EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

Triclocarban

For other uses than as a preservative

COLIPA n° P29

Adopted by the SCCP by written procedure on 1 June 2005

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1. BACKGROUND

Cosmetic products marketed in the European Union may only contain those preservatives which are listed in Annex VI of the Cosmetics Directive 76/768/EEC, "List of preservatives which cosmetic products may contain".

The preamble of the Annex states that preservatives marked with the symbol (+) may also be added to cosmetic products in concentrations other than those laid down in the Annex for other specific purposes apparent from the presentation of the products.

Triclocarban ((N-(4-chlorophenyl)-N'-(3,4-dichlorophenyl)urea), COLIPA[1] n° P29) bears the symbol (+) and can therefore be used in cosmetics at higher concentrations, as long as they are not employed as preservatives. Triclocarban is currently authorized as preservative up to a maximum concentration of 0.2 % (Annex VI, Part 1, No. 23).

In its opinion of 17 February 1999 concerning the restrictions on materials listed in Annex VI of Directive 76/768/EEC on cosmetic products, the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) stated that those substances indicated by (+) in Annex VI, when incorporated into cosmetic formulations for non-preservative functions, should be subjected to the same restrictions in usage levels and warnings as when used for preservative effects.

If a preservative marked with the symbol (+) is added for non-preservative purpose to a cosmetic product in a concentration higher than that laid down in the Annex VI, data to substantiate its safety should be submitted to the SCCP.

The European Commission has received a submission from industry proposing that Triclocarban is safe for use in rinse-off hand and body care products up to 1.5%

2. TERMS OF REFERENCE

1. *On the basis of provided data the SCCP is asked to assess the risk to consumers when Triclocarban is used for non-preservative purposes in cosmetic rinse-off hand and body care products up to a maximum concentration of 1.5%.*

2. *Does the SCCP recommend any further restrictions with regard to its use in cosmetic products?*

[1] COLIPA – European Cosmetics Toiletry and Perfumery Association

3. OPINION

Introduction

Following an appropriate evaluation Triclocarban (TCC) originally has been listed since 1982 as # 25 in part 2 of appendix VI of the Cosmetic Directive 76/762 and is further listed as a preservative in the. (2nd Corrigenda)-Directive 82/368/EEC. Since 1986 based on (Corrigenda)-Directive 86/199/EEC Triclocarban was listed as # 23 in the first part of Annex VI to the Cosmetic Directive and finally listed as substance/preservative with a permitted concentration of 0.2 % for the preservation of cosmetic products, but also with the remark for "other purposes (+)" in higher concentrations.

Since 1957/58 Triclocarban has been used in concentrations up to 1.5 % in bar soaps (see also Roman et al. 1958 [38]).

Triclocarban containing cosmetic products in which the substance has been used as an antimicrobial active ingredient, commonly bar soap, have been in the market for more than 45 years and thus have a long use history in Europe and the US; significant adverse effects have not been reported. Moreover, the existing data demonstrate that Triclocarban is of low acute and chronic toxicity and has an acceptable human safety profile for use in personal cleansing products. A human health risk assessment on the basis of the worst-case assumption of aggregate exposure demonstrates that the use of such products provides a high Margin of Safety.

As a carbanilide, it can be classified according to its antimicrobial mechanism as a membrane-active compound. The mode of action can be described as unspecific adsorption to cell membranes, interruption of the function of interstitial proteins and/or loss of the semi-permeability of the membrane, with discharge of ions and organic molecules. Bacteriostatic or bactericidal effects occur dependent on the concentration. In its standard application concentrations, Triclocarban inhibits primarily the growth of gram-positive bacteria, but also that of gram-negative bacteria. Unlike antibiotics, membrane-active antimicrobial substances such as Triclocarban are effective within a short period of time.

