repeated Dose	Single Dose	Repeated Dose
Plasma	0.317	0.209	0.093	0.089	16.175	7.898	7.139	5.206
Liver	0.068	0.037	0.017	0.015	3.433	1.831	1.361	1.212
Kidney	0.138	0.061	0.019	0.016	3.254	1.488	1.249	0.938
Lung	0.091	0.062	0.027	0.028	4.415	2.204	1.915	1.470

[1] Levels in µg of parental equivalents/gram of tissue.

Additional evidence of a lack of bioaccumulation or bioretention of triclosan is provided by the plasma AUC data generated in the definitive rat PK/ADME study [van Dijk, 1996 (82)]. In this study, the AUC value calculated following an oral dose of ^{14}C-labelled triclosan on Day 14 of a repeated triclosan exposure (diet) regimen was comparable to the AUC value calculated following a single oral dose (see Table 25). The similarity of the single dose and repeated dose AUC values suggests that the amount of triclosan absorbed is equal to that eliminated. Together with the similarity in plasma C_{max} values following repeated or single doses (shown in Table 24), the data show that accumulation of triclosan is unlikely to occur with repeated exposure.

It should be noted that tissue distribution data in the murine PK/ADME study showed that, at the oral dose of 200 mg/kg body weight/day for 14 days, levels of triclosan in the liver of male mice were approximately 2 times higher than levels in plasma [van Dijk, 1995 (81)].

While definitive studies examining tissue distribution over time have not been conducted for dermal application studies, measurements of plasma levels of triclosan in the 90-day study of newborn Rhesus monkeys washed with a soap solution containing 0.1% triclosan showed levels of 170 to 970 ng/mL triclosan in plasma. A comparison with C_{max} plasma levels of 500 to 700 ng/mL at the T_{max} of 12 hours after a single application suggests that there is no evidence of bioaccumulation/bioretention of triclosan after repeated dermal exposures.

In summary, both tissue distribution and plasma AUC data in hamsters and rats, respectively, provide evidence of a lack of bioaccumulation/bioretention of triclosan. Tissue distribution data in the mouse show that increased levels of triclosan are found in mouse liver compared to plasma.

3.3.9.2 Absorption

3.3.9.2.1 Oral Absorption

Overall, the data from studies in rodents and non-rodents suggest that triclosan is rapidly and highly absorbed from the gastrointestinal tract by all species, with maximum plasma concentrations typically being reached between 4 to 8 hours [van Dijk, 1995 (81); Lin and Smith, 1990 (86); Black *et al*., 1975 (27); Siddiqui and Buttar, 1979 (87); van Dijk, 1994 (80); Ciba-Geigy, 1976a (88); Stierlin, 1972a (89); Parkes, 1978b (90)]. A direct estimation of absorption was calculated from the comparison of AUC values between the oral and i.v. routes of administration in the rat. In this study, it was estimated that an oral dose was 70 to 80% absorbed in rats, based on i.v. and oral AUC values calculated from a 5 mg/kg body weight dose [Stierlin, 1972a (89)]. Likewise, in 1 dog study a comparison of urinary and faecal recovery following both oral and i.v. administration suggests an absorption rate of 50% in this species.

Autoradiography studies in mice, rats, and hamsters, also observed high levels of radioactivity in well-perfused organs within 30 minutes of an oral administration of triclosan [Kanetoshi *et al*., 1988 (94); Howes *et al*., 1989a (95); Howes *et al*., 1989b (96); Lin and Smith, 1990 (86); Stierlin, 1972a (89); Howes and Moule, 1989 (97)]. Altogether, these findings suggest that triclosan is highly absorbed following oral administration, with no species-related differences.

3.3.9.3 Distribution

A number of studies have been carried out to determine the distribution of ^{14}C-labelled triclosan in the tissues of mice, rats, hamsters, and monkeys.

In tissue distribution analyses conducted in mice following single and repeated doses of 2 or 200 mg/kg body weight/day of triclosan, levels of triclosan were comparable between liver and plasma [van Dijk, 1995 (81)]. However, with repeated dosing, radioactivity levels in the liver remained consistently higher than levels in plasma. In other studies in mice administered radiolabelled triclosan *via* the oral route, the sites with the highest levels of radioactivity were typically the stomach, GI tract, and gall bladder [Kanetoshi *et al*., 1988 (94); Howes *et al*., 1989a (96); Howes *et al*., 1989b (97)]. Organs/tissues that are well-perfused (*e.g.*, heart, lung, liver, kidney) also routinely displayed detectable levels of triclosan. High levels of radioactivity were still detected in the small intestine and gall bladder (the highest of all mouse tissues with levels of 1,130 µg Irgasan DP300 equivalents/g tissue) at 24 hours after a single oral dose of triclosan in ddY mice (100 µCi) [Kanetoshi *et al*., 1988 (94)]. Similar findings were observed following i.v. administration of triclosan in mice, with the highest levels of radioactive triclosan detected in the tissues of the liver, gut, and gall bladder [Stierlin, 1972a (89); Ciba-Geigy, 1977a (91)].

In rats administered triclosan *via* the oral route, the tissues with the highest levels of radioactivity included the pituitary gland, bladder, large intestine, kidneys, liver, stomach, and gingiva [Lin and Smith, 1990 (86); Stierlin, 1972a (89)]; however, after 24 hours following i.v. administration of triclosan, the only tissues with levels >1.3 µg/g were found to be liver, lung, and kidney [Stierlin, 1972a (89)]. In an examination of plasma, liver, and kidney levels of triclosan following oral doses of 2 or 200 mg/kg body weight/day, plasma and liver concentrations were comparable and kidney levels lower than plasma, even after repeated high doses of triclosan [van Dijk, 1996 (82)].

In the definitive PK/ADME study in hamsters, tissue distribution analyses showed that following either single or repeated doses of 2 or 200 mg/kg body weight/day of ^{14}C-labelled triclosan, the highest levels of triclosan were: plasma >> kidney ~liver ~ lung (see Table 30) [van Dijk, 1994 (80)]. Levels in the excretory organs kidney and liver as well as in lungs were about 3 to 6 times lower compared to plasma levels. In another study in the hamster, the highest levels of radiolabelled triclosan were in the gall bladder, stomach, and GI tract, with lower levels in the kidney and liver (no levels given) [Howes and Moule, 1989 (97)].

In monkeys washed daily with 0.1% triclosan for 90 days, triclosan species were detected in the lung (0.2 to 1.3 ppm), liver (0.1 to 0.5 ppm), kidney (0.1 to 0.9), and skin (0.6 to 1.4 ppm), confirming that triclosan distributes to the liver, lung, and kidney in primates [Hazleton Laboratories, 1979b (30)].

Overall, these data suggest that in rodent species the GI tract, kidney, liver, and gall bladder are the target organs for the disposition of triclosan. A significant contribution to this observation was postulated to result from biliary excretion leading to enterohepatic circulation. In the mouse, levels of triclosan in liver were higher than plasma following long-term repeated dosing.

3.3.9.3.1 Enterohepatic Circulation

Based on whole-body autoradiography studies that identified high levels of radioactivity in the gall bladder and GI tract, and data showing the presence of 2 peak concentrations in the plasma following single or repeated dosing, it has been suggested that mice and rats exhibit enterohepatic circulation [van Dijk, 1995 (81); van Dijk, 1996 (82); Howes *et al*., 1989a (94); Howes *et al*., 1989b (96); Kanetoshi *et al*., 1988 (94); Ciba-Geigy, 1977a (91);

Stierlin, 1972a (89)]. As such, these species would experience an enhanced local exposure to triclosan in the liver and GI tract. An i.v. study was conducted to investigate the potential for the enterohepatic circulation of triclosan in rats with biliary-fistula, [Ciba-Geigy, 1975b (98)]. Following a 5 mg/kg body weight i.v. dose, 73% of the dose was detected in the bile and then readministered into the duodenum. Of this re-administered dose, 38.8% (28.4% of the original dose) was found in the bile. Thus, these findings provide evidence that triclosan was reabsorbed from the GI tract. Although not definitive, additional evidence that supports the enterohepatic circulation in mice and rats comes from excretion data. Specifically, data from a number of studies have shown that triclosan is excreted primarily *via* the faecal route in mice and rats, regardless of the route of administration (*i.e.*, even following an i.v. dose) [Hazleton Laboratories, 1979b (30); Lin and Smith, 1990 (86); Howes *et al*., 1989b (96); van Dijk, 1995 (81); van Dijk, 1996 (82)]. These data suggest a strong GI component to the distribution of triclosan in these species, even following an intravenous dose. In contrast, although high levels of triclosan were detected in the gallbladder and GI tract of hamsters, there were no pharmacokinetic or distribution data comparable to those in mice and rats that would suggest any extensive enterohepatic circulation in this species [Howes and Moule, 1989 (97)].

3.3.9.3.2 Plasma Protein Binding

The plasma protein binding of triclosan in human, mouse, and hamster blood was investigated in an *in vitro* study [Sagelsdorff and Buser, 1995 (99)]. Plasma was incubated with Irgasan DP300 at final concentrations of 3.2, 6.4, and 16 µg/mL. An equilibrium dialysis method was used and the ratio of the bound to the unbound fractions was constant over the tested concentrations (*i.e.*, no saturation of binding was observed during testing). The plasma protein binding was determined to be 98.4 to 99.2, 98.1 to 98.7, and 98.7 to 99% in humans, mice, and hamsters, respectively [Sagelsdorff and Buser, 1995 (99)]. Thus, triclosan is highly bound in the plasma, with no species differences observed.

3.3.9.4 Metabolism

The metabolism of triclosan has been studied in a number of species including mice, rats, hamsters, dogs, and monkeys. An early study in the rat showed that metabolites of triclosan occurred mainly *via* aromatic hydroxylation of the ortho and meta positions to the ether bond of the benzene rings and to a smaller degree by scission of the ether bond. Scission of the ether bond produces 2,4-dichlorophenol (found in faeces and urine) and 4-chlorocatechol (excreted in the urine) [Tulp *et al*., 1979 (100)]. Two key studies have examined the metabolic pathway of triclosan in mice [van Dijk, 1995 (81)] and hamsters [van Dijk, 1994 (80)]. In the liver and skin, triclosan is primarily metabolised to glucuronide and sulfate conjugates, as well as other non-parent metabolites.

3.3.9.4.1 Biotransformation of triclosan

Triclosan is metabolised to parent sulfate and parent glucuronide conjugate compounds in all species examined to date. However, the relative ratio of these conjugates differs among species. The specific identity of the sulfate and glucuronide metabolites has been determined in mice, rats, and hamsters using TLC and gas-chromatography mass spectrometry (GC-MS) [van Dijk, 1995 (81); van Dijk, 1996 (82); van Dijk, 1994 (80)]. In addition to the parent compound and parent conjugates (glucuronide and sulfate), several non-parent metabolites and conjugates were detected [parent (M1), parent glucuronide (M7), parent sulfate (M4), non-parent metabolites (M2 and M3) and corresponding conjugates]. The non-parent metabolites are products of phase 1 metabolism, which are subsequently conjugated (phase 2 metabolites).

Following repeated dosing at target dose levels of 200 mg/kg body weight/day, there were more than 7-fold and 2-fold decreases in the levels of the non-parent metabolites in the urine of male mice (52.5 to 7.3%) [van Dijk, 1995 (81)] and hamsters (38.5 to 16.4%)

[van Dijk, 1994 (80)], respectively. Concomitant increases in the appearance of glucuronide conjugates in the urine were observed for both species, suggesting a decrease of phase 1 metabolism with repeated doses of triclosan.

As well as identifying and characterizing the triclosan metabolites in mice [van Dijk, 1995 (81)] and hamsters [van Dijk, 1994 (80)], much effort has been made in stand-alone or specific portions of studies to identify the predominant triclosan species in the urine, faeces, and plasma of mice [van Dijk, 1995 (81)], hamsters [van Dijk, 1994 (80)], and dogs [Hohensee and Berke, 1991 (85)]. These data are summarized in Table 31 below. In general, the sulfate, glucuronide, and free species are predominantly found in the plasma, urine, and faeces, respectively.

Table 31: Predominant Triclosan Species in Plasma, Urine, and Faeces of Animal Species Following Single and Repeated Dosing

Species	Route	Duration	Plasma	Urine	Faeces	Reference
Mouse (% of radio-activity recovered)	oral	single	Free: 64(M) Sulfate: 73(F)	Low dose: Glucuronide 23% (M)[2]; High dose: Free 65% (M); Glucuronide 70% (F)	Free 66-96	van Dijk, 1995 (81)
		repeated	Sulfate 78-90	Low dose: Free, 18-38%; High dose: Glucuronide, 63-75%	Free 68-96	
	i.v.	single	-	Free[3], 25-33%	Free 74-86	
		repeated	-	Free 43% (M); Glucuronide 32% (F)	Free 68-75	
Rat (ng/mL)	oral	single/ repeated	Sulfate 30 d=11,880 (M) 92 d=13,300 (M)	Free 2,308 (M) 57,350 (F)	Free 17,000 ng/g (M) 150,000 ng/g (F)	van Dijk, 1996 (82)
Hamster(%)	oral	single	Glucuronide 28-36	Glucuronide[1] 56-77	Free 55-87	van Dijk, 1994 (80)
		repeated	Glucuronide 24-56	Glucuronide 60-87	Free 57-91	
	i.v.	single	-	Glucuronide 27-58	Free 20-51	
		repeated	-	Glucuronide 33-56	Free 27-65	
Dog (ng/mL)	oral	30-day	Sulfate 9,688	Glucuronide 2,541	Free 46,367	Hohensee and Berke, 1991 (85)
Monkey (ppm, at 24 hr)	oral	single dose	Sulfate[1] 1.61-3.18	Glucuronide 21.3-78.8	Free 114-294	Parkes, 1978b (90)

Abbreviations: M, male; F, female
[1] Single, low-dose female had 43.3% free and 12.5% glucuronide
[2] Single, low-dose male had no detectable glucuronide
[3] Single, low-dose female had 39% glucuronide, male: 0% glucuronide.

A preliminary study was conducted to investigate the metabolism of triclosan in monkeys and dogs administered single oral doses of radiolabelled triclosan (5 mg/kg) [Ciba-Geigy, 1976a (88)]. Blood levels of radiolabelled triclosan compounds in dogs and monkeys were

6.08 µg/mL (T=3 hours) and 1.24 µg/mL (T=8 hours), respectively. The nature of the metabolites was revealed following incubation of samples with specific glucuronidase and arylsulfatase enzymes (*i.e.*, glucuronide and sulfate conjugates) and subsequent analysis by TLC. Prior to enzyme hydrolysis the levels of parent compound were less than 0.8%; however, following enzyme hydrolysis the levels of parent triclosan compound were greater than 80%.

Data on the systemic (not dermal) metabolism of dermally-applied triclosan are available from a 90-day bathing study conducted in Rhesus monkeys and its accompanying pilot study [Parkes, 1978a (29), Hazleton Laboratories, 1979b (30)]. In the pilot, single-dose study, 2 Rhesus monkeys (3 days old) were washed with a soap solution containing triclosan (1 mg/mL, volume was not disclosed) [Parkes, 1978a (29)]. Blood samples taken at 1, 3, 5, 8, 12, and 24 hours after washing showed that both glucuronide and sulfate conjugates were present and that no free, unconjugated triclosan was detectable. Blood levels reached plateau levels (0.43 to 0.68 ppm) by 8 to 12 hours after washing and were maintained for 8 to 24 hours. In the 90-day study, blood levels of total triclosan reached a plateau by 15 days and ranged from 0.17 to 0.97 ppm [Parkes, 1978a (29)]. Triclosan was present almost exclusively as glucuronide and sulfate conjugates in the blood; however, the glucuronide conjugate was predominant in samples from Days 1 to 2, after which point the sulfate conjugate predominated, such that sulfate conjugate levels in blood samples at the end of the study were 80 to 90% of the dose. Urinary concentrations ranged from 0.3 to 4.8 ppm and primarily contained the glucuronide conjugate, while faecal levels ranged from less than 0.1 to 10.5 ppm (primarily free triclosan). Overall, the monkey data show that triclosan is absorbed and metabolised to both glucuronide and sulfate conjugates following dermal application and that repeated dermal exposures to triclosan result in the formation of the sulfate conjugate of triclosan as the primary metabolite.

3.3.9.4.2 Dermal Metabolism

An *in vitro* diffusion skin cell model was used to assess the ability of the skin (rat and human) to metabolise triclosan applied using an ethanol:water (9:1) vehicle [Moss *et al*., 2000 (21)]. Glucuronidation and sulfation of triclosan were demonstrated to occur in this model (*i.e.*, the conjugates were found in the collecting reservoir following absorption through the skin), with the glucuronide being the primary metabolite at levels up to 1.4%. These findings were supported by *in vivo* studies with rats in which 0.4 and 1.5% of the parent glucuronide were reported to occur in the urine and skin, respectively, following the dermal application of triclosan [Moss *et al*., 2000 (21)]. These data show that triclosan is metabolised in the skin.

3.3.9.5 Excretion

The excretion of ^{14}C-labelled and ^{3}H-labelled triclosan was studied in mice, rats, guinea pigs, hamsters, dogs, and monkeys. These studies were the most frequently conducted of all triclosan pharmacokinetic studies. In particular, studies to ascertain the elimination half-life, the routes of excretion, and enterohepatic circulation were conducted for triclosan.

3.3.9.5.1 Elimination Half-Life

The terminal plasma half-life of orally administered ^{14}C-labelled triclosan and its metabolites was comparable in rats and mice, but much greater in hamsters (up to 3-fold). In mice, rats, and hamsters the half-life of radiolabelled triclosan is 9 to 12, 10 to 13, and 24 to 32 hours, respectively (Table 23) [van Dijk, 1995 (81); Lin and Smith, 1990 (86); van Dijk, 1996 (82); van Dijk, 1994 (80)]. In hamsters, the much longer half-life of radioactive triclosan is likely attributed to the long residence time of the non-parent M6 and M8/9 metabolites. Different routes of administration [*e.g.*, intraperitoneal (i.p.)] also have been associated with extended or longer half-lives. In rats given tritiated DP300 by i.p. injection, the half-life of radioactive triclosan was 18 hours as compared to 9 to 12 hours [van Dijk, 1996 (82)].

3.3.9.5.2 Routes of Excretion Following Oral Applications of Triclosan

In mice, rats, hamsters, and monkeys, unlabeled and radiolabelled ^{14}C-triclosan was used to assess the routes of excretion for triclosan following oral doses of triclosan. For studies using radioactive triclosan, the predominant route of excretion in mice and rats was the faeces, whereas in hamsters it was the urine. Based on a survey of the available data, the excretion rates for these species are presented in Table 32.

Table 32: Routes of Excretion of Triclosan Following Oral Dosing in Rodent Species

	Faeces (%)	Urine (%)	Reference
Mouse	25-89	26-44	Howes *et al.*, 1989a (95); Howes *et al.*, 1989b (96); van Dijk, 1995 (81)
Rat	81-84	4-12	van Dijk, 1996 (82); Lin and Smith, 1990 (86); Hong *et al.*, 1976 (24)
Hamster	0.1-0.3	60-80	Howes and Moule, 1989 (97); van Dijk, 1994 (80)

Routes of excretion in monkeys, have been shown to be urinary in a study using unlabelled triclosan administered to monkeys *via* oral gavage [Parkes, 1978b (90)], and faecal in a study using dermal applications (washing with soap containing 0.1% triclosan) [Hazleton Laboratories, 1979b (30)].

3.3.9.5.3 Routes of Excretion Following Dermal Applications of Triclosan

Excretion following dermal applications of triclosan showed that the faecal route predominated in rats, where triclosan was 70 to 90% excreted in the faeces compared to 3 to 4% eliminated in the urine [Ciba-Geigy, 1976b (25)]. Comparable data showing primarily faecal excretion of triclosan regardless of the formulation of the dose were found in other rat studies [Hong *et al.*, 1976 (24); Moss *et al.*, 2000 (21)].

In contrast to rats, rabbits exposed to triclosan in a dermally-applied solution or cream showed moderately greater urinary excretion compared to faecal elimination (47 to 53% *vs.* 38% of the applied triclosan solution excreted in the faeces; 29 to 48% in urine *vs.* less than 2% in faeces following the cream application) [Ciba-Geigy, 1976b (25)].