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Triclocarban (INCI name)

3.1.1.2. Chemical names

N-(4-chlorophenyl)-N'-(3,4-dichlorophenyl)urea
3,4,4'-Trichlorocarbanilide
urea, N-(4-chlorophenyl)-N'-3,4-dichlorophenyl)

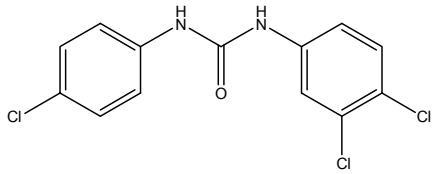
3.1.1.3. Trade names and abbreviations

Trade name : Preventol SB
COLIPA n° : P 29

3.1.1.4. CAS / EINECS number

CAS : 101-20-2
EINECS : 202-924-1

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{13}H_9Cl_3N_2O$

3.1.2. Physical form

White powder

3.1.3. Molecular weight

Molecular weight: 315.59

3.1.4. Purity, composition and substance codes

Mentioned in descriptive text, where applicable

3.1.5. Impurities / accompanying contaminants

Mentioned in descriptive text, where applicable

3.1.6. Solubility

0.11 mg/l at 20° C soluble in water

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow} : 4.2 (at 22.6 °C)

3.1.8. Additional physical and chemical specifications

Organoleptic properties	:	/
Melting point	:	250-255 °C (with decomposition)
Boiling point	:	> 300 °C
Flash point	:	/
Vapour pressure	:	< 1 mbar
Density	:	650 kg/m³ (bulk density)
Rel. vapour density	:	/
Viscosity	:	/
pKa	:	/
Refractive index	:	/
Stability	:	/

3.2. Function and uses

The most widespread use of Triclocarban is in antimicrobial bar and liquid soaps at concentrations up to 1.5 % and in body wash at concentrations of up to 0.2 %. It has further been used as an active ingredient in deodorant products such as sprays, roll-ons and sticks, shampoos and shaving creams and in some over the counter preparations like antiseptic foaming solution for topical application on skin and mucous membranes.

Triclocarban may be used as a preservative in cosmetic products at a maximum authorised concentration of 0.2% (Directive 76/768/EEC, Annex VI, part 1 n° 23).

3.3. Toxicological Evaluation

The long history of use and the in-use concentration provide the background of understanding that most of the toxicological results presented in the new submission have been established earlier, most of the conclusions have been published in original articles and in part in scientific peer reviewed journals.

3.3.1. Acute toxicity

Table 1: Summary of acute toxicity data

Study type	Species	Endpoint	Exposure	Result	References
Acute oral toxicity	Rat	LD_{50}	Single oral, neat product	> 2000 mg/kg bw	Bayer AG, 1991, Ref.: 1
	Mouse	LD_{50}	Single oral, neat product	> 5000 mg/kg bw	Marty and Wepierre, 1979, Ref.: 17
Acute dermal toxicity	Rabbit	LD_{50}	Single dermal 2 samples of neat product	>10000 mg/kg bw	Monsanto, 1979, Ref.: 21
Acute i.p. toxicity	Mouse	LD_{50}	Single intraperitoneal, neat product	2100 mg/kg bw	Marty and Wepierre, 1979, Ref.: 17

3.3.1.1. Acute oral toxicity

Several studies were conducted to assess the acute oral toxicity of Triclocarban to animals (Roman *et al*., 1957; Wright *et al.*, 1975; Marty and Wepierre, 1979; Monsanto, 1979; Bayer AG, 1991). In all studies, the acute oral toxicity of Triclocarban was reported to be larger than 2000 mg/kg bodyweight. Two exemplar studies are reported in this section.

Ref.: 38, 44, 17, 21, 1

Ten rats (5 of each sex) were administered a single oral dose of 2000 mg/kg bw Triclocarban (purity 98.8%) suspended in ethylene glycol 400 (Bayer AG, 1991). Animals were observed for mortality and clinical signs at 0.5, 1, 2 and 4 h after dosing and thereafter daily for 14 days. There were no signs of toxicity and no mortalities in any of the animals. The acute oral LD_{50} in rats was therefore >2000 mg/kg bodyweight. The study was GLP-compliant and followed OECD guideline 401.

Ref.: 1

Triclocarban dissolved in 1% carboxymethylcellulose was administered as a single oral dose at various test concentrations to NMRI (Swiss) mice. Animals were observed for 7 days, after which the LD_{50} for the study was determined. No mortalities were observed. The LD_{50} in mice was determined to be greater than 5000 mg/kg bw (Marty and Wepierre, 1979).