Triclosan levels in the urine and faeces of monkeys bathed daily from birth to 90 days with 15 mL of a soap solution containing triclosan (1 mg/mL) were found to range from 0.3 to 4.8 ppm in the urine and 0.1 to 10.5 ppm in the faeces [Hazleton Laboratories, 1979b (30)]. These data suggest that the faecal route may have dominated in the excretion of triclosan. For the purposes of comparison, it should be noted that the primary route of excretion in humans exposed to triclosan *via* the dermal route is urinary.

In summary, the primary route of excretion following dermal exposure to triclosan differs between species, with the faecal route predominating in the rat, but the urinary route predominating in the rabbit. Neither faecal nor urinary elimination appear to be strongly favoured in the case of primates based on the available data.

3.3.9.6 Summary of Pharmacokinetic and Toxicokinetic Data in Animals

Triclosan is rapidly absorbed (*via* the oral, i.p., and i.v.g. routes of administration) in all species. Specific studies conducted in rats indicated that the level of absorption was between 70 to 80% following oral administration in this species. Subsequent to absorption after oral administration, 2 peaks in plasma triclosan levels were detected in mice and rats at 1 and 4 hours, which is indicative of enterohepatic circulation. Evidence from both hamster and rat studies provide evidence of a lack of bioaccumulation/bioretention of triclosan following repeated oral doses.

Triclosan is widely distributed in organs and tissues, with well-perfused, and excretory, organs such as liver and kidney, as well as lung, heart, GI tract, and gall bladder showing highest levels following oral, dermal, or i.v. administration in rodents and monkeys. Levels of triclosan in mouse liver were higher than in plasma following repeated dosing with triclosan.

Following absorption, the parent triclosan compound was found to be metabolised to both glucuronide and sulfate conjugates. Although different ratios of the individual glucuronide and sulfate conjugates were observed among species, no unique species-specific metabolites have been identified to date. Repeated high-dose administration of triclosan was also shown to change the ratio of these 2 metabolites in hamsters, mice, and monkeys with the sulphate shown to predominate following chronic oral administration.

Triclosan was shown to be excreted primarily in the faeces of mice and rats following oral administration, while the predominant excretion route in hamsters and monkeys was *via* the urine. The faecal excretion route also predominated in rats following dermal applications of triclosan. In addition, evidence of enterohepatic circulation was found for rats and mice, based on autoradiography data, dual peak plasma concentrations, excretion data following i.v. dosing, and bile absorption studies. However, the data available for hamsters do not provide evidence for enterohepatic circulation in this species.

Some notable parallels and differences exist among the 3 rodent species, namely mice, rats, and hamsters, that are the most well-characterized species regarding triclosan nonclinical pharmacokinetics. For a target single-dose level of 200 mg/kg body weight, the maximal blood concentrations of both sexes were 213 to 267, 153 to 183 (only males), and 359 to 385 µg/g in mice, rats, and hamsters, respectively, with the resulting exposures for this dose of 4,505 to 6,322, 3,237 (average for males) and 4,298 to 6,010 µg*hr/mL (Table 23). Thus, in these single-dose studies, rats had the lowest blood levels, whereas mice and hamsters had comparable ranges in exposure levels. In contrast, in rodent chronic carcinogenicity bioassays of at least 18 months duration (*i.e.*, at least 18 months daily exposure in the diet) using different target dose levels, plasma levels in mice were slightly higher or comparable to plasma levels in rats (based on dose-normalized data) and much higher than plasma levels in hamsters (greater than 4- to 5-fold) (Table 29).

Evidence from pharmacokinetic studies suggests that the mouse liver is highly exposed to triclosan levels. Repeated dosing in mice led to an increased half-life for triclosan and its sulfate conjugate as measured in the kidney, and elevated liver triclosan levels (~2- to 3-fold) in male mice (with respect to levels measured in the plasma and kidney) [van Dijk, 1995 (98)]. The higher levels of triclosan in mouse liver than in plasma may be correlated with toxicology findings in mouse toxicology studies. Differences between mice, rats, and hamsters in hepatic levels of triclosan [van Dijk, 1994 (80); van Dijk, 1995 (81); van Dijk, 1996 (82)] also may correlate with differences in the incidence of liver-related findings in these species, with the mouse showing greater sensitivity to liver effects, but both the rat and hamster showing either a lower incidence or no liver effects (see Sections 3.3.5 and 3.3.7).

NOTE
Kinetic studies in HUMANS are described in section 3.3.11

3.3.10. Photo-induced toxicity

The data from these studies show that there is no evidence of photosensitisation in the guinea pig, mouse, and pig following exposure to triclosan. As in the case of the sensitisation studies in the guinea pig, these photosensitisation studies also were conducted prior to 1980, preceding the implementation of GLP regulations and OECD guidelines. In a photosensitisation study in the guinea pig, the majority of animals in all dose groups, including negative controls showed an inflammatory response 4 hours post-irradiation with unfiltered light; by 24 hours, none of the animals in the triclosan (1% in solution) or negative control groups showed an erythemic reaction [Thomann and Maurer, 1978 (7)]. It should be noted that a phototoxic response is expected with unfiltered light, as UVB is highly cytotoxic; hence the positive response in the negative control animals. No positive responses in any dose groups were reported following irradiation with filtered light. The authors concluded that there was no indication of photosensitisation activity with triclosan in the guinea pig. There was also no evidence of phototoxicity in the mouse or pig with triclosan (0.1% in methanol, or 0.1 and 1.0% in petrolatum) as reported in another study; however, detailed results were lacking in this study report [Urbach, 1973 (101)]. Based primarily on the guinea pig data, with supporting evidence from the mouse and pig data, there appears to be no potential for photosensitisation with triclosan.

Table 33: Findings from Photosensitisation Studies with Triclosan

Species (Strain)	Application Details	Major Findings	Reference, GLP and OECD Status
Guinea pig, Pirbright white	1.0% triclosan in solution, 0.1 mL single application. Irradiation: unfiltered light (UV-A, UV-B), 5 minutes, or filtered light (Pyrexfilters), 15 minutes	Unfiltered light: The majority of animals including controls showed an inflammatory response 4 hours post-irradiation in all dose groups. By 24 hours, none of the animals in the triclosan or negative control groups showed an erythemic reaction. Filtered light: No positive responses.	Thomann and Maurer, 1978 (7) Predates GLP and OECD
Mouse, Albino Skh:hair-less-1; Pig, Hanford Labco miniature swine	Triclosan (0.1% in methanol, 0.1 and 1.0% in petrolatum) Irradiation sources: UVC; UVB; UVA, and UVB.	No evidence of phototoxicity was observed.	Urbach, 1973 (101) Predates GLP and OECD

Summary of Photosensitisation Data

In summary, the results of these non-GLP studies indicate that there is no evidence for photosensitisation with triclosan in various formulations and concentrations (up to 1% in petrolatum) in the guinea pig, mouse, and pig.

3.3.11. Human data

Triclosan has been used in consumer products for over 30 years as an antibacterial agent and disinfectant. It is widely used in external-use, over-the-counter personal care products such as dentifrices, deodorants, soaps, creams, and lotions. Triclosan was approved for use at a level of 0.3% in cosmetics products in 1986 by the European Community Cosmetic Directive. The world-wide population of consumers exposed to triclosan includes both adults and children, with not-unexpected exposure in infants occurring *via* breast milk at low levels [Adolfsson-Erici *et al*., 2002 (102), Allmyr *et al.*, 2006 (103), Dayan, 2007 (104)].

Studies on triclosan levels in human blood samples from Sweden [Allmyr *et al.*, 2006 (103), Sandborgh-Englund et al. 2006 (116)] and from Australia [Allmyr *et al.* 2008 (AR1)] and a recent biomonitoring study with urine samples from the US [Calafat *et al.* 2008 (AR2) indicate widespread exposure of the general population to triclosan from various sources, including personal care products.

The following sections describe the general safety data for triclosan based on consumer use information and on results from clinical studies, the pharmacokinetics of triclosan in both adults and children following single and repeated oral and/or dermal exposures, and the irritation, sensitisation, and photosensitisation data for triclosan as evaluated in clinical tests. Estimates and findings of exposure levels to triclosan are presented in Sections 3.3.11.2.6 (for infants) and 3.3.11.2.7 of this opinion (and in the discussion).

3.3.11.1 Human Safety/Tolerability Data

3.3.11.1.1 Safety Data from Consumer Use Information

Consumer use information such as listings of adverse events for cosmetic products containing triclosan is generally unavailable.

No serious adverse events have been reported for triclosan-containing toothpaste.

The low number of reported non-serious adverse events for triclosan-containing toothpaste (frequency of 0.27 complaints/100,000 units sold) included reactions that were associated with the use of dentifrices in general, as identified during clinical testing (*e.g.*, dry mouth, mouth irritation/burning, sensitive teeth, altered taste or oral sensation, exfoliation). Other non-serious adverse events were those that represented a variety of much more infrequently reported effects (*i.e.*, usually reported by only one or two consumers, resulting in a reporting frequency of 0.09 complaints/100,000 units sold). These rare non-serious events, which may not have been related to the use of the triclosan-containing toothpaste, included stomach ache, belching, alopecia, anxiety, headache, black or coated tongue, dizziness, excess saliva, upper respiratory infection, increased urge to urinate, and shortness of breath.

Thus, based on consumer use information for triclosan-containing toothpaste, which represents a wide-spread use that includes the potential for systemic exposure through the oral route, data indicate that triclosan can be used safely and with good tolerability at levels that also are found in personal care products.

3.3.11.1.2 *Safety/Tolerability Data from Clinical Studies*

In addition to the indications of good tolerability and safety from historical and consumer use of personal care products containing triclosan, a number of clinical studies have investigated the safety and tolerability of triclosan.

Table 34: Findings from Human Safety Studies for Triclosan

No. Subjects	Dosing Regimen	Major Findings	Reference
153	Twice daily brushing, for 12 weeks, with 0.2% triclosan in toothpaste (75 subjects) or NaF/silica toothpaste (78 subjects).	For triglycerides, the triclosan group differed from the placebo group ($p<0.01$) at 3 weeks, but not at 12 weeks. There was no difference between treatment and control groups for any of the other liver function tests.	Colgate-Palmolive, 1994 (105)

No. Subjects	Dosing Regimen	Major Findings	Reference
112	Normal daily use of a toothpaste containing 0.2% triclosan and 0.5% zinc citrate for 52 weeks, followed by a 13-week washout period.	The data showed increases in white blood cells and lymphocytes throughout the study, with decreases in mean corpuscular haemoglobin/concentration. Biochemical analyses showed increases in sodium, cholesterol (total, LDL, and HDL), uric acid, and creatinine and decreases in bilirubin at all time points in the study in either males or females or both sexes. These changes were not attributed to triclosan.	Safford, 1991 (106)
112 (86 subjects completed study)	Normal daily use (according to subject's normal brushing habits) of a toothpaste containing 0.2% triclosan and 0.5% zinc for 12 weeks, with an extension of the study to 52 weeks (followed by a 13-week observation period)	Blood samplings at baseline and 4, 12, 24, 38, 52, and 65 weeks. Standard haematology and clinical biochemistry results showed some statistically significant changes periodically (compared to baseline values), but no pattern to the changes. Some significant correlations were found between parameters and triclosan blood levels, but these were inconsistent over the course of the study in terms of time or sex of subject. Therefore, these changes were not attributed to triclosan toothpaste use. Oral irritation data including subjective "complaint level" for the whole trial period were summarised and found to be similar to effects experienced in similar trials involving toothpaste. There were no withdrawals from the trial due to adverse reactions.	Barnes, 1991 (107)
3,000	Normal dentifrice use, for 3 years, with silicon dioxide-based toothpaste containing 0.243% fluoride and 0.3% triclosan, or 0.243% NaF, or 0.331% NaF.	No treatment-related changes were reported for any parameter measured, including haematology, clinical chemistry, and urinalysis parameters. The data shows that the use of toothpaste containing 0.3% triclosan produces no adverse effects compared to triclosan-free toothpaste formulations.	Fishman, 1994 (108)
20	0, 1, 5, 9, 12, 15, 18, 21, 24, 27, or 30 mg triclosan in capsule, depending on study phase. Single-dose and repeat-dose depending on study phase (up to 30 days).	There were 6 dropouts (5 triclosan and 1 placebo) due to adverse effects (skin reactions - 3 subjects including 1 placebo subject; increase in liver enzymes - 2 subjects; and, antrum gastritis - 1 subject). These findings were not considered to be attributable to triclosan, as they are not uncommon in pharmacological studies. Based on vital signs, ECG, lung function, neurological examination and most laboratory parameters, triclosan was considered to be well-tolerated. There were slight increases in liver enzyme values including SGPT, SGOT, and gamma-GT.	Lucker *et al.*, 1990 (109)

Four human studies have evaluated the safety of triclosan in toothpaste products. These studies tested triclosan-containing toothpaste preparations in study populations over periods ranging from 12 weeks to 3 years at concentrations of 0.2% triclosan [Colgate-Palmolive, 1994 (105)], 0.2% triclosan with 0.5% zinc citrate [Safford, 1991 (106); Barnes, 1992 (107)], and 0.3% triclosan with 0.243% fluoride [Fishman, 1994 (108)]. Endpoints measured included blood chemistry and haematological parameters in all 4 studies. In addition, urinalysis parameters were evaluated in 1 study [Fishman, 1994 (108)]. In all studies, there were no changes indicative of overt toxicity. Reported changes in haematological and/or clinical chemistry parameters that did occur could not be attributed to the use of the triclosan-containing toothpaste.

In a separate study of pharmacokinetics and tolerability, consumption of escalating daily doses of triclosan in capsule form (0 to 30 mg/capsule) by 20 volunteers was reported to be without overt effects on ophthalmic, neurologic, cardiac, and lung function evaluations [Lucker *et al.*, 1990 (109)]. There were slight increases in liver enzyme values of the treated subjects, but this is in contrast to the four human studies (described above) that reported a lack of findings in liver enzyme parameters. Five of the treated participants dropped out during the course of the study; however, the adverse events reported in these subjects could not be attributed to triclosan.

One of the safety studies [Safford, 1991 (106)] reported on the concentrations of triclosan in the blood following the use of a toothpaste containing 0.2% triclosan and 0.5% zinc citrate. In this study of 112 participants, the average triclosan concentration in the blood of 15.5 ng/mL was reported to rise to 31.2 ng/mL after a period of 4 weeks on study. This concentration was reported to subsequently level off with further use of the triclosan-containing dentifrice.

In summary, the human safety studies reviewed showed no signs of overt toxicity in over 3,000 subjects that used triclosan-containing toothpaste for 12 weeks to 3 years.

3.3.11.2 Human Pharmacokinetics and Metabolism

A total of over 30 pharmacokinetic studies investigating the absorption, metabolism and excretion of triclosan in humans have been reviewed. In these studies, several different routes of administration, including oral exposure to triclosan-containing products (*e.g.*, toothpaste), oral ingestion of capsules, aqueous solutions, and dental slurries (*i.e.*, following brushing with triclosan-containing toothpaste), and percutaneous exposure (*in vivo* and *in vitro*) have been investigated. Overall, ingested triclosan is almost completely absorbed, whereas oral cavity and percutaneous exposure to triclosan-containing products (*e.g.*, toothpaste, soap, cream, *etc*.) results in limited absorption. Following all routes of administration, absorbed triclosan is nearly totally converted to glucuronic and sulphuric acid conjugates (varied relative proportions), with only trace amounts of the parent compound detected in the plasma, and the predominant route of excretion for triclosan is the urine, with the majority of the compound appearing as the glucuronide conjugate.

3.3.11.2.1 Pharmacokinetics

The pharmacokinetic parameters assessed for triclosan in humans include the C_{max}, the time required to reach T_{max}, the AUC values for plasma concentrations *versus* time, and the elimination half-life (t½) of plasma concentrations. These parameters were assessed following several different routes of administration, including oral exposure to triclosan-containing toothpaste (expelled dental slurry), and oral ingestion of triclosan-containing capsules, aqueous solutions, and dental slurries (*i.e.*, ingestion of dental slurry following brushing with triclosan-containing toothpaste). In general, the pharmacokinetic studies reviewed herein analysed samples using HPLC or GC with electron capture detector.

The pharmacokinetic data for triclosan, as measured in children and adults following various routes of administration, are summarized in Table 35.

Table 35: Summary of Plasma Pharmacokinetics of Triclosan in Children and Adult Subjects

Route	Subject	Dose/Duration	Parameters[1]				Reference
			C_{max} (ng/mL)	T_{max} (h)	AUC[2] (ng*h/mL)	t½ (h)	
Oral (capsules)	Adults	1 mg/single dose	23.3	5.00	208	13.4	Lucker *et al.*, 1990 (109)
		15 mg/day for 36 days	330.9	4.08	4,296	19.0	
Oral (aqueous solution)	Adults	10 mg/single dose	974.1	1.6	11,237	19.9	Concordia Research Laboratories, 1997a (110)
		4 mg/day for 21 days	191.2	16.0	2,788		Colgate-Palmolive, 1989 (113)

Route	Subject	Dose/Duration	Parameters[1]				Reference
			C_{max} (ng/mL)	T_{max} (h)	AUC[2] (ng*h/mL)	t½ (h)	
		4 mg/single dose	218	1.5	2,600	21	Sandborgh-Englund *et al.*, 2006 (116)
	Children[3]	3.0 mg/single dose	495.9	1.8	6,545	16.8	Colgate-Palmolive, 1997a (111)
			N/A	N/A	N/A	12.7	Concordia Research Laboratories, 1997b (112)
Oral (dental slurry)	Adults	6 mg/single dose	574.2	3.0	7,235	20.0	Concordia Research Laboratories, 1997a (110)
		18 mg/day for 14 days (dental slurry swallowed after brushing)	878.0	N/A	218,856	N/A	BIBRA International, 1997 (114)
Oral (toothpaste)	Adults	4 mg/day for 21 days	26.7	12.0	329	N/A	Colgate-Palmolive, 1989 (113)
		18 mg/day for 14 days (dental slurry expelled after brushing)	145.5	N/A	34,855	N/A	BIBRA International, 1997 (114)
		3.75 mg/single dose	242.9	4.0	2,818	14.6	Colgate-Palmolive, 1997b (115)
		11.25 mg/day for 12 days (3.75 mg x 3 brushings daily)	384.0[4,5]	2.0	2,844[6]	N/A	Colgate-Palmolive, 1997b (115)

N/A, not available
[1] C_{max} =maximal concentrations in blood and tissues, T_{max} =the time required to reach the peak concentration, AUC= the plasma area under the concentration-time curve, $T_{1/2}$= the elimination half-life of the radioactivity
[2] When both $AUC_{(0\rightarrow\infty)}$ and $AUC_{(0\rightarrow 24h)}$ values were reported, the former was used.
[3] Children ranged from 8 to 12 years of age. Due to limited blood collection intervals in the study, limited or no C_{max} and T_{max} data were available, and no AUC values were calculated for the available data [Concordia Research Laboratories, 1997b (112)].
[4] Sampling was conducted following the first of 3 brushings on Day 12 of the multiple-dose phase of the study.
[5] Plasma concentrations ranged from 353 to 384 between hours 1 to 7 of the study.
[6] AUC value is dose-normalized (mean $AUC_{(0\rightarrow 24)}$ corrected for number of brushings on that day (AUC_{24}/3)

As outlined in Table 35, C_{max} and AUC values obtained from adult subjects generally increased with increasing dose levels, with C_{max} values ranging from 23.3 to 974.1 ng/mL, and AUC values ranging from 208 to 11,237 ng*h/mL for single doses ranging from 1.0 mg/dose to 10 mg/day (corresponding to approximately 0.017 and 0.17 mg/kg body weight, respectively, for a 60 kg adult). For multiple doses ranging from 4 to 18 mg/day (corresponding to 0.067 and 0.30 mg/kg body weight/day for a 60 kg adult), C_{max} values ranged from 26.7 to 878 ng/mL, and AUC values ranged from 329 to 21,8856 ng*h/mL. Corresponding T_{max} values ranged from 1.5 to 5.0 hours and from 4.08 to 16.0 hours for single and multiple doses, respectively. t½ values obtained from adult subjects remained fairly constant across all the studies reviewed, ranging from 13.4 to 21.0 hours.

There are limited pharmacokinetic data for children, and no direct comparisons to adults were possible, given differences in doses and dosing formulations in all of the studies, with the exception that elimination was determined to be essentially the same for children and adults. Two single oral dose (3.0 mg triclosan from aqueous solution) studies were

conducted in children [n = 11 children, aged 8 to 12 years in Colgate-Palmolive, 1997a (111) and n = 4 children, aged 9 to 12 years in Concordia Research Laboratories, 1997b (112)]. The mean elimination rate constants (Kel) for each study were calculated to be 0.0453 h^{-1} (n = 11) (3) and 0.062 h^{-1} (n = 4) [Concordia Research Laboratories, 1997b (112)]. These values are similar to those calculated in adult subjects exposed to single oral doses of triclosan from aqueous solution (Kel = 0.043 h^{-1}), dental slurry (Kel = 0.043 h^{-1}) and toothpaste use (Kel = 0.0742 h^{-1}) [Concordia Research Laboratories, 1997a (110)]. Thus, the rate of elimination is comparable in children and adults.

Pharmacokinetic parameters have also been assessed in the saliva of adult subjects exposed to 4 mg triclosan from toothpaste (not outlined in Table 35). Throughout the 2-hour period following tooth brushing, mean saliva triclosan concentrations and Ke and t½ values revealed first-order kinetics [Gilbert, 1987 (117)].

Intra-subject comparisons were made following single and multiple oral doses of triclosan [Lucker *et al.*, 1990 (109), Colgate-Palmolive, 1997b (115)], and following ingestion of triclosan-containing dental slurry and triclosan aqueous solution [Concordia Research Laboratories, 1997a (110)]. The mean AUC values for the single- and dose-normalized multiple-dose exposures were not different (dose-normalized AUC values for multiple-dose exposures not shown in Table 35), suggesting a linear disposition for triclosan after repeated brushing [Colgate-Palmolive, 1997b (115)]. Dose-normalized AUC and C_{max} values were similar following ingestion of triclosan-containing dental slurry and triclosan aqueous solution, suggesting that each route of administration results in similar levels of exposure to triclosan [Concordia Research Laboratories, 1997a (110)].

To investigate differences in exposure levels following different formulae of administration, inter-subject comparisons were made between triclosan-containing toothpaste (dental slurry expelled) use *versus* ingestion of the dental slurry [BIBRA International, 1997 (114)], and between triclosan-containing toothpaste (dental slurry expelled) use *versus* ingestion of triclosan aqueous solution [Colgate-Palmolive, 1989 (113)]. As would be expected, based on AUC and C_{max} values, ingestion of triclosan aqueous solutions (at levels simulating the maximum absorption of triclosan that would be possible from a triclosan-containing toothpaste) resulted in higher levels of exposure to triclosan compared with the use of triclosan-containing toothpaste (expectorated) [Colgate-Palmolive, 1989 (113)]. Similarly, higher AUC and C_{max} values were observed in subjects who ingested the dental slurry compared with those who expelled the slurry following brushing with triclosan-containing toothpaste [BIBRA International, 1997 (114)]. For each study, there were no differences between groups with respect to time to reach steady-state plasma triclosan concentrations [4 days in the 14-day study (BIBRA International, 1997 (114), and approximately 14 days in the 21-day study (Colgate-Palmolive, 1989 (113)].

In summary, AUC and C_{max} values appear to increase with increasing doses of triclosan. There does not appear to be a correlation between the duration of triclosan exposure (measured in days) from tooth brushing and the levels of exposure (*i.e.*, AUC values); however, the formula of administration does appear to affect both AUC and C_{max} values. While the ingestion of triclosan-containing dental slurry and triclosan aqueous solution each results in similar levels of exposure to triclosan, both routes of exposure result in higher levels when compared with the use of triclosan-containing toothpaste followed by expectoration. These data show that there is limited absorption under normal conditions of toothpaste use (*i.e.*, expectoration and rinsing) compared with oral ingestion of triclosan-containing solutions (see absorption section).

Bioaccumulation/Bioretention

In the absence of data showing tissue levels of triclosan following single and repeated exposures, evidence of a lack of bioaccumulation or bioretention of triclosan is provided by an examination and comparison of plasma triclosan levels in a number of studies.

The plasma $AUC_{0\text{-}inf}$ level of triclosan was 2,818 ng*h/mL following single use of dentifrice containing 0.3% triclosan (3.75 mg dose, with ingestion of the dentifrice) in a study of 21 adult subjects [Colgate-Palmolive, 1997b (115)] (see Table 35). In the repeated dose phase of the same study, subjects brushed 3 times daily for 12 days with ingestion of the dental slurry (3.75 mg/dose). After 12 days of brushing, the plasma AUC levels following a single dose was calculated to be 2,844 ng*h/mL after normalizing for the number of doses ($AUC_{0\text{-}24h}$ = 8,833 ng*h/mL per 3 doses per 24 hours). There was no significant difference between the single- and repeated-dose AUC values, suggesting a complete elimination of the daily triclosan dose, and no increase in the triclosan level during repeated brushing/ingestion.

Additional supporting evidence for a lack of bioaccumulation or bioretention of triclosan is shown in several studies in which there was no further increase in plasma triclosan levels once steady-state blood concentrations had been reached. Triclosan levels in plasma were comparable from Days 7 to 21 in a study in which subjects either ingested 20 mL of an aqueous solution containing 0.01% triclosan or brushed twice daily with 1 g of a dentifrice containing 0.2% triclosan (with expectoration and rinsing) (plasma levels ranged from 15 to 21 ng/mL in the dentifrice group) [Colgate-Palmolive, 1989 (113)]. In a longer-term parallel, double-blind 12-week study with dentifrice containing 0.2% triclosan, blood samples showed comparable mean levels of 16 and 14 ng/mL at 3 and 12 weeks, respectively [Lin, 1989 (135)]. In a 52-week tooth brushing study using dentifrice containing 0.2% triclosan, total triclosan levels in plasma were consistently in the range of 27 to 40 ng/mL from 4 weeks to the end of the 52-week exposure period [Safford, 1991 (106)]. Altogether, the data from these 3 studies show consistency in plasma triclosan levels following the use of dentifrice containing 0.2% triclosan. In a review of the data from Colgate-Palmolive, 1989 (113), Lin, 1989 (135), and Colgate-Palmolive, 1997b (115), this conclusion was extended to dentifrices containing triclosan at levels up to 0.3% [Bagley and Lin, 2000 (142)]. In addition, the data suggest that there is no accumulation of triclosan levels as reflected in comparable plasma levels over the time course of each study, suggesting a lack of evidence of bioaccumulation of triclosan.

In the absence of studies examining tissue concentrations over time, relatively invariable plasma concentrations of triclosan provide evidence of a lack of bioaccumulation following dermal application in human studies. Plasma levels of total triclosan ranged between 85 and 101 ng/mL between Days 5 to 20 in males and 41 to 47 ng/mL in females over the same time period in which triclosan exposure occurred through the use of hand wash containing 1% triclosan [Ciba Specialty Chemicals, 2002 (134)]. These data suggest a balance between absorption and elimination and a lack of bioaccumulation following dermal absorption.

In summary, plasma AUC data for triclosan following either single or repeated oral dosing indicate a lack of accumulation of triclosan. The blood or plasma AUC is a measurement of exposure to a given dose of drug or substance, encompassing both amount absorbed and amount eliminated. If the AUC of a 24-hour dose interval in a continuous dosing regimen is equal to the AUC of an equivalent single dose, the data would indicate a lack of accumulation (*i.e.*, the complete elimination of the daily triclosan dose, with the absorption equal to the elimination from the body in a given single-dose interval). In addition, the steady and relatively invariable plasma levels of triclosan in long-term dosing (brushing) studies and in dermal application studies further suggest that triclosan does not accumulate in the body.

3.3.11.2.2 Absorption

The absorption of triclosan was measured following several different routes of administration, including oral exposure to triclosan-containing products (*e.g.*, toothpaste),

oral ingestion of capsules, aqueous solutions, and dental slurries (*i.e.*, following brushing with triclosan-containing toothpaste), and percutaneous exposure (*in vivo* and *in vitro*).

Absorption Following Oral Administration

Several studies of oral absorption have been conducted for exposures *via* capsules, solutions, dental slurries, mouthwash, or toothpaste. Overall, triclosan administered *via* the oral route in capsules is almost completely absorbed, as indicated by urinary elimination data [Lucker *et al.*, 1990 (109); Stierlin, 1972b (121)]. Following exposure to 15 mg/day of triclosan (corresponding to 0.25 mg/kg body weight/day) for 36 days, 90% of the administered dose was eliminated in the faeces (10%) and urine (80%), as shown in an intra-subject study [Lucker *et al.*, 1990 (109)]. Similarly, more than 98% of the administered dose was recovered in the urine (87%) and faeces (11%) following a single 200.5 mg dose of radiolabelled triclosan in a gelatine capsule (corresponding to 3.3 mg/kg body weight) [Stierlin, 1972b (121)].

Two separate studies examined the effects of the formulation of the administered triclosan on oral absorption [Concordia Research Laboratories, 1997a (110); Colgate-Palmolive, 1989 (113)]. A study comparing oral ingestion of triclosan from dental slurry (following brushing with triclosan-containing toothpaste) with oral ingestion of triclosan from aqueous solution revealed that the onset and rate of absorption of triclosan was faster for the aqueous solution compared with the dental slurry [Concordia Research Laboratories, 1997a (110)]. Results from a study comparing triclosan-containing toothpaste use (expectoration of dental slurry) with ingestion of triclosan aqueous solution confirmed that the amount of triclosan absorbed from normal toothpaste use (including expectoration and rinsing) is extremely low (*i.e.*, 5 to 10% of the dose, which corresponds to 9 to 14% of the amount absorbed and excreted following ingestion of an equivalent amount of triclosan in aqueous solution) due to decreased ingestion [Colgate-Palmolive, 1989 (113)]. The results of these studies show that normal toothpaste use would be expected to result in low levels of total absorption together with a slow onset and rate of absorption compared with oral ingestion of triclosan in an aqueous solution.

Oral retention of triclosan following the use of triclosan-containing products (toothpaste and mouth rinse) was examined in 2 studies [Gilbert, 1987 (117); Lin, 2000 (118)]. In one study, saliva samples were collected after the first of two daily brushings with triclosan-containing toothpaste (2 mg triclosan per brushing). Mouth rinse samples were collected following the use of a mouth rinse formulated to recover triclosan after the second brushing. The results indicated that approximately 25% of the triclosan dose is retained in the mouth following tooth brushing, with the remainder being recovered on the toothbrush, expectorated and rinsed out. The use of a non-triclosan mouth rinse following brushing decreases oral retention further, with approximately 14% of the retained triclosan (*i.e.*, of the 25%) being recovered by the mouth rinse [Gilbert, 1987 (117)]. In a separate study, 4.5 mg of triclosan was administered in a mouth rinse twice daily for 21 days. Oral retention of triclosan was measured to be 4 to 13% of the daily dose, and buccal absorption of triclosan was estimated to be 2 to 4% of the daily dose [Lin, 2000 (118)].

Absorption Following Percutaneous Administration

The main findings from *in vivo* and *in vitro* percutaneous absorption studies for triclosan are summarized in Table 36, with discussion in the paragraphs that follow.

Table 36: Findings from Human *In Vivo* and *In Vitro* Percutaneous Absorption Studies for Triclosan

Subject	Method	Major Findings	Reference
In Vitro Studies			

Subject	Method	Major Findings	Reference
In vitro	Breast or abdominal skin (0.64 cm^2) was obtained and exposed to a tritiated triclosan solution for up to 24 hours.	Non-GLP. After 24 hours, approximately 6.3% of the applied dose appeared in the receptor fluid, and 46%, 24%, and 22% remained on the skin surface, in the epidermis and dermis, and stratum corneum, respectively. Of the radioactivity in the receptor fluid at 24 hours, 0.8% of the applied dose was present as triclosan, 3.5% as triclosan glucuronide, and 0.2% as triclosan sulfate. Of the radioactivity in the skin at 24 hours, 12% of the dose was recovered as triclosan, 3% as triclosan glucuronide, and 3% as triclosan sulfate.	Moss *et al.*, 2000 (21)
In vitro	Female epidermal skin samples from cosmetic surgery, mounted in diffusion cells; single application of 0.2% triclosan in w/o emulsion; leave-on.	GLP-compliant. Conducted comparable to OECD Guideline No. 428. After 24 h, 95% recovery of the applied dose of triclosan. Period of penetration was between 8 and 24 h following a lag phase of approximately 8 h. The rate of skin permeation reached ~0.008 µg/cm^2/h. After 24 h, 3.9±0.4% of the applied dose of triclosan had penetrated into the receptor fluid (0.14±0.01 µg/$cm^{2)}$. As measured at 24 h, triclosan in the surface material was 76% of the applied dose (65% in the 24-h surface wipe and 11% in the first 3 tape strips of skin). Succeeding tape-strips (strips 4-20) contained 6.8% of the applied dose (0.25 µg/$cm^{2)}$, and 7.4% of the dose was recovered from the remainder of the sample of skin (0.28±0.05 µg/$cm^{2)}$. Percutaneous absorption was calculated to be 11.3% (0.42 µg/$cm^{2)}$ based on receptor fluid plus remainder of skin after removal of tape strips 1-20.	Ciba Specialty Chemicals, 1998a (130)
In vitro	Female epidermal skin samples from cosmetic surgery mounted in diffusion cells; single application of dishwashing liquid (0.2% triclosan); rinse-off after 30 minutes.	GLP-compliant. Conducted comparable to OECD Guideline No. 428. After 24 h, there was 89% recovery of the applied dose of triclosan. Period of penetration was between 2 and 6 h following a lag time of about 2 h. The rate of skin permeation reached ~0.01 µg/cm^2/h. After 24 h, 2.3±0.3% of the applied dose had penetrated into the receptor fluid (0.093±0.01 µg/$cm^{2)}$. As measured at 24 h, triclosan in the surface material was 4.3% of the applied dose (<1% of wiped off surface); 70% had been recovered in the 30-minute rinsate. Succeeding tape-strips (strips 4-20) contained 3.0% of the applied dose (0.25 µg/$cm^{2)}$, and 9.7±1.7% of the dose was recovered from the remainder of the sample of skin (0.39±0.06 µg/$cm^{2)}$. Percutaneous absorption was calculated to be 12.0% (0.483 µg/$cm^{2)}$ based on receptor fluid plus remainder of skin after removal of tape strips 1-20.	Ciba Specialty Chemicals, 1998b (131)
In vitro	Female epidermal skin samples from cosmetic surgery mounted in diffusion cells; single application of a deodorant formulation (0.2% triclosan); leave-on.	GLP-compliant. Conducted comparable to OECD Guideline No. 428. After 24 h, 84% recovery of the applied dose of triclosan. Period of penetration was between 8 and 24 h following a lag phase of 6-8 h. The rate of skin permeation reached ~0.002 µg/cm^2/h. After 24 h, 0.85±0.13% of the applied dose of triclosan had penetrated into the receptor fluid (0.033±0.006 µg/cm^2). As measured at 24 h, triclosan in the surface material was 64% of the applied dose (40% in the 24-h surface wipe and 24% in the first 3 tape strips of skin). Succeeding tape-strips (strips 4-20) contained 13.2% of the applied dose (0.50 µg/cm^2), and 6.8% of the dose was recovered from the remainder of the sample of skin (0.27±0.09 µg/cm^2). Percutaneous absorption was calculated to be 7.65% (0.303 µg/$cm^{2)}$ based on receptor fluid plus remainder of skin after removal of tape strips 1-20.	Ciba Specialty Chemicals, 1998c (132)

Subject	Method	Major Findings	Reference
In vitro	Female epidermal skin samples from cosmetic surgery, mounted in diffusion cells; single application of a soap solution (0.02% triclosan); rinse off after 10 minutes.	GLP-compliant. Conducted comparable to OECD Guideline No. 428. After 24 h, there was 91% recovery of the applied dose of triclosan. Period of penetration was between 2 and 6 h (following a short lag time of <1 h). The rate of skin permeation reached ~0.001 µg/cm^2/h between 2 and 6 hours. There was marked decrease in penetration between 6 and 8 h, reaching a plateau by 24 hours. After 24 h, 2.3±0.25% of the applied dose of triclosan had penetrated into the receptor fluid (0.0096±0.0011 µg/cm^2). As measured at 24 h, triclosan in the surface material (with stratum corneum) was 9%. Succeeding tape-strips (strips 4-20) contained 4.3% of the applied dose (0.018 µg/cm^2) and 4.9% (0.021±0.0042 µg/cm^2) of the dose was recovered from the remainder of the sample of skin. Percutaneous absorption was calculated to be 7.2% (0.0306 µg/cm$^{2)}$ based on receptor fluid plus remainder of skin after removal of tape strips 1-20.	Ciba Specialty Chemicals, 1998d (133)
In vitro	Single *versus* 6 applications in 3 days of 0.25 mL of an 8% (w/v) conventional soap (freshly prepared or equilibrated over 1 week) or non-soap detergent suspension (freshly prepared) each containing 0.08% (w/v) [^{3}H]DP300 was applied to the lower back (20 cm^2) for 2 minutes.	Pre-dates GLP. Autoradiography of skin at 10 minutes after a single application of the various [^{3}H]DP300 soap suspensions showed very low silver grain densities on the stratum corneum and in the epidermis, low or very low densities in the upper dermis, and very low or nil densities in the lower dermis. No grains were seen in the follicles or sebaceous glands. At 48 h after the single application, no silver grains were seen, except in the corneum after application of the fresh soap preparation. Scintillation counting showed no significant differences between the soap vehicles or in the single *vs*. repeated applications.	Black *et al.*, 1975 (27)
In Vivo Studies, Percutaneous Absorption Only			
3 subjects (2 treatment, 1 control)	10.0g soap containing 0.75% Irgasan® DP 300 used for full body bathing for 75 days.	Blood levels reached a plateau (7.0 to 19.1 ng/mL) immediately (*i.e.*, within 2 hours following first bath) and did not accumulate throughout study.	Ciba-Geigy, 1972a (119)
125 male and female	21-day hand washing study with diluted derma-san containing 0.25% Irgasan® DP 300 or scrub containing 1.0% Irgasan® DP 300. 7-day withdrawal period.	Blood levels increased as duration of study increased for 0.25% (28 to 68 ng/mL) but not 1.0% Irgasan® DP 300 (87 to 94 ng/mL). Dose-dependent increase in blood levels was observed. Blood levels return to near baseline (16 ng/mL, baseline = <10 ng/mL) by the end of withdrawal period. Irgasan was present in the plasma in a conjugated form (not specified): no free Irgasan detected.	Ciba-Geigy, 1973b (120)
2 females	0.34 and 0.51 mg radiolabelled GP 41 535/kg body weight applied to area of skin measuring 8 x 8 cm and covered for 24 h with occlusive dressing (1 dose/subject).	Only a very small percentage of the administered dose was absorbed and was excreted completely; 2 to 7% was recovered in the urine, 0.5 to 2% in the faeces and the remainder in the dressing. The blood concentrations of GP 41 353 were ≤0.01 µg/mL at all time points measured (1, 3, 5, 9 and 24 h after application).	Stierlin, 1972b (121)
9 males	Group of 3 subjects received single intravenous dose of ^{14}C-irgasan® CH3565; group of 6 subjects received single application (52 µg) of ^{14}C-irgasan® CH3565 suspended in Ivory soap to 13 cm^2 area of forearm for 24 h.	Following intravenous administration, the majority of CH 3565 was accounted for in the urine (65.4%±13.5% of the injected dose) and faeces (20.6%±10.4%). The half -life of CH 3565 was approximately 10 h. Following percutaneous application, an average of 8.9%±3.2% of the CH 3565 dose was absorbed percutaneously.	Maibach, 1969 (123)