Ref.: 17

3.3.1.2. Acute dermal toxicity

Two different samples of Triclocarban containing varying levels of dichloro- and tetrachlorocarbanilide (i.e., sample 1 contained 6 - 8% 4,4'-dichlorocarbanilide and 6 - 8% 3,3',4,4'-tetrachlorocarbanilide; sample 2 contained 15 - 20% 4,4'-dichlorocarbanilide and 15 - 20% 3,3',4,4'-tetrachlorocarbanilide) were applied in increasing doses at 0.2 fractional log intervals to the closely clipped, intact skin of New Zealand white rabbits of both sexes (Monsanto, 1979). After application, the treated areas were covered with plastic strips and the animals placed in wooden stocks for periods up to 24 hours. Thereafter animals were assigned to individual cages and observations were made for toxic symptoms. No mortalities were observed and no necropsies performed. The acute dermal LD_{50} to rabbit for both samples was found to be >10000 mg/kg bodyweight. Although the study was not conducted according to regulatory guidelines, it was considered to be scientifically valid.

Ref.: 21

Intraperitoneal

Triclocarban diluted in 1% carboxymethylcellulose was administered as a single intraperitoneal dose at various concentrations to NMRI (Swiss) mice. The animals were then observed for 7 days. Signs of toxicity were slow to appear, the first deaths occurring only after 24 hours. The LD_{50} (i.p.) was determined to be 2100 mg/kg bodyweight (Marty and Wepierre, 1979).

Ref.: 17

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2. Irritation and corrosivity

Table 2: Summary of animal skin irritation data

Study type	Species	Endpoint	Exposure	Result	References
Skin irritation	Rabbit	Erythema/eschar and oedema formation	4h semi-occlusive, neat product	Not irritating PII = 0.0	Bayer AG, 1992b, Ref.: 3
	Rabbit	Erythema/eschar and oedema formation	24h occlusive, neat product	Not irritating PII = not available	Monsanto, 1979, Ref.: 21
	Guinea pig and rabbit	Erythema/eschar and oedema formation	24 and 48 h open patch, neat product	Not irritating PII = not available	Morikawa *et al*., 1974, Ref.: 25

3.3.2.1. Skin irritation

The skin irritation potential of Triclocarban was evaluated in studies with rabbits and guinea pigs (Morikawa *et al.*, 1974, Lautier, *et al*., 1978; Bayer AG, 1992d; Monsanto, 1979).

Ref.: 25, 15, 5, 21

Triclocarban (500 mg, purity 98.8%) moistened with polyethylene glycol 400 was applied to a hypoallergenic patch placed on the dorso-lateral area of the trunk of three New Zealand white albino rabbits for 4 hours (Bayer AG, 1992b). The treated skin area was approximately 6cm^2 in size. After the exposure period, the dressing and patches were removed and the exposed skin areas were carefully washed with water without altering the existing response. For each animal, the Draize scores describing the degree of erythema/eschar and oedema formation were recorded approximately 24, 48, and 72h after application. According to the Draize scoring system, the irritation index was determined to be 0.0, indicating that under the test conditions Triclocarban was not irritating to the skin. The study was conducted in compliance with GLP and according to OECD guideline 404.

Ref.: 3

The same result was obtained when finely ground powder of two different samples of Triclocarban containing varying levels of dichloro- and tetrachlorocarbanilide (i.e., sample 1 contained 6 - 8% 4,4'-dichlorocarbanilide and 6 - 8% 3,3',4,4'-tetrachlorocarbanilide; sample 2

contained 15 - 20% 4,4'-dichlorocarbanilide and 15 - 20% 3,3',4,4'-tetrachlorocarbanilide) and 25% corn oil suspensions of these powders were applied to the clipped intact skin of albino rabbits for 24h. The application was under fully occlusive conditions by covering the application sites with plastic strips to retard evaporation and avoid contamination. Irritation response was observed over several days. According to the Draize scoring, all Triclocarban-based test materials were evaluated to be not irritating to rabbit skin (Monsanto, 1979).