Subject	Method	Major Findings	Reference
1 female and 3 male	10 g of surgical scrub product containing 0.75% by weight of Irgasan® DP 300 was applied to the surface area of both hands of each subject (2 washings/day for 3 minutes each for a total of 2 weeks).	The amount of DP-300 in blood increased immediately after washing, reaching a plateau at the 15 ng/mL level in the blood after the fourth day of hand washing (8 washes). 24 h after discontinuation of the hand washing, 2 subjects had no detectable amounts of Irgasan® DP 300 in the blood and by 48 h after discontinuation the third had no detectable amounts of Irgasan® DP 300 (1 subject dropped out of the study due to illness). Therefore, small amounts of Irgasan® DP 300 are absorbed through the skin and blood levels rapidly drop upon discontinuation of application.	Ciba-Geigy, 1972b (124)
6 male	Single dermal application of 5 g of CGP. 433 cream (equivalent to a dose of 150 mg triclosan). Applied to back of each subject (surface area = 900 cm^2).	Urinary excretion of free triclosan ranged from 0.006 to 0.041% of the administered dose during the 48-h urine collection period. Urinary excretion of free plus glucuronide conjugated triclosan ranged from 2.52 to 6.47% of the administered dose during the 48-h urine collection period. Results indicate that triclosan is slightly absorbed percutaneously.	Caudal *et al*., 1974 (125)
6 subjects	3 different soap formulations (Ivory, Dial base and Colgate base soap), each with and without 2% solution of CH3565 were contained in glass boats. Each subject (2 sets of subjects) had a glass boat taped to each forearm (one with CH3565, the other without). The applications covered a surface area of 15 cm^2 for 6 h (single application).	Analysis of the remaining contents of the boats and the washings from the skin revealed 100% recovery of 2% CH3565 from all 3 soap formulations for the first set of subjects. For the second set of subjects, recovery was 96% for the Ivory and Colgate soap bases. The computed average recovery of each formulation was 98%, suggesting that an average of 2% of the CH3565 was absorbed.	Schenkel and Furia, 1965 (126)
4 subjects	Total of 10 mL of Approve® skin cleanser (containing 0.75% triclosan) lathered on hands and forearms for a total of 8 minutes (single application).	Absorption of triclosan was minimal and occurred over a period of 8 h, at which time peak plasma concentrations of free plus conjugated triclosan ranged from 15 to 31 ng/mL. Plasma concentrations of free triclosan ranged from <3 to 4 ng/mL. Mean total urinary excretion of free plus conjugated triclosan was 627±101 µg over a period of 48 hours. Urine concentrations of free triclosan ranged from <3 to 16 ng/mL.	Thompson *et al*., 1975a (127)
4 subjects	Application of 1.0 mL of 0.5% GP 41353 Patient Skin Prep (equivalent to 5 mg of triclosan) onto a 100 cm2 area of normal and abraded abdominal skin, the former with and without an occlusive dressing (three 12-hour applications, separated by 4 weeks).	In the absence of an occlusive dressing the absorption of triclosan was below the limit of detection (plasma levels <15 ng/mL). The presence of the occlusive dressing enhanced absorption (plasma levels of free plus conjugated triclosan 112 to 192 ng/mL 4 to 8 h after application, declined slowly over 32 to 96 hours). Urinary excretion of free plus conjugated triclosan accounted for 6 to 14% of the dose without occlusive dressing, and 40 to 58% of the dose with occlusive dressing. These values decreased to <2% by 72 to 96 h after application. Absorption was not markedly increased by abrasion by application and removal of cellulose tape.	Thompson *et al*., 1975b (128)

Subject	Method	Major Findings	Reference
Single: 6 males 8-day: 6 males (4 Caucasian, 2 African American); 31-day: 11 males (5 Caucasian, 6 African American, including the 2 from the 8-day)	Single *versus* 8-day (22 applications) *versus* 31-day (91 applications) scrub study. 10 mL of GP 41353 Surgical Hand Scrub containing 50 mg triclosan applied to hands and forearms per 5-minute application.	Single: 10 to 24 hours following single application, maximum plasma concentration of free plus conjugated triclosan reached 62 to 143 ng/mL and returned to baseline over a period of 56 to 96 h. 2.6 to 6.6% of the applied dose was excreted in the urine as free plus conjugated triclosan (elimination half-life 14.4 to 38.4 h). 8-day: Plasma concentration of free plus conjugated triclosan still increasing on Day 8 (maximum concentrations ranging from 490 to 715 ng/mL and elimination half-lives ranging from 1.4 to 2.1 d for Caucasian subjects; maximum concentrations ranging from 1,640 to 1,780 ng/mL and elimination half-lives ranging from 11.3 to 15.6 d for African American subjects). Plasma concentrations of free triclosan ranged from <3 to 13 ng/mL for all subjects. 31-day: maximum plasma concentration of free plus conjugated triclosan (740 to 1,030 ng/mL) was reached at Days 12 to 15 for Caucasians. Plasma concentration of free triclosan was 16 and 6 ng/mL on Days 12 and 19, respectively. African American subjects were categorized as fast- or slow-eliminators. Slow eliminators had maximum plasma concentrations of free plus conjugated triclosan ranging from 3,400 to 4,080 ng/mL and baseline values were still not attained by Day 78. The fast-eliminators had max concentrations ranging from 554 to 690 ng/mL and baseline values were attained by Day 50. Plasma concentrations of free triclosan for slow- and fast- eliminators, respectively, were <3 and 9 ng/mL on Day 12 and <3 and 10 on Day 19.	Thompson *et al*., 1976 (129) (single *versus* 8-day) Thompson, 1975 (122) (8-day *versus* 31-day)
6 healthy males, 15 leukaemia patients, additional 4 subjects	Healthy males used 1% DP300 soap bar for 11 months. Leukaemia patients bathed daily with 1% DP300 soap bar for 5 weeks. 4 subjects applied an aerosol anti-perspirant containing 0.1% DP300 daily for 4 weeks.	DP300 was not readily absorbed through the skin following repeated topical application of hygienic products. Maximum blood levels of total triclosan were 44, 500, and 18 ng/mL for healthy subjects using soap bar, leukemic subjects using soap bar, and subjects using anti-perspirant, respectively. Blood levels of free triclosan did not exceed 8 ng/mL for healthy subjects using soap bar, or subjects using anti-perspirant. Leukemic subjects using soap bar had blood levels of free triclosan as high as 500 ng/mL. Maximum urine levels of total triclosan were 1,106, >1,001, and 890 ng/mL for healthy subjects using soap bar, leukemic subjects using soap bar, and subjects using anti-perspirant, respectively.	Hong *et al*., 1976 (24)
7 healthy males and 6 healthy females completed study	Volunteers washed their hands with ~3-5 mL of the test material (commercial hand wash containing 1% triclosan) 6 times/day, ~every 2 h during the day, for a lathering time of 15 seconds/washing followed by thorough rinsing, for 20 consecutive days.	Steady-state plasma levels of free and total (free + conjugated) triclosan were measured. Only about 10% of total triclosan was present as free (unconjugated) triclosan. Total plasma triclosan levels were consistently higher in males than females. At steady-state (on Day 20), triclosan levels in plasma were: 95.2 ng/mL in males and 47.4 ng/mL in females; 73.1 ng/mL for both sexes combined. Steady-state plasma levels were estimated to have been reached by Day 7. Plasma levels rapidly decreased after cessation of hand washing. Elimination half-life:1.4 d. There was considerable inter-individual variability in plasma levels. Investigators concluded that use of the 1% hand wash formulation resulted in low levels of systemic exposure (lower than oral route).	Ciba Specialty Chemicals, 2002 (134)
In Vivo Studies, Combined Oral and Percutaneous Absorption			

Subject	Method	Major Findings	Reference
20 volunteers (10/group)	Group 1: placebo soap, talc, antiperspirant + triclosan-containing toothpaste (0.215%). Group 2: triclosan-containing soap, talc, antiperspirant (concentrations of triclosan not specified) + triclosan-containing toothpaste (0.215%). Exposure for up to 56 d.	Total triclosan plasma levels did not generally exceed 100 ng/mL, and levels quickly diminished below detection level upon cessation of treatment (*i.e.*, by Day 64). The amount of unconjugated triclosan levels in all samples was <10 ng/mL. Triclosan was present in plasma mainly as its glucuronide-conjugated metabolite. Wide inter-subject variation prevented meaningful comparisons between groups 1 and 2.	BIBRA International, 1988 (137)
68 Caucasian, 54 African-Americans, and 45 Asian subjects, 18-65 years	Group 1: placebo soap and underarm deodorant + triclosan-containing toothpaste (0.28%). Group 2: soap (0.75%, 2X/day), underarm deodorant (0.39% 1X/day) + triclosan-containing toothpaste (0.28%). Daily exposure for 3 months.	Plasma levels of triclosan at Weeks 3, 6, and 13 showed slight, significantly increased blood levels of total triclosan and of glucuronide-conjugated triclosan in Group 2 (triclosan-containing toothpaste plus triclosan-containing hygiene products) compared to Group 1 (triclosan-containing toothpaste alone) [*e.g.*, 23.79 *vs*. 18.99 ng/mL total triclosan at Week 13]. Note: sulfate-conjugated triclosan levels were difficult to interpret due to inefficiencies in the analysis. These data show percutaneous absorption of triclosan from personal hygiene products.	Beiswanger and Tuohy, 1990 (138)

Absorption Following *In Vitro* Percutaneous Administration

^{3}H-labelled triclosan has been used to examine percutaneous absorption in a number of *in vitro* studies. Percutaneous penetration of 30.3% of the total applied dose of triclosan in an ethanol/water solution was measured 24 hours after application of the dose to human skin samples in a diffusion cell system [Moss *et al*., 2000 (21)]. Results from studies using skin samples in diffusion cells showed limited percutaneous absorption following application of triclosan to the skin surface in any of the formulations used. The amount of triclosan tested in most of these studies (0.2%) is within the range of anticipated concentrations in consumer products (from 0.15 to 0.3%). The low concentration in the soap solution study (0.02%) was intended to simulate actual-use conditions (*i.e*., a soap solution of 0.2% triclosan would be diluted with water when applied to the skin). Appropriate rinse steps were included in the rinse-off product studies. As recommended by SCCP guidelines (SCCP, 2006), calculations of dermal absorption included the amounts of triclosan recovered in the dermis and epidermis layers (*i.e*., the "remaining sample of skin" after removing the stratum corneum, represented by tape strips 1 to 20 in these studies) and the amount recovered in the receptor fluid. Table 37 presents a summary of the values from the *in vitro* studies conducted in human skin samples. The results from the autoradiography study [Black *et al*., 1975 (27)] indicated no significant differences in the low levels of percutaneous absorption between different soap vehicles or between single *versus* multiple applications [Black *et al*., 1975 (27)]. Overall, the dermal absorption of triclosan was shown in these *in vitro* studies to be lower in human skin than in rat (7.2 to 30.3% *versus* 41.2% in studies in rats, including the data from the study using ethanol/water formulation)].

Table 37: Summary of Dermal Absorption Values in Human Skin Samples from *In Vitro* Studies for Triclosan

Test Formulation	% Triclosan in test material	Dermal Absorption (SCCP)[1]		Reference
		%	µg/cm^2	
w/o Emulsion	0.2%	11.3	0.420	Ciba Specialty Chemicals, 1998a (130)
Dishwashing	0.2%	12.0	0.483	Ciba Specialty Chemicals,

Test Formulation	% Triclosan in test material	Dermal Absorption (SCCP)[1]		Reference
		%	µg/cm²	
formulation				1998b (131)
Deodorant formulation	0.2%	7.7	0.303	Ciba Specialty Chemicals, 1998c (132)
Soap solution	0.02%	7.2	0.0306	Ciba Specialty Chemicals, 1998d (133)
Ethanol/water formulation	--	30.3%	--	Moss *et al.*, 2000 (21)

[1] Dermal absorption = amount measured in the dermis, epidermis (without stratum corneum) and the receptor fluid (SCCP, 2006). Thus, for data from the *in vitro* studies, dermal absorption = "remainder of skin" + receptor fluid

Absorption Following *In Vivo* Percutaneous Administration

The *in vivo* percutaneous absorption of triclosan following single applications of triclosan-containing products (*e.g.*, cream and soap) has been consistently reported as only a small proportion of the applied dose (*i.e.*, generally ≤9%) [Stierlin, 1972b (121); Maibach, 1969 (123); Caudal *et al.*, 1974 (125); Schenkel and Furia, 1965 (126); Thompson *et al*., 1975a (127)].

Additional studies were conducted to determine blood and urine levels of triclosan (as a measure of absorption) following single and multiple percutaneous applications of triclosan in different vehicles including soaps, creams, other skin cleansers and surgical scrubs. Together, these data support findings indicating that percutaneous triclosan absorption is minimal [Caudal *et al.*, 1974 (125); Thompson *et al*., 1975a (127); Hong et al., 1976 (24)]. In general, blood levels of triclosan increased immediately after percutaneous application [Ciba-Geigy, 1972a (119); Ciba-Geigy, 1972b (124)], were enhanced by the presence of an occlusive dressing [Thompson *et al*., 1975b (128)], and were proportional to the dose applied [Ciba-Geigy, 1973b (120)]. Percutaneous absorption of triclosan from the use of triclosan-containing soap, underarm product and talc was also shown to be detectable in subjects already using triclosan-containing toothpaste [BIBRA International, 1988 (137); Beiswanger and Tuohy, 1990 (138)].

3.3.11.2.3 Metabolism

The metabolism of triclosan was investigated for several different routes of administration, including oral exposure to triclosan-containing products (*e.g.*, toothpaste), oral ingestion of capsules and aqueous solutions, and percutaneous exposure (*in vivo* and *in vitro*). Table 38 identifies the studies and their designs based on the route of administration of triclosan.

Table 38: List of Metabolism Studies of Triclosan

Route of Administration	Study Design	Reference
Oral (capsules)	Single *versus* multiple dose (intra-subject comparisons)	Lucker *et al.*, 1990 (109)
Oral (toothpaste)	Treatment *versus* placebo (inter-subject comparisons)	Lin, 1989 (135)
	Treatment *versus* placebo (inter-subject comparisons); effect of mouth rinse	Colgate-Palmolive, 1988 (136)
	Toothpaste *versus* toothpaste plus percutaneous (inter-subject comparisons)	BIBRA International, 1988 (137); Beiswanger and Tuohy, 1990 (138)
Oral (mouth rinse)	Treatment *versus* placebo (inter-subject	Lin, 2000

Route of Administration	Study Design	Reference
	comparisons)	(118)
Percutaneous (*in vivo*)	Treatment *versus* placebo (intra-subject comparisons) (soap bar use)	Wagner and Leshar, 1977 (139)
	Low *versus* high dose (hand washing study)	Ciba-Geigy, 1973b (120)
Percutaneous (*in vitro*)	Human *versus* rat	Moss *et al*., 2000 (21)

Biotransformation of triclosan

In general, the studies outlined in Table 38 indicate that, due to a pronounced first-pass effect, only trace amounts of the parent compound (*i.e*., unconjugated or free triclosan) are detected in the plasma. As a result of the first-pass effect, there is near total conversion of triclosan to glucuronide and sulphate conjugates [Lin, 1989 (135) and 2000 (118)].

Studies indicate that the relative amounts of glucuronide- and sulphate-conjugated triclosan present in the plasma vary depending on the plasma steady state concentrations of total triclosan. Up to study day 14, the main metabolite in plasma was the glucuronide conjugate (97% of total triclosan) in patients receiving 15 mg/day with only trace amounts of sulphate [Lucker *et al*., 1990 (109)]. Similarly, the glucuronide conjugate predominated in plasma samples from subjects brushing daily with triclosan-containing toothpaste (0.6%) [Colgate-Palmolive, 1988 (136)]. However, as steady state concentrations were reached (C_{max} = 330 ng/mL) during a 36-day exposure to the capsules, the absolute amount of glucuronide conjugates remained relatively constant, whereas sulphate conjugates increased to approximately 53% [Lucker *et al.*, 1990 (109)].

Two studies comparing oral exposure to triclosan-containing toothpaste alone and in combination with percutaneously applied triclosan-containing products (*e.g*., soap, talc, anti-perspirant/underarm deodorant) revealed that, in each study and for both groups, circulating metabolites were composed primarily of glucuronide for the duration of the study (8 and 13 weeks) [BIBRA International, 1988 (137); Beiswanger and Tuohy, 1990 (138)]. Plasma concentrations of total triclosan generally did not exceed 100 ng/mL in the 8-week study and ranged from approximately 20 to 30 ng/mL in the 13-week study. In the 13-week study [Beiswanger and Tuohy, 1990 (138)], the group receiving oral plus percutaneous exposure had increased blood levels of total triclosan and glucuronide-conjugated triclosan (approximately 24 to 30 and 26 to 29 ng/mL, respectively) compared with the group receiving oral exposure only (approximately 19 to 22 and 19 to 21 ng/mL, respectively) throughout the study (Weeks 3, 6, and 13).

In summary, the relative ratio of glucuronide to sulphate conjugates alters with repeat dosing (*i.e*., as plasma steady-state triclosan levels are reached) *versus* single dosing. Generally, lower plasma concentrations of total triclosan are associated with glucuronide conjugates as the predominant metabolite. With increasing plasma levels of total triclosan, there is an increase in circulating sulphate conjugates, which can reach levels greater than those attained by glucuronide conjugates, given high enough plasma levels of total triclosan.

Metabolism Following Percutaneous Administration

Glucuronidation and sulphation were demonstrated to occur in an *in vitro* diffusion skin model used to assess the ability of skin to metabolise triclosan [Moss *et al*., 2000 (21)]. Of the radioactivity in the receptor fluid (*i.e*., following absorption through the skin) at 24 hours, 3.5% of the applied dose was present as triclosan glucuronide and 0.2% was present as triclosan sulfate.

A study of percutaneous absorption from 21 days of hand washing with triclosan-containing soap or scrub showed that triclosan was nearly completely converted to a conjugated form detectable in plasma (no free triclosan was detected) [Ciba-Geigy, 1973b (120)]. *In vivo* percutaneous absorption studies have measured plasma concentrations of free plus conjugated triclosan as well as free triclosan following single and multiple applications of triclosan-containing products (*e.g.*, soap, anti-perspirant, skin cleanser, hand scrub) [Thompson *et al*., 1975a (127); Thompson, 1975c (122); Thompson *et al*., 1976 (129); Hong *et al*., 1976 (24)] (see Table 36). In these studies, although the specific conjugates were not differentiated (*i.e*., glucuronic acid and sulphuric acid conjugates), results showed that the majority of circulating triclosan was in the conjugated form. In a small study, 6 subjects used triclosan-containing soap for 45 days, with plasma steady-state concentrations ranging from 100 to 2,580 ng/mL. Plasma from subjects with lower steady-state levels contained primarily glucuronide conjugates, whereas plasma from subjects with higher steady-state levels contained primarily sulphate conjugates [Wagner and Leshar, 1977 (139)].

3.3.11.2.4 Excretion

The routes of excretion of triclosan were measured following single and multiple oral doses (triclosan capsules or mouthwash solution), single and multiple percutaneous applications (soap), and intravenous administration. Elimination half-life values for triclosan were outlined in the pharmacokinetic section (see Table 35) and are discussed in more detail below.

Elimination half-life

The elimination half-life values for orally-administered triclosan are comparable from study to study, irrespective of the formulations used, the duration of exposure, and the age of the subjects. For adults receiving single and multiple administrations of triclosan capsules, the elimination half-life values are 13.42 and 18.97 hours, respectively [Lucker *et al.,* 1990 (109)]. In adults, single doses from aqueous solutions and dental slurries have elimination half-life values approximating 11 to 20 hours [Concordia Research Laboratories, 1997a (110); Sandborgh-Englund *et al.*, 2006 (116)], and single use of triclosan-containing toothpaste has a value of 14.6 hours [Colgate-Palmolive, 1997b (115)]. In children, single doses of aqueous solution result in elimination half-lives up to 16.8 hours [Colgate-Palmolive, 1997a (111); Concordia Research Laboratories, 1997b (112)]. One study reported an elimination half-life value of approximately 10 hours based on excretion data following intravenous administration of triclosan [Maibach, 1969 (123)]. This value suggests a slightly shorter elimination half-life for intravenously injected triclosan compared with values obtained from oral studies, which may be reflecting the fact that absorption is by-passed following intravenous administration.

The elimination half-life value for dermally-applied triclosan used at 1% in a hand wash formulation was 1.4 days, based on combined data from men and women [Ciba Specialty Chemicals, 2002 (134)]. *In vivo* percutaneous absorption studies for triclosan conducted in the early 1970s had suggested that differences exist in the rate of elimination between Caucasians and African Americans [Thompson, 1975c (122); Thompson *et al*., 1976 (129)]. In an 8-day study conducted with triclosan-containing hand scrub, the elimination half-lives ranged from 33.6 to 50.4 hours for Caucasians and from 271.2 to 374.4 hours for African Americans [Thompson, 1975c (122)]. In a similar 31-day study, this difference in rate of elimination was observed between some but not all the African American and Caucasian subjects [Thompson *et al*., 1976 (129)]. Despite these findings, a subsequent study was designed specifically to evaluate any race differences (Caucasians *vs.* African Americans *vs.* Asians) in the metabolism of triclosan [Beiswanger and Tuohy, 1990 (138)]. This study was conducted with triclosan-containing toothpaste, soap and deodorant, and revealed no metabolic differences between these populations.

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The elimination half-life value for dermally-applied triclosan used at 1% in a hand wash formulation was 1.4 days, based on combined data from men and women [Ciba Specialty Chemicals, 2002 (134)]. *In vivo* percutaneous absorption studies for triclosan conducted in the early 1970s had suggested that differences exist in the rate of elimination between Caucasians and African Americans [Thompson, 1975c (122); Thompson *et al*., 1976 (129)]. In an 8-day study conducted with triclosan-containing hand scrub, the elimination half-lives ranged from 33.6 to 50.4 hours for Caucasians and from 271.2 to 374.4 hours for African Americans [Thompson, 1975c (122)]. In a similar 31-day study, this difference in rate of elimination was observed between some but not all the African American and Caucasian subjects [Thompson *et al*., 1976 (129)]. Despite these findings, a subsequent study was designed specifically to evaluate any race differences (Caucasians *vs.* African Americans *vs.* Asians) in the metabolism of triclosan [Beiswanger and Tuohy, 1990 (138)]. This study was conducted with triclosan-containing toothpaste, soap and deodorant, and revealed no metabolic differences between these populations.

Excretion Following Oral Administration

Following both single and multiple oral doses of triclosan in a gelatine capsule, the predominant route of excretion is the urine (57 to 87% of the administered dose), with much smaller amounts appearing in the faeces (10 to 33% of the administered dose) [Lucker *et al.*, 1990 (109); Stierlin, 1972b (121); Ciba-Geigy, 1976c (140)]. In a study using single doses of aqueous solutions containing triclosan, the major fraction was excreted within 24 hours of exposure, with between 24 and 83% (median 54%) of the oral dose excreted within the first 4 days after dosing [Sandborgh-Englund *et al.*, 2006 (116)]. In the same study, the median urinary excretion half-life was 11 hours and relative renal clearance was 57% of the total dose. Following single oral administration of radioactive triclosan (in capsule form), nearly 100% of the radioactivity detected in the urine was reported to be the glucuronide conjugate [Stierlin, 1972b (121); Ciba-Geigy, 1976c (140)], whereas in the faeces, 30 to 40% was recovered as the free unchanged triclosan compound [Stierlin, 1972b (121)].

Excretion Following Percutaneous Administration

The predominant route of excretion following percutaneous application of triclosan is the urine (2 to 14% of the applied dose) [Stierlin, 1972b (121); Caudal *et al.*, 1974 (125); Thompson *et al*., 1975b (128)], with much smaller amounts appearing in the faeces (0.5 to 2% of the applied dose) [Stierlin, 1972b (121)]. Excretion data revealed that, following single and multiple applications (2 to 3 times/day for 45 days), all the triclosan appearing in

the urine was present as the glucuronide conjugate [Wagner and Leshar, 1977 (139); Caudal *et al.*, 1974 (125)], whereas the majority of the faecal triclosan was present as the free unchanged compound [Wagner and Leshar, 1977 (139)].

Excretion Following Intravenous Administration

Excretion data obtained from an intravenous study were consistent with those obtained from the oral studies. Following injection of radiolabelled triclosan, the majority of the dose (approximately 65%) was excreted in the urine, while approximately 21% was excreted in the faeces [Maibach, 1969 (123)], suggesting biliary excretion into the gut.

3.3.11.2.5 Plasma Protein Binding

An *in vitro* study was conducted with serum derived from a healthy donor's blood to estimate the binding of ^{14}C-labelled GP 41353 to serum proteins (specifically albumin) using equilibrium dialysis. At concentrations ranging from 0.36 to 7.9 µg/mL, 99.8% of the drug was bound to serum proteins. There was no indication of saturation of binding sites, and the ratio of the unbound fraction to the bound fraction remained constant with increasing concentrations. At concentrations ranging from 0.095 to 9.1 µg/mL, 98.9% of the drug was bound to albumin, with no indication of saturation of binding sites. It was therefore concluded that, in plasma, the majority of the drug binds to albumin. Equilibrium dialysis showed that dialysis time is 60 minutes and that GP 41353 is bound to protein more firmly than it adheres to the membrane [Wagner, 1973 (141)].

3.3.11.2.6 Exposure of Infants by breast milk

Distribution into Breast Milk

Infant exposure to triclosan was estimated based on levels of triclosan found in 5 random samples of breast milk in a small study [Adolfsson-Erici *et al*., 2002 (102)]. In this study, the concentrations of triclosan in breast milk were reported to range from less than 20 to 300 µg/kg lipid weight. Based on breast milk fat intake rates of 0.0268 kg breast milk fat/day and using the most conservative (highest) concentration for intake estimates, the exposure of infants to triclosan *via* breast milk was calculated to be approximately 8.04 µg/day (0.00804 mg/day). Assuming a small infant of 2 kg weight, the exposure to triclosan from breast milk was calculated to be 4.02 µg/kg bw/day.

Another study determined triclosan levels in plasma and milk from 36 Swedish nursing mothers with and without known exposure from personal care products [Allmyr et al., 2006]. In this study triclosan was found in breast milk at concentrations ranging from < 0.018 to 0.95 ng/g (levels comparable on a fresh weight basis for milk to those determined by Adolfsson-Erici et al.). Allmyr et al. (Ref 103) calculated that the daily intake of triclosan would be <11–570 ng/day for an infant weighing 4 kg with an estimated milk intake of 150 ml/kg/day. As breast milk levels are lower than those in plasma of nursing mothers (ratio M/P <1), the infant is exposed to a considerably smaller dose of triclosan via the breast milk compared to the dose in the mother.

A recent risk assessment estimated the maximum daily consumption of triclosan by infants through breast milk, based on the most conservative values for breast milk concentration [Dayan, 2007 (104)]. The study based its calculations on the average triclosan concentration in the 5 breast milk samples with the highest triclosan levels, out of 62 samples, as well as a value for infant milk intake covering 97.5% of the population with the highest intake on a volume/kg body weight basis, *i.e.*, 1-month-old infants. The maximum daily infant consumption of triclosan through breast milk was calculated to be 7.4 µg/kg bw/day.

3.3.11.2.7. Triclosan levels in urine and plasma

Information on exposure to triclosan can be derived from biomonitoring and similar studies. Urinary triclosan concentrations were assessed in a representative sample of the U.S. general population from the 2003-2004 National Health and Nutrition Examination Survey (NHANES) by Calafat et al. (2008) **[**AR2**].** They analyzed 2,517 urine samples by means of sensitive analytical methods and detected triclosan in about three-quarters of urine samples: The geometric mean and 95th percentile concentrations were 13.0 µg/L (12.7 µg/g creatinine) and 459.0 µg/L (363.8 µg/g creatinine), respectively. Concentrations differed by age and socioeconomic status but not by race/ethnicity and sex. Specifically, the concentrations of triclosan appeared to be highest during the third decade of life and among people with the highest household incomes.
Dose estimates for triclosan based on the NHANES measurement data have not been reported by Calafat et al. since the conversion of measured spot urine concentrations to daily doses is not trivial because of several uncertainties related to variable dilution caused by wide variations in fluid intake and excretion and lack of information on route of exposure (i.e. oral, dermal, inhalation) and duration. Three methods proposed to estimate the dose from measured spot urine concentrations in the absence of total urine volume data have been recently used by the US EPA in computations for an aggregate risk assessment on triclosan (US EPA 2008; AR9).
SCCP considers these dose estimates for consumers in the US as useful additional information in their evaluation on the safety of triclosan.

There are three smaller studies on plasma levels in consumers, two in Swedish and one in Australian subjects: Sandborgh-Englund et al. [ref. 116] found triclosan in plasma at 0.1–8.1 ng/ml of 10 subjects, of which 5 were exposed and 5 not exposed to triclosan via personal care products. In that study there was no apparent difference between the two groups. Allmyr et al. 2006 (103) detected a broader range of plasma concentrations (0.010–38 ng/ml) in 36 Swedish nursing mothers, with higher concentrations in both plasma and milk of individuals with known exposure to triclosan via personal care products. Recently, Allmyr et al. 2008 [AR1] reported on their analysis of human blood samples collected in Australia between 2002 and 2005, and pooled according to age, gender and region. The dataset suggests that the exposure to triclosan among different groups of the Australian population is relatively homogeneous [Allmyr et al. 2008]. In comparison to their previous measurements in human plasma from Sweden, triclosan concentrations were a factor of 2 higher in Australian serum than in Swedish plasma.

3.3.11.2.8 Summary of Human Pharmacokinetic Data

Triclosan is very well absorbed following oral ingestion (up to 98% of the dose). However, under normal conditions of toothpaste use (*i.e.*, expectoration and rinsing) or following percutaneous application of several different personal care products, there is only limited absorption (approximately 5 to 10% of the dose *via* either of these routes of administration). Based on plasma levels and percentage of dose absorbed, it is clear that low exposures to triclosan occur following either toothpaste use or soap/hand wash use and that, with repeated exposures using either route, low steady-state levels of triclosan are reached after approximately 7 to 10 days.

Regardless of the formulation administered, only trace amounts of the parent compound are detected in the plasma following exposure to triclosan-containing products. Due to a pronounced first-pass effect, there is near total conversion of absorbed triclosan to glucuronic and sulphuric acid conjugates. The relative proportions of these metabolites vary depending on the plasma steady state concentration of total triclosan, with higher concentrations resulting in a shift from predominantly glucuronide- to predominantly sulphate-conjugates. Following ingestion, percutaneous application, or intravenous administration of triclosan, the predominant route of excretion is the urine, in which

triclosan is present as the glucuronide conjugate. In contrast, triclosan excreted in the faeces is present as the free unchanged compound.

Pharmacokinetic data, in particular, AUC values after single or repeated oral exposures (*e.g.*, after toothpaste use), as well as plasma levels after dermal (soap application) exposures, indicate a lack of evidence of bioaccumulation of triclosan.

3.3.11.3 Human Irritation and Sensitisation

Several irritation, sensitisation, and photosensitisation studies have been conducted with triclosan in humans. Data regarding the irritation and/or sensitisation potential of triclosan was also available from studies that reported results of routine patch tests with a series of compounds in patients presenting with contact dermatitis.

3.3.11.3.1 Irritation/Corrosivity

The irritation potential of triclosan was determined in tests on human skin and human mucous membranes. The main findings of these studies are presented in Table 39.

Table 39: Findings from Irritation Studies with Triclosan in Humans

No. of subjects	Application Details	Major Findings	Reference
N=106 females.	Repeated Insult Patch Test. Soap formulation applied to the back for 48 hours, twice a week for 5 weeks. Concentration of triclosan not reported (test material described as "soap bar" and "P-300 Anti-Bac. Deo. Soap (NDA #16-486)")	There was no evidence of primary irritation following any of the 48-h patch tests (a total of 1155 patch tests).	Colgate-Palmolive, 1972 (147)
N=20 volunteers	Hand washing Study. Volunteers with no known allergic reactions to hand washing agents. A commercially available 2% triclosan detergent was used (commercially available). Subjects washed with 5 mL for 1 min, rinsed, and applied a further 5 mL for 2 min of washing before rinsing and drying thoroughly. This procedure was performed 5 times at 20 min intervals.	Five of 20 subjects (25%) complained of irritation consisting of itching and soreness. In all cases, the irritation reaction was delayed by 6 to 8 h after the use of the triclosan-containing detergent. In 4/5 cases, the reaction lasted 24 to 36 h. In the worst case, the complaint was of a burning sensation and slight swelling that persisted for 2 days. It is important to note that this study did not use a control group treated with a detergent formulation without triclosan and it was inconclusive whether the irritation reaction was due to triclosan or to some other component of the detergent formulation.	Bendig, 1990 (162)
N=10 subjects	Finn Chamber Patch test. Tests were conducted on the forearms of subjects. Single application of patches saturated with either 1.0% SLS, 0.3% triclosan, 1.0% SLS and 0.3% triclosan, or distilled water.	No skin reactions were observed with triclosan alone or distilled water. SLS resulted in erythema of the skin of all subjects. SLS and triclosan together did not result in erythema. Pre-treatment with triclosan did not reduce erythema resulting from exposure to SLS.	Barkvoll and Rolla, 1994 (143)
N=19 subjects	Occluded patch test, double-blind study. 1 cm of each of 4 toothpastes was applied to the forearm of each subject for 24 hours. Concentration of ingredients in toothpastes was not reported.	One triclosan-containing toothpaste (triclosan/copolymer/SLS/ propylene glycol) produced mild to severe irritant reactions in 16 of 19 subjects, while the other (triclosan/zinc citrate/SLS/ polyethylene glycol) did not elicit any reaction.	Skaare *et al.*, 1997a (144)

No. of subjects	Application Details	Major Findings	Reference
N=14 or 15 subjects	Five mouth rinses were tested in groups of subjects: A, 1.5% sodium lauryl SLS B, 1.5% SLS/0.5% zinc citrate; C, 1.5% SLS/polyethylene glycol (1:8); D, 1.5% SLS/0.15% triclosan/0.3% zinc citrate; E, 1.5% SLS/0.15% triclosan	Mouth rinses containing triclosan (D, E) resulted in decreased frequency and severity of erythemic reactions, compared to mouth rinses without triclosan (A, B, C). Mouth rinse D elicited only 2 erythema reactions and E elicited 5 reactions. These 5 subjects also had mild desquamation.	Skaare *et al.*, 1997b (145)
Experiment I: N=10 subjects Experiment II: N=28 subjects	Irritation tests in oral mucosa (daily exposures). Experiment I (5 min/d for 5 d): Solidox Fluor® (F) (1.5% SLS) or Solidox G® (G) (1.5% SLS/0.3% triclosan/0.75% zinc citrate). Experiment II (2 min twice daily for 4 d): A, 1.5% SLS; B, 3% SLS/0.3% triclosan/ 0.75% zinc citrate; C, detergent-free toothpaste (negative control).	None of the subjects using Solidox G® (triclosan-containing toothpaste) showed oral mucosal desquamation, *versus* 7/10 subjects with positive results using toothpaste without triclosan. Comparison of results overall showed that triclosan eliminated the effects of 1.5% SLS (Experiment I) and reduced the severity of the effects of 3% SLS (Experiment II).	Skaare *et al.*, 1996 (146)

Abbreviations: SLS = sodium lauryl sulfate

The ability of triclosan to cause irritation to human skin or mucous membranes was evaluated in human volunteers. Triclosan (0.3%) was shown not to induce skin irritation in single patch tests in 10 subjects [Barkvoll and Rolla, 1994 (143)]. There was also no evidence of primary skin irritation in repeated patch tests with a bar soap formulation containing triclosan (concentration of triclosan not specified) in 106 female subjects [Colgate-Palmolive, 1972 (147)]. In this study, a total of 1,155 patch tests were conducted, including a challenge application 14 days after the 10^{th} patch test in each subject. Although irritation effects were observed in a hand washing study using a commercial detergent containing a high concentration (2%) of triclosan, the irritation effects could not clearly be attributed to triclosan [Bendig, 1990 (162)]. Thus, taken altogether, the data indicated that triclosan showed low skin irritation potential in clinical irritation tests and, possibly, skin irritation in a commercial detergent formulation containing a high concentration of triclosan (2%).

The effect of triclosan on skin and oral mucosa irritation produced by SLS was investigated in four studies. In the same patch test study in which triclosan was shown not to produce skin irritation, it was also shown to eliminate SLS-induced irritation [Barkvoll and Rolla, 1994 (143)]. In another study, the effects of triclosan in toothpaste formulations on SLS-induced irritation was tested in skin, with inconclusive, but suggestive, results with respect to a protective effect of triclosan [Skaare *et al.*, 1997a (144)]. Studies of oral mucosal response to various mouth rinse or toothpaste formulations containing SLS (1.5 or 3%) with/without triclosan (0.15% or 0.3%) showed that formulations containing triclosan reduced or eliminated the severity and frequency of SLS-induced oral mucosal erythema and desquamation compared to formulations without triclosan [Skaare *et al.*, 1997b (145); Skaare *et al.*, 1996 (146)]. Taken altogether, the data from these studies indicate that triclosan has a protective effect against SLS-induced skin or oral mucosa irritation. While the effects of triclosan alone have not been evaluated in stand-alone tests in oral mucosa because triclosan was typically tested in combination with SLS, the lack of irritant effects of triclosan-containing test formulations in oral mucosa studies, together with the protective effects of triclosan against SLS-induced irritation, indicate that triclosan is not a mucosal irritant.

In summary, the skin and oral mucosa irritation studies evaluating the effects of triclosan alone, or in combination with SLS, indicate that triclosan is not a skin or oral mucosal irritant.

3.3.11.3.2. Sensitisation

Table 40: Findings from human induction studies

No. of Subjects	Application Details	Major Findings	Reference
106 females	Repeated Insult Patch Test. Induction: Soap formulation applied to the back for 48 hours, twice a week for 5 weeks (occluded). Challenge: application 14 d after 10th application. Concentration of triclosan not reported in P-300 Anti-Bac. Deo. Soap.	There was no evidence of sensitisation potential in any of the 106 volunteers.	Colgate-Palmolive, 1972 (147)
144 males	Modified Draize test (patch test). Induction: triclosan in petrolatum was applied for 48-72 h per application, 10 times over 3.5 weeks. Challenge: After 14 d, using a patch for 72 h.	There was no evidence of sensitisation potential in any of the tests conducted with induction/challenge combinations of 5%/5% (none of 61 subjects tested), 20%/1% (none of 83 subjects tested).	Marzulli and Maibach, 1973 (148)
20 males / females	Modified Maximisation Test. Preparation: 5% sodium lauryl sulphate under occlusion for 24 h. Induction: Application of 20% triclosan in petrolatum at the same site on Days 1, 3, 5, 7, and 9. Challenge was 14 d later, using 1%, 2%, or 5% triclosan.	Induction phase: 17/20 subjects showed signs of skin irritation (erythema, slight oedema, moderately painful). However, 0/20 test volunteers and all control volunteers showed no positive patch test reactions up to 7 days post-challenge.	Lachapelle and Tennstedt, 1979 (18)
150, sex of subjects sex not specified	Repeat Insult Patch Test (100 volunteers) and the Prophetic Patch Test (50 volunteers). Applications (0.5 mL) of triclosan in solution or as a slurry were for 24 h (no further details were provided).	There was no evidence of skin sensitisation in any of the 150 volunteers (note that the concentration of triclosan tested was not provided).	DeSalva *et al.*, 1989 (1)

In total, triclosan was tested in 420 healthy subjects using variations and modifications to the Patch, Draize, and Maximisation Test methods at induction concentrations of up to 20% and challenge concentrations of up to 5%. There were no positive reactions in any of the test subjects, including after repeated patch testing [*e.g.*, Colgate-Palmolive, 1972 (14); DeSalva *et al.*, 1989 (1)]. No positive challenge results were observed, leading investigators to conclude that triclosan has a very low sensitisation potential. Taken altogether, the results from these studies indicate that triclosan has very low sensitisation potential in healthy subjects.

Comment
SCCP considers human induction studies as unethical

Triclosan has also been tested in patients with contact dermatitis or suspected cosmetic allergy. The results of routine patch testing with triclosan as one of a series of preservative or antimicrobial ingredients tested in these patients are shown in Table 41. The data show that triclosan has a low potential to cause positive skin reactions in this sensitive population.

Table 41: Findings from Patch Testing with Triclosan in Patients with, or Suspected of Having, Contact Dermatitis

No. of Patients Tested	Major Findings	Reference
5,202 (cosmetic intolerance)	Patch test. Seven (7) of 5,202 patients (0.1%) had a positive reaction to triclosan. In particular, 1 of 156 patients with a known "pure" cosmetic allergy showed a positive reaction (0.6%). Note: concentration of triclosan was not reported.	Broeckx *et al*., 1987 (149)
627 (suspected contact dermatitis)	Patch test. No positive reactions to triclosan (2% in petrolatum) were observed (0%).	De Groot *et al*., 1986 (150)
11,406 (consecutive patients)	Patch test. Twenty-nine (29) of 11,406 patients (0.3%) had a positive reaction to triclosan (2% in petrolatum). Note that 59 of 11,406 (0.5%) patients had a questionable/irritative reaction. Investigators considered the sensitisation rate to be low or very low.	Schnuch *et al*., 1998 (151)
179 (suspected cosmetic allergy)	Patch test. Two (2) of 179 patients (1.1%) had a positive reaction to triclosan (2% in petrolatum)	De Groot *et al*., 1985 (152)
3 (patients known to have used cream containing 3% triclosan)	All 3 patients in these case studies tested positive in patch tests (2% triclosan in petrolatum) at 48, 72, and 96 h.	Veronesi *et al*., 1986 (153)
2,295 (suspected allergic contact dermatitis)	Patch test. Triclosan was described as having a low sensitisation rate, based on the observed rate of 0.8% positive reactions to triclosan (2% in petrolatum).	Perrenoud *et al*., 1994 (154)
2,002 (consecutive patients)	Patch test. In total, 0 of 432, 0 of 470, and 2 of 1,100 patients tested positive to 0.5% triclosan in Vaseline, 1.0% triclosan in ethanol, and 2.0% triclosan in Vaseline, respectively (*i.e.*, 0%, 0%, and 0.18% positive reactions, respectively).	Wahlberg, 1976 (155)
103 (suspected contact dermatitis)	Patch test. Three (3) of 103 patients (2.9%) tested positive. Of the 3 positive reactions, 2 patients were known to have used cream containing 3% triclosan (the 3rd patient's history of use of triclosan was unknown). Note: triclosan concentration tested was not reported.	Steinkjer and Braathen, 1988 (156)
1,796 (eczema patients with suspected contact allergy)	Patch test (chamber method). One (1) of 1,796 patients (0.06%) tested positive to 1% triclosan.	Hannuksela *et al*., 1976 (156)
1,234 (consecutive patients with eczema)	Patch test. There were reported to be 1 to 2% positive reactions in 1,234 patients tested with 2% triclosan in petrolatum.	Mitchell *et al*., 1982 (157)
713 (suspected cosmetic dermatitis)	Patch test. One (1) of 713 patients (0.14%) with suspected cosmetic dermatitis tested positive to triclosan. Note: triclosan concentration tested was not reported.	Adams and Maibach, 1985 (158)
745 (suspected sun-related skin disease)	Photopatch test trial. One (1) of 745 patients (0.13%) tested positive for a contact allergic reaction to 2% triclosan in petrolatum; 2 of 745 (0.27%) tested positive for a photoallergic reaction.	Wennersten *et al*., 1984 (160)

Case reports of contact dermatitis due to triclosan use have been relatively rare (Campbell and Zirwas 2006, AR3), although a few cases have been reported following the use of a cream formulation containing a high concentration of triclosan (3%) together with 0.02% flumethasone pivalate. Three such cases were reported by Veronesi *et al*. [1986 (153)], and 2 by Steinkjer and Braathen [1988 (159)]. The reason for a third case of reported allergic contact reaction to triclosan was not discovered [Steinkjer and Braathen, 1988 (159)]. All 6 of these patients were found to have positive results in patch tests using 2% triclosan in petrolatum (see Table 41). Investigators concluded that low concentrations of triclosan in cosmetic products do not cause contact dermatitis; however, sensitisation may occur following the use of products containing higher concentrations [Veronesi *et al*., 1986 (153)].

In general, the clinical test results have shown that triclosan has a very low sensitisation potential. This is apparent in the interpretation of results from extensive testing in patients with known or suspected allergic contact dermatitis. In total, over 14,000 consecutive patients have been tested for reaction to triclosan (typically tested at a concentration of 2% in petrolatum), with the range of positive results being 0.1 to 0.3% of the tested population. Additionally, triclosan testing in patients with known or suspected cosmetic allergy or intolerance has shown positive reaction rates ranging from 0.06 to 0.8% of a total of 11,887 tests conducted in this population.

3.3.11.3.3 Photo-Induced Toxicity

The potential of triclosan to induce photosensitisation reactions was determined in a number of studies. The major findings of these studies are presented in Table 42.

Table 42: Findings from Phototoxicity and Photosensitisation Studies with Triclosan in Human Subjects

Application Details	Major Findings	Reference
N=5 males. 100 µL (triclosan, 0.1% in methanol, 0.1 or 1.0% in petrolatum) applied to a 30 cm^2 area on the back. Exposed to light sources 1 hour after application; readings at 24 and 48 h.	There was no evidence of phototoxicity.	Urbach, 1973 (101)
N=104 females. Subjects had undergone repeated insult patch testing (soap formulation with concentration of triclosan not reported; 10 applications and a challenge after 14 d – all negative). Photochallenge: 24 h after challenge and again 7 days after 1st photochallenge.	There was no evidence of photosensitising potential following the photochallenge application. After the second UV light photochallenge, one subject had a minimal reaction, considered to be the result of scratching, which was completely negative by the 48-h reading.	Colgate-Palmolive, 1972 (147)
Phototoxicity (n=10): triclosan (2.5% in petrolatum) applied to skin for 1 hour, followed by irradiation. Readings at 4-6 and 24 h. Photoallergy (n=25): triclosan (10% in petrolatum) applied to the same site for five 48-hour intervals under occlusion. Site was irradiated after each application. A new site was photopatch tested 2 weeks after the last 48-h test.	There was no evidence of phototoxicity or photoallergenicity.	Kligman, 1969 (161)
Modified Draize test (patch test). Induction: triclosan in petrolatum was applied for 48-72 h per application, 10 times over 3.5 weeks. Elicitation: After 14 d, using a patch for 72 h. Three (3) minimal erythema doses (MED) of Kromayer light were used during the induction phase, and 10 MED filtered through window glass during the elicitation phase.	There was no evidence of photosensitisation in induction/challenge combinations using 1%/1%, 5%/1%, 20%/1%, and 20%/5% concentrations of triclosan (*i.e.*, 0/51, 0/60, 0/52, and 0/24 positive responses in the tests, respectively, for a total of 0/187 subjects).	Marzulli and Maibach, 1973 (148)
Photopatch test trial. 745 (patients with suspected sun-related skin disease (photodermatitis)) were tested for reaction to triclosan (2% in petrolatum) as part of a standard series of tests.	2 of 745 (0.27%) tested positive for a photoallergic reaction.	Wennersten *et al.*, 1984 (160)
Patch and photopatch test. Triclosan (2% in petrolatum) was tested; details not provided. 103 patients with suspected contact dermatitis.	No positive photoallergic reactions.	Steinkjer and Braathen, 1988 (159)

Six studies investigated the photosensitising potential of triclosan. In one of the larger studies using a soap formulation containing triclosan (concentration not reported), only one positive reaction was observed following a second photochallenge with the soap formulation; however, this reaction was considered to be the result of scratching [Colgate-Palmolive, 1972 (147)]. There was no evidence of photosensitising potential of triclosan in two of the other studies that tested over 100 subjects per study, and only two of 745

patients tested positive for a photoallergic reaction in the largest study (0.27% of patients) [Wennersten *et al.*, 1984 (160)]. There was also no evidence of phototoxicity or photoallergenic potential in two smaller studies (n#25) with triclosan at concentrations of up to 10% in petrolatum or 0.1% in methanol.
In summary, data from the photosensitisation and patch testing studies performed with triclosan indicate that it is unlikely to produce phototoxicity or photosensitisation in human skin at levels used in personal care products. Triclosan was tested at concentrations of up to 10% in petrolatum in the photosensitisation studies.

3.3.12. Special investigations

Special investigations have been conducted to study potential neurotoxic and nephrotoxic effects of triclosan in rats. In the 14-day neurotoxicity study, clinical signs, organ weights, and brain and nerve histopathology were examined. In the nephrotoxicity study, kidney tissue function from triclosan-treated rats was assessed *in vitro*. In addition to these studies, the effects of triclosan in rodent liver have been evaluated in rats, mice, and hamsters. Studies have been conducted to determine the effect of triclosan on liver morphology (*e.g.,* size, hepatocyte necrosis, hepatocyte proliferation, changes in hepatocellular organelles) and on biochemical parameters (*e.g.*, protein content, cytochrome P450 content and activity, and fatty acid oxidation activity).

3.3.12.1 Effects of Triclosan in the Brain

A 2-week neurotoxicity study was conducted with triclosan in the rat [Ciba-Geigy, 1973a (163)], the main findings of which are provided in Table 43. No histopathological changes were observed in the brain or sciatic nerve of treated or control animals. There were no differences in brain weights between treated and control animals. Clinical signs included decreased movement and muscular tone, polydipsia, and polyuria at dose levels of 300 mg/kg body weight/day and higher. The results of this study indicate a NOEL of 100 mg/kg body weight/day for triclosan in the rat. There was no evidence of neuropathology at any dose level, as examined in the brain and sciatic nerve tissues. The investigators concluded that triclosan produces no specific neurotoxic effects in the rat; however, the reasons for observations of clinical signs consistent with possible neurotoxicity (*e.g.*, hypoactivity, decreased muscular tone) are unclear.

Table 43: Findings from a Two-Week Oral Neurotoxicity Study with Triclosan in the Rat

Species (Strain)	Dosing Regimen (mg/kg bw/day)	Major Findings	Reference, GLP and OECD Status
Rat, albino SIV 50	0, 100, 300, 1,000, or 2,000 mg/kg bw/day *via* oral administration (specific route not reported) for 14 days	17 deaths in high-dose group (5 of these were sacrificed due to moribund condition). Decreased body weights in high-dose group. Dose-dependent inhibition of movement, decreased muscular tone, polydipsia and polyuria at dose levels of 300 mg/kg bw/day and higher. Clinical signs were most severe in the high-dose group and also included spastic respiration for several animals. No difference in brain weights between treated and controls (no other organ weights were measured). No histopathological changes in the brain or sciatic nerve of treated or control animals were observed (no other tissues evaluated). NOEL: 100 mg/kg bw/day	Ciba-Geigy, 1973a (163) Predates GLP and OECD

3.3.12.2 Effects of Triclosan in Kidney

The nephrotoxic effects of triclosan have been investigated in a non-GLP study (published report) [Chow *et al.*, 1977 (164)], described in Table 44 by means of *in vitro* and *in vivo*

methods. Data from renal cortical slice incubation assays that measured *p*-aminohippurate (PAH) and *N*-methylnicotinamide accumulation as a measure of nephrotoxicity indicate that triclosan may have potential to cause kidney damage, although blood urea nitrogen (BUN) measurements in the same animals did not show indications of overt nephrotoxicity. The reason for the discrepancy between the *in vitro* results (where triclosan was added to the incubation medium) and *in vivo* results (where triclosan was administered to rats) is unclear, but may be related to the amounts of triclosan that reached the kidney, potential formation of a metabolite that selectively alters PAH accumulation, or the relative sensitivities of the assays.

Table 44: Findings from a Nephrotoxicity Study for Triclosan

Species (Strain)	Dosing Regimen	Duration of Treatment	Major Findings	Reference, GLP and OECD Status
Rat (Wistar)	625, 1,250, or 1,875 mg/kg bw 24 h prior to kidney cortical slice assay	Single dose	*In vitro* assay: There was decreased accumulation of both PAH and NMN. *In vivo* study: No change in blood urea nitrogen concentration at 24 h. Time-course study of renal cortical function in high-dose rats showed initial decreases in PAH accumulation that recovered to near-control levels by 72 h. *N*-Methylnicotinamide (NMN) accumulation was not significantly different from control up to 72 h. The significant decreases in PAH accumulation were dose-dependent.	Chow *et al.*, 1977 (164) Predates GLP and OECD

In addition to these early toxicity data, a later (1994) GLP study conducted in hamsters showed that doses of 350 or 900 mg/kg body weight/day in the diet induced renal tubular epithelium proliferation that was evident at 7 and 13 weeks in the 13-week study [See Table 45, Persohn, 1994 (167)]. The authors concluded the increased labelling index (LI) in kidney tubular epithelium was a compensatory response to cell damage in the kidney. However, no histopathology results were available for confirmation of the existence of cell damage.

In summary, findings in rat and hamster kidney studies of decreased kidney function and of increased cell proliferation that may be secondary to cellular damage suggest that triclosan may induce kidney damage at relatively high-dose levels.

3.3.12.3 Effects of Triclosan in Rodent Liver

The effects of triclosan on liver morphology, hepatocyte proliferation, and biochemical parameters, including the activities of cytochrome P450 enzymes, have been investigated in a number of studies in mice, rats, and hamsters. The major findings of these studies are presented in Tables 45 and 46.

3.12.3.1 Cell Proliferation in Rodent Liver

The effect of triclosan on hepatocyte replicative DNA synthesis has been examined in studies conducted in the CD-1 mouse, Sprague-Dawley rat, and Syrian hamster at doses ranging from 25 to 900 mg/kg body weight/day [Eldridge, 1993 (165); Persohn and Molitor, 1993 (166); Persohn, 1994 (167)]. In both the mouse and hamster cell replication studies, the effect of triclosan on replicative DNA synthesis was monitored by using the proliferating cell nuclear antigen (PCNA) technique, the results of which are summarized in Table 45.

Table 45: Findings from Cell Proliferation Studies for Triclosan

Species (Strain)	Dosing Regimen	Duration of Treatment	Major Findings	Reference, GLP and OECD Status
Mouse (CD-1)	Oral (diet) doses of 0, 25, 75, 200, 350, or 900 mg/kg bw/d	90 days (45 day interim data also available)	In general, labelling index (PCNA-stained S-phase cells) increased with dose and time, and was slightly greater in males *vs.* females. Cell proliferation was significantly increased at 90 days by 4.8-, 3.5-, 11-, and 15-fold in M at doses of 75, 200, 350, and 900 mg/kg/d, respectively, and by 1.5-, 3.3-, 6.1-, and 7.1-fold in F. Control group labelling indices were 0.035±0.016 and 0.042±0.036 in males and females, respectively. Cell proliferation was not increased at 25 mg/kg bw/d. Liver morphology changes showed dose-related hepatocyte hypertrophy starting at 25 mg/kg, together with necrosis of individual hepatocytes. Hypertrophy was reported as the most consistent and prominent change at 45 d, along with large areas of necrosis in the 350 and 900 mg/kg groups. Necrosis was accompanied by proliferating cells (bile duct epithelial cells, fibroblasts, Kupffer cells). Findings at 90 days were comparable to those at 45 d, except that necrosis was occasionally more severe, with panlobular necrosis in the most severe cases. Mean scores for liver necrosis at 90 days were 0, 0, 0.2, 0.6, 1.2, and 1.8 for males in the 0, 25, 75, 200, 350, and 900 mg/kg groups and 0, 0, 0, 1.2, 0.6, and 1.6 for females, respectively (scale of 0-4). Lipofuscin staining, lipid vacuolization, biliary hyperplasia were minimal.	Eldridge, 1993 (165) GLP and OECD not specified, but original animal study was GLP-compliant and consistent with OECD
Rat (Sprague-Dawley)	Oral (diet) doses of 0, 300, 1,500, or 6,000 ppm (actual doses of 0, ~25, ~125, and ~500 mg/kg bw/d)	2, 4, 7, 14, or 42 days (1 group received 6,000 ppm for 14 days followed by 28 days of recovery)	No deaths occurred, and no clinical signs of toxicity were observed. Feed consumption was initially decreased in high-dose animals, but was subsequently increased *vs.* controls. Body weight gains were initially decreased, but subsequently normal in the high-dose group. Absolute and relative liver weights were increased at 6,000 ppm compared to controls. Decreased numbers of hepatocyte nuclei per microscopic field together with increased liver weights indicated hepatic hypertrophy in high-dose animals. Triclosan did not increase cell proliferation at doses of up to 6,000 ppm in the diet, for treatment durations of up to 42 days. Slight but significant decreases in cell proliferation at the high dose were observed after at least 7 days of treatment. There were no effects in the lower dose groups. No histopathology results were available.	Persohn and Molitor, 1993 (166) GLP: not specified OECD: comparable
Hamster (Syrian)	Oral (diet) doses of 0, 75, 200, 350, 750, or 900 mg/kg bw/d	13 weeks (7-week data also available)	No increases in hepatic labelling indices (LI) *vs.* control were observed at the high dose of 900 mg/kg bw/d at 13 weeks. Kidney tubular epithelial cell nuclear mean LI were examined at 200, 350, and 900 mg/kg bw/d doses at 13 weeks. Kidney LI was significantly increased in males at 350 and 900, but not 200 mg/kg bw/d (3.7 and 6X greater than control, respectively). Kidney LI was significantly increased in high dose females at 13 weeks (8.8X greater than control). No histopathology results were available.	Persohn, 1994 (167) GLP-compliant. OECD not specified, but original animal study was consistent with OECD

The cell proliferation data demonstrate that triclosan treatment produces a dose-related increase in cell replication in male and female mice. Morphological examination of liver sections from this study revealed various histological changes including hepatocyte hypertrophy and necrosis in both sexes of the higher dose groups. In contrast, DNA synthesis data in male rats, and in male and female hamsters showed that triclosan did not increase hepatic replicative DNA synthesis in either sex after 7 or 13 weeks of treatment [Persohn and Molitor, 1993 (166); Persohn, 1994 (167)] (Table 45). Indeed, a significant decrease in hepatocyte proliferation relative to control was observed in male rats treated with ~500 mg/kg body weight/day for at least 7 days, and in male hamsters treated with 900 mg/kg body weight/day for 7 weeks. Altogether, the data show that cell proliferation following administration of triclosan is observed in mice, but not in rats or hamsters.

3.3.12.3.2 Liver Morphology and Biochemical Studies for Triclosan

Several investigations into changes in liver morphology and selected biochemical parameters induced by triclosan have been conducted. Of the six studies, the findings of which are presented in Table 46, three [Molitor *et al*., 1992 (168); Molitor and Persohn, 1993 (169); and Thomas, 1994 (170)] can be considered to be "pivotal" in the scope of the endpoints examined within the investigation, even though 2 of the 3 did not contain statements of GLP compliance.

Table 46: Findings from Liver Morphology and Biochemical Studies for Triclosan

Species (Strain)	Dosing Regimen	Duration of Treatment	Major Findings	Reference, GLP and OECD Status
Mouse (CD-1)	Oral (diet) doses of 0, 18, 54, 258, or 951 mg/kg bw/d (males) or 0, 20, 271, or 1,105 mg/kg bw/d (females)	14 days + recovery (28 days)	Increases in protein and cytochrome P450 (P450) content, and enzyme activities were dose-dependent and reversible. Highlights of the results include (high-dose data reported as % of control): increased lauric acid hydroxylation (up to 833%), ethoxyresorufin *O*-deethylase activity (up to 502%), and testosterone hydroxylation (up to 619%); peroxisomal fatty acid beta-oxidation (~340% in M and F) and pentoxyresorufin *O*-dependentylase (up to 2,390 and 1,580% in males and females, respectively). Immunoblot analyses showed increases in CYP3A1/2 proteins (842 and 5,851% in M and F, respectively) and in CYP4A proteins (~800% in M and F). Electron microscopy results show dose-dependent and reversible effects of an increase in smooth endoplasmic reticulum (ER) membranes, reduction and disorganization of rough ER membranes, and increase in lipid vacuolization in hepatocytes. Dose-dependent increases in numbers of peroxisomes ("moderate" to "striking" increases in numbers) at doses of ≥54 mg/kg bw/d.	Molitor *et al*., 1992 (168) GLP: not specified OECD: not applicable

Species (Strain)	Dosing Regimen	Duration of Treatment	Major Findings	Reference, GLP and OECD Status
Rat (Sprague-Dawley)	Oral (diet) doses of 0, 23, 108, or 518 mg/kg bw/d	14 days + recovery (28 days); 42 days	Increased, but reversible, absolute and relative liver weights in high-dose group. Increases in enzyme activities and cytochrome P450 content were generally dose-dependent and reversible. Highlights of the results include (high-dose data reported as % control): lauric acid hydroxylation (up to 281%); pentoxyresorufin *O*-dependentylase activity (up to 1,143%). Peroxisomal fatty acid beta-oxidation (FAO) was unaffected. Immunoblot analyses showed dose-dependent, large, and reversible increases in CYP2B1/2 enzymes (at least >100X control at 6,000 ppm); only slight or small increases in CYP3A1/2 and CYP4A proteins (209% and170% of control, respectively). Electron microscopy findings show reversible proliferation of smooth endoplasmic reticulum, increased cytoplasmic lipid vacuoles, dilated mitochondrial cristae, transient formation of matrical plates in peroxisomal matrices in high-dose rats.	Molitor and Persohn, 1993 (169) GLP: not specified OECD: not applicable
Hamster (Syrian)	Oral (diet) doses of 0, 50, 310, or 800 mg/kg bw/d in males, or 0, 46, 314, or 960 mg/kg bw/d in females	14 days + recovery (28 days)	Increased, but reversible, absolute and relative liver weights in high-dose females. Increases in enzyme activities and cytochrome P450 content were generally dose-dependent and reversible. Highlights of the results showed increases in: cytochrome P450 content; ethoxyresorufin *O*-deethylase and pentoxyresorufin *O*-dependentylase activities; 7α-hydroxytestosterone production in females; 15 α-HT, 16β-HT, and androstenedione production, lauric acid hydroxylation. Peroxisomal fatty acid beta-oxidation was unchanged. Immunoblot analyses showed no increases in levels of CYP1A- and CYP3A-related proteins, but slight to mild increases in CYP4A-related proteins (levels in males were 140% greater than controls, but increases in females were slight, with no overall change in total CYP4A levels). Electron microscopy findings show no changes to hepatocytes, including the number and size of peroxisomes.	Thomas, 1994 (170) GLP: compliant OECD: not applicable
Mouse (ddY); Rat (Wistar)	Daily i.p. doses of 50 or 100 mg/kg bw/d	3 days	Triclosan induced aminopyrine *N*-demethylase (APND) activity slightly, but significantly, in mice at a dose of 100 mg/kg bw/d. Slight (<100% increase *vs.* control), but significant increases in biphenyl 4-hydroxylase, APND, and phenacetine O-deethylase activities at 100 mg/kg bw/d in rats. Triclosan induced moderate increases (100-130%) in biphenyl 2-hydroxylase, ethoxycoumarin *O*-deethylase, and *p*-nitrophenerole *O*-deethylase (*p*-NPOD) activities at 100 mg/kg bw/d in rats. *p*-NPOD activity was also increased slightly at 50 mg/kg bw/d in rats.	Kanetoshi *et al.*, 1992 (4) GLP: not reported OECD: no comparable guidelines

Species (Strain)	Dosing Regimen	Duration of Treatment	Major Findings	Reference, GLP and OECD Status
Rat (Wistar)	*In vitro* concentrations of 0.1, 1, 10, or 100 µM	Male rats were pre-treated with cytochrome P450 inducers prior to preparation of microsomes	Ethoxyresorufin *O*-deethylase (EROD) and pentoxyresorufin *O*-dependentylase (PROD) activities were inhibited by 93 and 86%, respectively at a concentration of 10 µM triclosan. Inhibition of phenacetine *O*-deethylase and 4-nitrophenol hydroxylase activities was 5-60% *vs*. control levels at 10 µM and up to 80% *vs*. controls at 100 µM. Testosterone 6β-hydroxylase and lauric acid hydroxylase activities were inhibited by up to 50% at 100 µM. Ki values for EROD and PROD activities were 0.24 and 1.48 µM, respectively. The effect on EROD activity was consistent with competitive inhibition, whereas the effect on PROD indicated noncompetitive inhibition.	Hanioka *et al*., 1996 (171) GLP: not specified OECD: not applicable
Rat (Wistar)	0, 58, 116, or 232 mg/kg bw/d	5 days	Increases in biochemical parameters were generally dose-related, reaching significance at the high dose in rats. Increases included: microsomal protein, cytochromes P450 and b5, and NADPH-cytochrome C reductase. Benzyloxyresorufin *O*-debenzylase (BROD) and pentoxyresorufin *O*-dependentylase (PROD) activities were induced up to 22- and 20-fold, respectively. Levels of other P450 enzymes were either 2.4- to 4.9-fold increased at the high dose, or were unchanged. Immunoblotting data show levels of CYP 2B proteins (associated with PROD activity) were increased from 10.8 up to 34-fold.	Hanioka *et al*., 1997 (172) GLP: not specified OECD: not applicable

A series of 3 critical studies were conducted in mice, rats (males only), and hamsters to examine the effects of triclosan on selected biochemical and morphological liver parameters following dietary administration of triclosan for 14 days [Molitor *et al*., 1992 (168); Molitor and Persohn, 1993 (169); Thomas, 1994 (170)]. Biochemical investigations included measurements of levels of protein and cytochrome P450 (P450) content and P450 enzyme activities. Morphology was examined using electron microscopy (EM).

As can be seen from Table 46, and summarized in Table 47, there exist differences in responses between species. There was little difference between sexes, although females appeared slightly less sensitive to the effects of triclosan than males. With regard to morphology changes, the most notable was a dose-dependent, "moderate" to "striking" increase in the numbers of peroxisomes in mouse liver electron micrographs [descriptors taken from the original report by Molitor et al., 1992 (168)]. In contrast, there were no increases in numbers of peroxisomes in rats and hamsters. Liver weight and microsomal P450 content were strongly increased in mice, mildly increased in higher dose rats and relatively unaffected in hamsters. Similarly, induction of various enzyme activities was most pronounced and occurred at lower doses in mice when compared to other species. Cyanide-insensitive fatty acid β-oxidation activity (palmitoyl-CoA oxidation), a diagnostic indicator of peroxisome proliferation, showed a dose-dependent increase in mice, but not in rats or hamsters. Cytosolic glutathione *S*-transferase activity was mildly increased in mice, less so in rats, and slightly depressed in hamsters. Lauric acid 12-hydroxylation activity, as catalysed by isoenzymes of the cytochrome P450 CYP4A gene subfamily, was strongly increased in mice but less so in rats and hamsters. Lauric acid 11-hydroxylation activity, as catalysed by isoenzymes of the cytochrome P450 CYP1A, CYP2B, and CYP2C families, was again strongly increased in mice, but less so in hamsters. No effects were found in rats. Ethoxyresorufin *O*-deethylase (EROD) activity showed a mild dose-dependent increase in mice, with lesser increases in hamsters, and was decreased in rats. A strong induction of 7-pentoxyresorufin *O*-dependentylase (PROD), a CYP2B marker, was found in mice and rats; hamsters showed significantly less potent induction of this activity.

The pattern of regio- and stereo-selective testosterone hydroxylation was used as a tool to assess treatment-related effects on the activities of several isoenzymes of the microsomal P450 enzyme family. Only in the mouse was total testosterone hydroxylation significantly induced. At the highest dose level of 951 mg/kg body weight/day, a 6-fold dose-dependent increase was observed. The most prominent changes in the testosterone hydroxylation profile were increased production of the 2β-, 6β-, 15β-, and 16β-hydroxy metabolites. Production of the 2β- and 6β-hydroxy metabolites is associated with the expression of cytochrome CYP3A, while 16β-hydroxylation is catalysed by the CYP2B cytochrome family. Rats showed a different pattern of testosterone hydroxylation after triclosan administration. Production of the 16β-hydroxy metabolite was most prominent and, except for a mild induction of 15α-hydroxylation which is associated with cytochromes CYP2C12 and CYP2C13, no other hydroxy metabolite was produced in significant amounts. Hamsters were much less sensitive to P450 enzyme induction than mice. The only significant change observed in the hydroxylation profile was a small increase in 17-oxidation to form androstenedione.
Immunoblot analyses using monoclonal antibodies generated against, and specific for, inducible isoenzymes of rat liver cytochrome P450 were performed to further clarify the nature of triclosan-induced changes in liver enzymes. Mice demonstrated a slight dose-dependent decrease in the content of the isoenzymes of the CYP1A gene subfamily. Rats had an increased content of this isoenzyme family and hamsters a marginal increase. Triclosan strongly induced isoenzymes of the CYP3A subfamily in mice (8.4x control levels), produced a small increase (2X) at the highest dose in rats, and produced a decrease of this enzyme in female hamsters, with no effect in male hamsters. CYP4A induction was also extremely strong (7.8x at the highest dose) in mice and much less so in the rat and hamster (1.6x and 2.4x at the highest dose, respectively).

The effect of triclosan on P450 enzyme activities was also investigated in 3 published reports of an *in vivo* study in mice and *in vitro* and *in vivo* studies in rats [Kanetoshi *et al.,* 1992 (5); Hanioka *et al*., 1996 (171); Hanioka *et al*., 1997 (172)]. Both of the rat studies support the findings in the critical 1993 rat investigation [Molitor and Persohn, 1993 (169)]. In the *in vitro* study, the effects of triclosan on P450 enzyme activities were investigated using liver microsomes from rats pretreated with one of several P450 inducers (3-methylcholanthrene, phenobarbital, pyridine, dexamethasone, or clofibrate) [Hanioka *et al*., 1996 (171)]. The data from this study, which assessed the effect of triclosan on the activity levels of P450 enzymes previously increased by P450 inducers, suggest that triclosan competitively inhibits enzymes of the cytochrome P450 1A family, as indicated by inhibition of EROD and phenacetin *O*-deethylase activities, and non-competitively inhibits cytochrome P450 2B enzymes, as evidenced by its effect on PROD activity. Interaction of triclosan with cytochrome P450 2B1/2 enzymes was also suggested by the pattern of induction of various P450 enzyme activities in the published *in vivo* rat study which investigated the effects of 5 days of dosing with triclosan on P450 enzyme activities [Hanioka *et al*., 1997 (172)]. Data from the *in vivo* study show slight to no effects of triclosan administered for 3 days in mice on a variety of P450 activities, but did show increases in P450 activities in rats [Kanetoshi *et al.,* 1992 (5)]. The reason for the inconsistency with the pivotal 1992 mouse biochemical investigation is unclear, but may be related to the length of time of dosing or the difference in the route of administration (intraperitoneal in this published report *vs.* dietary administration in the pivotal report). Table 47 summarizes the effects of triclosan on selected biochemical parameters.

Table 47: Summary of Effects on Selected Biochemical Parameters Following 14-Day Dietary Administration of Triclosan

End-Point	Male Rat [1]		Male Mouse [2]		Female Mouse [2]		Male Hamster [3]		Female Hamster [3]	
	Effect	Minimum Effect Level (mg/kg bw/day)	Effect	Minimum Effect Level (mg/kg bw/day)	Effect	Minimum Effect Level (mg/kg bw/day)	Effect	Minimum Effect Level (mg/kg bw/day)	Effect	Minimum Effect Level (mg/kg bw/day)
Liver weight (absolute)	↑	518	↑	50	↑	270	none		↓	960
Protein content, microsomal	none		↑	20	↑	270	↑	800	↑	960
Cytochrome P450 content	↑	518	↑	50	ND		↑	310	↑	314
Glutathione S-transferase	↑	518	↑	250	ND		↓	800	↓ (sl.)	960
Fatty acid β-oxidation	none		↑	50	↑	270	none		none	
Lauric acid 11-hydroxylation	none	518	↑	20	ND		↑	800	↑	960
Lauric acid 12-hydroxylation	↑	518	↑	50	ND		↑	310	↑	960
Ethoxyresorufin *O*-deethylase	↓	23	↑	20	ND		↑	310	↑	314
Pentoxyresortufin *O*-depentylase	↑	108	↑↑	20	↑↑	20	↑	310	↑	314
Testosterone hydroxylation (total)	none		↑	20	ND		none		none	
1α-hydroxylase	none		ND		ND		ND		ND	
2α-hydroxylase	none		ND		ND		ND		ND	
6α-hydroxylase	none		none		ND		ND		ND	
7α-hydroxylase	none		↑	250	ND		none		↑	*
15α-hydroxylase	↑	518	ND		ND		↑	*	↑	*
16α-hydroxylase	none		↑	50	ND		ND		ND	
2β-hydroxylase	↑	*	↑↑	20	ND		none		none	
6β-hydroxylase	↑	*	↑↑	20	ND		none		none	
15β-hydroxylase	none		↑	50	ND		none		none	
16β-hydroxylase	↑	108	↑	950	ND		none		none	
Androstenedione	↑	*	↑	20	ND		↑	350	↑	*

Table 47: Summary of Effects on Selected Biochemical Parameters Following 14-Day Dietary Administration of Triclosan

End-Point	Male Rat [1]		Male Mouse [2]		Female Mouse [2]		Male Hamster [3]		Female Hamster [3]	
	Effect	Minimum Effect Level (mg/kg bw/day)	Effect	Minimum Effect Level (mg/kg bw/day)	Effect	Minimum Effect Level (mg/kg bw/day)	Effect	Minimum Effect Level (mg/kg bw/day)	Effect	Minimum Effect Level (mg/kg bw/day)
Cyt. P/-450 protein levels										
CYP1A	↑	108	↓	50	↓	270	none		none	
CYP2B	↑	23	ND		ND		ND		ND	
CYP3A	↑	518	↑↑	20	↑↑	20	none		↓	314
CYP4A	↑ (sl.)	518	↑↑	20	↑↑	20	↑	800	none	

[1] Rat data from Molitor and Persohn, 1993 (169).
[2] Mouse data from Molitor *et al*., 1992 (168)
[3] Hamster data from Thomas, 1994 (170).
* Not statistically significant but dose-response effect observed.
ND, not done
sl., slight

3.3.12.3.3 Summary of Liver Morphology and Biochemistry Data for Triclosan

The data showing strong increases in peroxisomal fatty acid beta-oxidation, 11- and 12-hydroxylation of lauric acid, and levels of CYP4A proteins (diagnostic for peroxisome proliferation), and increases in numbers and size of peroxisomes provide biochemical and EM evidence that triclosan has peroxisome proliferator-type activity in mouse liver at doses ≥50 mg/kg body weight in males. In contrast, key events associated with PPARα agonists, such as lauric acid 12-hydroxylation and an increase in total cytochrome P450, were significantly increased in the high dose group in rats; however, the magnitude of the increase was less than that seen in mice. The hamster data provide only limited evidence of any peroxisome proliferator activities for triclosan, with increased CYP4A proteins and lauric acid hydroxylation activity occurring only at high doses [Thomas, 1994 (170)]. However, the lack of induction of peroxisomal fatty acid oxidation and of morphological evidence suggests that triclosan is not a peroxisome proliferator in hamster liver.
Mice and rats, but not hamsters, produced a dose-related increase in PROD, which was significant at all doses in the mouse [Molitor *et al.*, 1992 (168)], but only significant in the highest dose group in rats [Molitor and Persohn, 1993 (169)]. Activation of PROD is typically associated with induction of CYP2B which is induced by phenobarbital. However, as is discussed more fully in Section 3.13.3, several other known PPARα agonists also significantly increase induction of PROD. In examining the evidence for peroxisome proliferator-like effects of triclosan in mice, it is interesting to note that the effects of the known peroxisome proliferator clofibrate in rats treated for 3 days at the dose of 400 mg/kg body weight/day were shown to include induction of PROD (3.7X), 4-nitrophenol hydroxylase (2.9X), and lauric acid hydroxylase (12.5X) activities compared to untreated rats [Hanioka *et al*., 1996 (171)].
Overall, both biochemical and morphological evidence from special investigations of the effects of triclosan in rodent liver serve to differentiate the response to triclosan in mice as that of a peroxisome proliferator.

3.3.12.4. *Effects of Triclosan on Rat Thyroid*

Effects of triclosan on thyroid hormone levels in rats have been investigated in two recent studies, one with 4-day oral exposure (10, 30, 100, 300, 1000 mg/kg bw/d) in weanling female Long-Evans rats (Crofton et al., 2007, AR4), the other with oral administration (3, 30, 100, 200, 300 mg/kg bw/d) from postnatal day (PND) 23 to 53 in male Wistar rats (Zorilla et al., 2009, AR10).

Short term oral exposure in female rats resulted in dose dependent decreases in serum thyroxine levels: serum T4 was decreased 28, 34 and 53% following treatment with 100, 300 and 1000 mg/kg bw/day triclosan, respectively. No significant changes were seen at 10 and 30 mg/kg bw/day triclosan in female weanling rats. The authors of this study (Crofton et al. 2007) suggest that decreases in T4 may result from increases in the sulfation or glucuronidation via PXR-linked genes. This view is consistent with triclosan-induced up-regulation of liver enzymes documented in other studies that have been described above (section 3.3.12.3. and Tab. 3.3.12.3.2-1 and Tab. 3.3.12.3.2-2).

The purpose of the second study was to determine effects of triclosan on pubertal development and thyroid hormone levels in the male rat. After 31 days of exposure, triclosan significantly decreased serum thyroxine (T4) in a dose-dependent manner at 30 mg/kg bw/day and higher (Zorilla et al., 2009). The active thyroid hormone triiodothyronine (T3) was decreased significantly only at 200 mg/kg bw/day, and thyroid stimulating hormone (TSH) was not statistically different from controls at any dose. Liver weights were increased at 100 mg/kg bw/day triclosan and above suggesting that induction of hepatic enzymes have contributed to the altered T4 and T3 levels. The authors did not consider the levels of change in glucuronidation (UDPGT) activity at 30 mg/kg as sufficient to explain the observed decrease in T4 levels; however, sulfation activity was not assessed. Triclosan did not alter the age at onset of puberty (assessed by preputial separation) or the

development of androgen-dependent tissues, even though there was a 60% decrease in androgen serum levels in the 200 mg/kg dose group.

In conclusion, alterations in thyroid hormone levels induced by triclosan in juvenile male rats did not lead to any apparent functional consequences. Thus, the lowest observed effect level for a decrease in T4 (30 mg/kg bw/day) is regarded as biochemical effect marker, but neither this nor the no observed effect level (3 mg/kg bw/day) are used for a risk assessment for triclosan since they have not been linked to an adverse effect.

Moreover, it is important to acknowledge major differences in the thyroid hormone physiology and regulation between rats and humans (SCCNPF 2004, AR7). Since the rat is a very sensitive model for chemical induced changes in the thyroid hormone axis, limitations exist with respect to extrapolation of rat data to human physiology/pathophysiology.

3.3.13. Safety evaluation (including calculation of the MoS)

3.3.13.1 Consumer Exposure Assessment

For cosmetics, consumer exposure, measured as systemic exposure dose (SED) is typically based on dermal absorption data. In the case of triclosan, because of exposure through toothpaste use and mouthwash, oral exposure data are also relevant.

Calculations were made for individual products and for product groups in which triclosan is used according to the industry submission, i.e. most prevalently in toothpaste, deodorant, hand and body soaps (referred to as common-use products) and for products in which triclosan is used less frequently, such as facial cosmetic products, body lotion, and mouthwash (referred to as marginal-use products).
For the purpose of SED calculations for oral formulations (toothpaste, mouthwash) it was assumed that triclosan is 100% bioavailable (see Table 48).

In the dossier that was submitted, calculations were based on current-use triclosan concentrations in different product types as given by the applicant, which, for some product categories, are below the maximally allowed content of 0.3% triclosan. However, the SCCP was requested to evaluate the safety of triclosan at the currently authorised level. Therefore, SEDs from both the current-use and the maximally allowed concentrations of triclosan are given in the calculations below.

Table 48: SED Calculation for Oral Products

Product	Assumed bioavailability (%)[1]	Amount applied (mg)[2]	Retention[3]	Frequency of application (times/d)[4]	Triclosan content (%)	BW (kg)	SED (mg/kg bw/d)[5]
Toothpaste	100	2,750 mg per day	0.17	NA	0.3	60	0.0234
Mouthwash	100	10,000 mg per use	0.1	3	0.2[6]	60	0.1000
Mouthwash	100	10,000 mg per use	0.1	3	0.3[7]	60	0.1500

Abbreviations: BW, body weight; d, day; NA, not applicable; SED, systemic exposure dose
[1] For the purposes of these calculations (i.e., for oral products), it was assumed that the bioavailability of triclosan was 100%.
[2] Amount of application value was taken from Table 2, Section 6-2 in SCCP, 2006.
[3] Retention value was taken from Table 2, Section 6-2 in SCCP, 2006.
[4] Frequency of use per day value for toothpaste was taken from Table 3, Section 6-2 in SCCP, 2006, which takes into account frequency of application. Frequency of use per day value for mouthwash was taken from Table 2, Section 6-2 of the SCCP, 2006 guidance.

[5] Formula: SED = (Bioavailability in % x amount of product applied (mg) x Frequency x Retention factor x amount of triclosan in product) / BW.
[6] Current use concentration as given by the applicant
[7] maximally authorised concentration

For dermal formulations, SED calculations were based on percutaneous absorption data from *in vitro* human studies (Table 37). SED calculations for individual personal-care products containing triclosan were carried out based on dermal absorption values (µg/cm^2) from *in vitro* percutaneous absorption studies conducted with deodorant and w/o formulations containing 0.2% triclosan and dilute soap solution formulation containing 0.02% triclosan.
In each calculation for dermal products, an extrapolated value for flux (µg/cm^2 absorption) was used, based on the assumption that skin penetration is by passive diffusion, such that flux would be proportional to the concentration of triclosan applied to the skin. For hand soap and body soap, the conversion of the µg/cm^2 dermal absorption value to a current-use value for amount of triclosan in the product type assumed a 10-fold dilution of 0.3% triclosan. For both soaps, a retention factor was not used due to the inclusion of a rinse-off step in the relevant *in vitro* percutaneous absorption study. The results of these calculations are provided in Tables 49 and 50, below, for leave-on and rinse-off products, respectively.

Table 49: SED Calculation for Leave-On Products

Product	24-h dermal absorption based on 0.2% triclosan (µg/cm^2) [1]	Triclosan content (%)	Calculated 24-h dermal absorption based on triclosan content (µg/cm^2) [2]	SA (cm^2) [3]	F (per d)	Conver-sion (mg/µg)	R	BW (kg)	SED (mg/kg bw/d) [5]
Deodorant stick	0.303	0.3	0.455	200	1	1x10^{-3}	1	60	0.0015
Body Lotion	0.420	0.15[6]	0.315	15670	1	1x10^{-3}	1	60	0.0823
Body Lotion	0.420	0.3[7]	0.630	15670	1	1x10^{-3}	1	60	0.1646
Face powder	0.420	0.2[6]	0.420	565	1	1x10^{-3}	1	60	0.0040
Face powder	0.420	0.3[7]	0.630	565	1	1x10^{-3}	1	60	0.0060
Blemish Concealer	0.420	0.15[6]	0.315	57	1	1x10^{-3}	1	60	0.0003
Blemish Concealer	0.420	0.3[7]	0.630	57	1	1x10^{-3}	1	60	0.0006

Abbreviations: BW, body weight; d, day; F, frequency of application; h, hour; R, retention; SA, surface area of application; SED, systemic exposure dose
[1] Dermal absorption values based on *in vitro* data using 0.2% in deodorant formulation (for deodorant stick) and 0.2% water/oil emulsion (for body lotion, face powder, and stick concealer).
[2] Calculation: (Absorption from 0.2% triclosan applied in the relevant *in vitro* study) x (triclosan content for the product/0.2%) = Absorption from 0.3% triclosan. This assumes that skin penetration is by passive diffusion, such that flux would be proportional to the concentration of triclosan applied to the skin.
[3] Area of application values were taken from Table 1, Section 6-2 in SCCP, 2006. The skin area for blemish concealer was assumed to be 1/10th of the face.
[4] Frequency of application values for use per day were taken from Table 2, Section 6-2 in SCCP, 2006. Frequency of application values for face powder and stick concealer were assumed to be once per day.
[5] Formula: SED = (24-h dermal absorption in µg/cm^2 based on use levels of triclosan in product x surface area of application in cm^2 x frequency of application per day x retention x conversion factor) / BW.
[6] Current use concentration as given by the applicant
[7] Maximally authorised concentration

Table 50: SED Calculation for Rinse-Off Products

Product	24-h dermal absorption based on 0.02% triclosan (µg/cm²) [1]	Calculated 24-h dermal absorption based on 0.03% triclosan (µg/cm²) [2]	SA (cm²) [3]	F (times/d) [4]	Conversion (mg/µg)	BW (kg)	SED (mg/kg bw/d) [5]
Hand soap	0.0306	0.046	860	10	$1x10^{-3}$	60	0.0066
Shower gel/body soap	0.0306	0.046	17,500	2	$1x10^{-3}$	60	0.0268

Abbreviations: BW, body weight; d, day; F, frequency of application; h, hour; SA, surface area of application; SED, systemic exposure dose

[1] Dermal absorption value based on *in vitro* data using 0.02% soap solution.

[2] Calculation: (Absorption from 0.02% triclosan applied in the relevant *in vitro* study) x (0.03%/0.02%) = Absorption from 0.03% triclosan solution. This assumes that 1) a 10X dilute solution of 0.3% triclosan is applied and 2) skin penetration is by passive diffusion, such that flux would be proportional to the concentration of triclosan applied to the skin.

[3] Area of application values were taken from Table 1, Section 6-2 in SCCP, 2006.

[4] Frequency value for shower gel/body soap use per day was taken from Table 2, Section 6-2 in SCCP, 2006. Frequency for hand washing was not provided in SCCP, 2006, so was assumed to be 10 times per day for this calculation.

[5] Formula: SED = (24-h dermal absorption in µg/cm² based on 0.03% triclosan x surface area of application in cm² x frequency of application per day x conversion factor) / BW.

The combined SED for common-use triclosan-containing personal care products and marginal-use triclosan-containing personal care products was also calculated. The SED calculations are presented in the Table below.

Summary of Triclosan SED Values from the Use of Personal Care Products		
Type of Product(s)	**Triclosan content (%)**	**SED**
Toothpaste	0.3	0.0234
Hand Soap	0.3	0.0066
Body Soap/shower gel	0.3	0.0268
Deodorant (Stick)	0.3	0.0015
Mouthwash	0.2	0.1000
Mouthwash	0.3	0.1500
Face powder	0.2	0.0040
Face powder	0.3	0.0060
Body lotion	0.15	0.0823
Body lotion	0.3	0.1646
Stick-type concealer	0.15	0.0003
Stick-type concealer	0.3	0.0006
Common-Use Products (toothpaste, hand soap, body soap/shower gel, deodorant stick)	0.3	0.0583
Marginal-Use Products (mouthwash, body lotion, face powder, stick concealer)	0.15-0.2	0.1866
Marginal-Use Products (mouthwash, body lotion, face powder, stick concealer)	0.3	0.3212
All Products (toothpaste, hand soap, body	0.15-0.3	0.2449

Summary of Triclosan SED Values from the Use of Personal Care Products		
Type of Product(s)	**Triclosan content (%)**	**SED**
soap/shower gel, deodorant stick, mouthwash, body lotion, face powder, stick concealer)		
All Products (toothpaste, hand soap, body soap/shower gel, deodorant stick, mouthwash, body lotion, face powder, stick concealer)	0.3	0.3795

Abbreviations: SED, systemic exposure dose

Internal exposure and absorption of triclosan under simulated use conditions by humans can be approximated also from *in vivo* studies with volunteers that applied triclosan-containing personal care products for a prolonged time period (see table 36). Precise information on the use-pattern and the level of triclosan content of the formulations was available in the study by Beiswanger and Tuohy (1990), in which 182 subjects used a toothpaste (0.28 % triclosan), a bar soap (0.75% triclosan) and a deodorant (0.39% triclosan) for 13 weeks. The results of this study indicated that all subjects reached a stable plateau plasma level after 3 weeks of use of the toothpaste, deodorant, and soap. The results showed plasma levels of: 19-23 ppb (exposure to toothpaste only), and 29-31 ppb (exposure to toothpaste, deodorant, and soap).

3.3.13.2 Safety Assessment

The human plasma levels can be compared to the plasma level (of 28,160 ng/ml) reported in studies with rats that received triclosan doses at the NOAEL of 12 mg/kg bw/day (see Tab. 27) to derive plasma level based MoS.

Types of Products Used	**SED** (mg/kg bw/d)	**MoS Based on Rat NOAEL of 12 mg/kg bw/d**	**MoS Based on Plasma Levels**
Toothpaste	0.0234	513	1408
Toothpaste, deodorant stick, and hand soap	0.0315	381	939
Common-Use Products 0.3% triclosan (toothpaste, hand soap, body soap/shower gel, deodorant stick)	0.0583	206	Not done (no human plasma data available)
All Products 0.15 – 0.3% triclosan (toothpaste, hand soap, body soap/shower gel, deodorant stick, mouthwash, body lotion, face powder, blemish concealer)	0.2449	49	Not done (no human plasma data available)
All Products 0.3% triclosan (toothpaste, hand soap, body soap/shower gel, deodorant stick, mouthwash, body lotion, face powder, blemish concealer)	0.3795	32	Not done (no human plasma data available)

From the calculations above it can be deduced that the use of triclosan in all products is not safe because of the magnitude of the aggregate exposure. This conclusion is reached regardless whether current use levels as given by the applicant or the maximally authorised use level is considered. However, the use in the common-use products (toothpaste, hand soap, body soap/shower gel and deodorant stick) is considered safe. The exposure to triclosan from face powder and blemish concealer (up to 0.3% triclosan) is low and not considered to be of concern in addition to exposure from common-use products. However, the use of triclosan in body lotions and mouthwashes results in high exposures and is not recommended.

Safety of Triclosan in Children and Neonates

The very low levels of exposure as measured in breast milk indicate that maternal use of triclosan immediately post-partum is unlikely to be a safety concern for neonates (see 3.3.11.2.6). Infant exposure to triclosan from breast milk has been shown to be significantly lower than exposure to the mother, based on a comparison of triclosan concentrations in breast milk and plasma [Allmyr *et al*., 2006 (103)].
No measured exposure data for babies and young children following use of consumer products containing triclosan was identified in the literature, except spot urine measurements in the age group 6-11 years from the NHANES study [Calafat et al. 2008; AR3]. Based on the conversion of spot urine concentrations to estimated dose, this subpopulation had a lower aggregate exposure to triclosan than children of 11-19 years and adults [US EPA 2008 (AR9)]. The rapid increase in maturation of glucuronidation ability within the first year and the maturity of the sulfation pathway, indicate that capabilities of children to metabolise triclosan through glucuronidation or sulfation are likely comparable to those of adults. Also, glomerular filtration rates normalised to body weight approach adult values by around 6 months of age and renal tubular function matures to near-adult values by around 1 year of age (Alcorn and McNamara, 2002). Accordingly, studies have shown that elimination is comparable in adults and children (see Section 3.11.2.1).

3.3.14. Discussion

Physico-chemical properties
Trichlosan is a phenol and a weak acid (pKa 8.1). This and its partition coefficient (logPo/w 4.8) facilitate transfer of the protonated (non-ionized) form of triclosan across lipid membranes.

General toxicity
Triclosan is not acutely toxic *via* the oral route of administration, with high oral intubation LD_{50} values in the range of 3,750 to 5,000 mg/kg body weight in mice and rats, and an oral capsule LD_{50} value of greater than 5,000 mg/kg body weight in dogs. SCCP considers the NOAEL as 12 mg/kg bw/d due to haematoxicity and decreased absolute and relative spleen weights (Mid Dose Females) in the long term toxicity study in rats.

Irritation / sensitisation
The irritation/corrosivity data from either irritation studies in the hamster, guinea pig, and rabbit, or skin toxicity studies conducted in the mouse, rat, monkey, and dog suggest that triclosan may cause slight reversible skin irritation at concentrations of 0.5 to 5% under experimental conditions. Triclosan at concentrations of 1 to 10% produced only slight, reversible irritation in the rabbit eye. Data from human use evaluating the skin and oral mucosa irritation effects of triclosan alone, or in combination with SLS, indicate that triclosan 0.3% is not a skin or oral mucosal irritant.
In the guinea pig no sensitisation with triclosan in various formulations and concentrations (up to 10% in petrolatum) was found. However, clinical experience has shown that triclosan does have a low sensitisation potential in humans. In over 14,000 patients patch tested with triclosan (typically tested at a concentration of 2% in petrolatum), the range of positive

results was 0.1 to 0.3% of the tested groups. When tested in patients with known or suspected cosmetic allergy or intolerance positive reaction rates ranged from 0.06 to 0.8% of a total of 11,887 tests conducted. Possible photocontact allergy has been rarely reported.

Dermal and oral absorption
Data from percutaneous absorption studies indicate that triclosan is well absorbed through the skin in all species tested with the extent of absorption being dependent on the formulation in which it was delivered. In the rat, percutaneous absorption was approximately 23 to 28% of the applied dose of triclosan in ethanol, ethanol/ water, soap suspension, or a cream formulation.
Triclosan is highly absorbed following oral administration, with no species-related differences, and in humans this is up to 98% of the dose. However, under normal conditions of toothpaste use (*i.e.*, expectoration and rinsing) or following percutaneous application of several different personal care products, absorption is more limited (approximately 5 to 10% of the dose *via* either of these routes of administration).
See also brief summary of in vivo data under "Kinetics"

Mutagenicity / genotoxicity
The genotoxic potency of triclosan has been investigated in a number of tests which can be broadly sub-divided in non-regular and normal (regulatory accepted) tests. Most of the tests are rather old and performed before the introduction of OECD guidelines. Consequently, the latter tests are not performed under currently accepted protocols. Since, next to non-standardised protocols, the tests have limited value but may occasionally give supportive evidence. Only two of the (non-regular) tests indicate a putative genotoxic potential of triclosan: Irgasan DP 300 (triclosan) induced mutations in an *in vitro* gene mutation assay in yeast. This positive result is not confirmed in an appropriate gene mutation test in mammalian cells. The same compound also induced mutations in a mouse spot test. However, in a similar experiment with lower and thus less toxic concentrations this result could not be confirmed.

Triclosan was investigated in (regular) genotoxicity tests covering the 3 endpoints: gene mutations, structural and numerical chromosome aberration. Triclosan exposure did not result in gene mutations in bacteria or mammalian cells nor did it induce UDS *in vitro* in primary hepatocytes. Triclosan induced chromosome aberrations in V79 cells, but was tested negative in assays with CHO cells. The positive result could not be confirmed in an *in vivo* micronucleus test in bone marrow cells of mice. Consequently, triclosan can be considered to have no relevant genotoxic potential in vivo.

Carcinogenicity
Three rodent lifetime bioassays have been conducted to evaluate the carcinogenic potential of triclosan. Triclosan produced hepatic effects and hepatic tumours in mice, but little evidence of toxicity and no tumours in rats. Hamsters showed increased liver toxicity relative to the rat, but no tumours.

According to the EU classification system, triclosan is not considered classifiable as a carcinogen. It should be noted that triclosan is a peroxisome proliferator in mice liver.

Reproductive/developmental Toxicity
Triclosan was not teratogenic nor a reproductive toxicant in a full complement of reproductive and developmental toxicity studies conducted in mice, rats, and rabbits conducted at doses of up to 350 mg/kg body weight/day.

NOAEL (NOEL) values from the definitive GLP studies were summarized in Table 21. It is important to note the determination of the foetal NOAEL value for each study was based on foetal variation effects that were most likely secondary to general maternal toxicity, and not direct effects of triclosan *per se*. It is also worth noting that the low NOAEL value for foetal effects in the mouse study (25 mg/kg body weight/day) is likely attributable to the sensitivity of the maternal mice to the liver effects of triclosan, also observed in the repeated dose and carcinogenicity studies in mice.

Kinetics
Numerous human and animal studies are available on the toxicokinetics of triclosan following oral and dermal exposure to single and repeated doses. The studies cover all important aspects, i.e. absorption, distribution, metabolism and excretion. Upon oral administration absorption of triclosan from the gastrointestinal tract is rapid and extensive in both humans and animals. But, limited buccal absorption was seen in humans following normal toothpaste use (up to 14% of the amount that would be absorbed upon ingestion of an equivalent dose). Upon dermal application in humans, absorption was at least 3% to 7%, and at least 14% in one volunteer.
Triclosan is rapidly distributed in the organism following oral or dermal exposure. The main metabolic pathways in humans and animals involve glucuronidation and sulfation by phase-2 enzymes. The half-life of elimination for orally administered triclosan ranged from 13 to 29 h in humans compared to 10 to 15 h in rats, 8-12 in mice and 25 to 32 h in hamsters. The major route of excretion in humans, hamsters, rabbits and primates is via urine, with excretion via faeces being of secondary importance in these species. The reverse situation is observed in rats, mice and dogs where biliary excretion is more important than renal excretion. The human oral and dermal data provide no evidence for a bioaccumulation potential. Likewise, the kinetic data in rats and hamsters provide no evidence for a bioaccumulation in these species, whilst in mice retention of triclosan (and/or metabolites) appears to occur in liver.
In conclusion, kinetics of triclosan are qualitatively similar, but the observed quantitative differences between humans and several animals make human data the first choice for the safety evaluation of triclosan-containing consumer products.

Other aspects
Recently, the US EPA (2008) utilized population-based biological monitoring data for triclosan (available from the NHANES study) to assess the co-occurrence of uses to develop an aggregate exposure assessment. Because of some uncertainties in converting spot urine concentrations to estimated dose, three conversion methods were used. Calculated exposure was then compared to the selected oral NOAEL of 30 mg/kg/day (from the chronic toxicity study in baboons). Based on the results at the mean and 99th percentile, the aggregate risks to triclosan from all (personal care and other consumer products) uses did not trigger a risk of concern. The mean MOEs ranged from 4,700 to 19,000. The MOEs at the 99th percentile ranged from 260 to 1,500 [US EPA 2008, AR9].

Exposure estimates based on biological monitoring data from the US are considered by SCCP as useful additional information in their overall evaluation on the safety of triclosan.

The difference in SCCP and US-EPA evaluations of triclosan may be explained as follows:

- USA-EPA chose a NOAEL of 30 mg/kg/d whereas SCCP selected a NOAEL of 12 mg/kg/d (based on haemotoxicity) as the critical effect level against which human exposure to triclosan is compared (for subsequent MOE or MOS calculations). The SCCP approach is in line with the evaluation of triclosan by EFSA for its use in food contact materials.
- US-EPA has estimated triclosan exposure in the US population on the basis of biomonitoring data from spot urine samples. Although this approach probably reflects exposure from current use concentrations in various products on the US market, it cannot be applied directly to the evaluation regarding the safe use of triclosan in cosmetic products by SCCP, since:
    - The current use concentrations in the USA may have been lower than the maximal triclosan concentration limit of 0.3% as preservative in cosmetic products in the EU, the safety of which SCCP was asked to evaluate according to this mandate (Question 1)
    - In estimating human exposure, the SCCP followed its Notes of Guidance to calculate systemic exposure doses (SED) from triclosan-containing products (at 0.3%) applied

orally and dermally. This may be viewed as a worst-case scenario. The alternative approach, i.e. MOS calculations that are based on plasma levels (measured under simulated use-conditions) were only available for certain products, not for all triclosan-containing products. Representative biomonitoring data are not available for the European population.

It is important to note that the two evaluations followed different objectives: While US-EPA in principle looked at real exposure that occurred in the population to derive a conclusion about a possible concern, the SCCP is asked to evaluate the safety of a hypothetical maximum exposure according to the authorised concentrations and applications in the cosmetic legislation.

4. CONCLUSION

Taking into account the provided toxicological data, the SCCP considers that the continued use of triclosan as a preservative at the current concentration limit of maximum 0.3% in all cosmetic products is not safe for the consumer because of the magnitude of the aggregate exposure.

However, its use at a maximum concentration of 0.3% in toothpastes, hand soaps, body soaps/shower gels and deodorant sticks ("common-use products" as defined by the applicant) is considered safe. Any additional use of triclosan in face powders and blemish concealers at this concentration is also considered safe but the use of Triclosan in other leave-on products (e.g. body lotions) and in mouthwashes is not considered safe for the consumer due to the resulting high exposures.

Importantly, before a final conclusion on the safety of triclosan in cosmetic products can be reached, the potential development of resistance to triclosan and cross-resistance by certain micro-organisms must be assessed. This aspect is not covered in this document and will be discussed in a separate opinion.

Inhalation exposure to triclosan from spray products (e.g. deodorants) was not assessed.

5. MINORITY OPINION

Not applicable

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