Ref.: 21

In a further study, 0.03 ml of Triclocarban diluted in acetone at concentrations of 0.5%, 1% and 3% was applied to a depilated circle of skin (1.5 cm diameter) on the back of male albino guinea pigs and male white albino rabbits by an open patch methodology. The sites of topical application were observed for the occurrence of skin reaction 24 and 48 h after application. The intensity of skin reaction was graded according to the Draize irritation grading scale for erythema and oedema. No effects were seen in any animals at any dose. Triclocarban was therefore considered to be non-irritating to guinea pig and rabbit skin under the conditions of the study (Morikawa *et al*., 1974).

Ref.: 25

Human data

The cumulative skin irritation effect of a liquid hand soap containing 0.15% Triclocarban has been investigated in a 3-Patch Application Test (i.e., 3-PAT) (Procter and Gamble, 1991). Ten to twelve human volunteers were treated with patches on the lateral surface of the upper arms under fully occlusive conditions (max 8 patches per person, 4 per arm). Test material was applied for 24 hours, 3 times a week at the same skin site, for a total of one week. Twenty four hours after removal of the patches (48 hours after a weekend), the skin was graded for irritation according to a 0 – 4 scoring scale. The liquid hand soap was tested as a 1%, 0.25% and 0.10% aqueous solution in distilled water resulting in respectively, 0.0015%, 0.0004% and 0.00015 % Triclocarban under the patches.

Ref.: 32

In a separate, similar study, the cumulative skin irritation potential of bar soaps containing 1.5% and 1.35% Triclocarban was investigated in a 3-PAT (Procter and Gamble, 1986). Ten to twelve human volunteers were treated with patches on the upper arms or backs under fully occlusive conditions. The test material was applied for 24 hours, 3 times a week at the same skin site, for a total of one week. A control material of which historical patch test data including market experience data were available was included into the test. Twenty four hours after removal of the patches (48 hours after a weekend), the skin was graded for irritation according to a 0 – 4 scoring scale. The bar soaps containing 1.5% and 1.35% Triclocarban were tested as a 2% aqueous solution in distilled water resulting in 0.03% and 0.027% Triclocarban under the patches, respectively. The mean average skin grading scores were 1.36 and 1.32, respectively. These scores are all indicative of a slightly irritating effect. The skin irritation profile of the test substances was similar to those of the control product.

Ref.: 28

The irritancy potential of Triclocarban was tested with human skin when held in continuous contact over 21 days. Petrolatum (0.1 ml) containing 0, 1, 3, 6, 9% Triclocarban was applied to the same site on the backs of ten normal white human males (Maibach *et al.*, 1978). The patches were removed daily for grading and reapplied under occluded conditions over 21 consecutive days. One subject show some irritation response to the 3% Triclocarban beginning 10 days after patching but did not show a response at the higher Triclocarban (6 and 9%) concentrations. The observed skin irritation reactions never exceeded the grade of 1.
The significance of the effects was questionable as it was not dose related. In conclusion, 9% Triclocarban was considered to be minimally irritating.

Ref.: 16

The phototoxicity of 9% Triclocarban was evaluated according to the method of Marzulli and Maibach (Maibach *et al.*, 1978). Petrolatum containing 9% Triclocarban was applied to both forearms of 10 Kaukasian males for 1 hour. The test sites on one forearm were then irradiated with a Wood's Light Inspectolite for 45 min at 10 cm. Over 90% UV radiation output was at 365 ± 5 nm; total UV output at the test distance of 3000 joules/cm^2. No positive responses were observed in any of the test subjects.

Ref.: 16

3.3.2.2. Mucous membrane irritation

Table 3: Summary of animal eye irritation data

Study type	Species	Endpoint	Exposure	Result	Reference
Eye irritation	Rabbit	Effects on cornea, iris, and conjunctivae	0.1 mL (42mg TCC)	Non irritating EII = 0.0	Bayer AG, 1992b, Ref.: 3
	Rabbit	Effects on cornea, iris, and conjunctivae	20mg of neat powder 2 samples	Non irritating EII_1 = 7.3 EII_2 = 6.6	Monsanto, 1979, Ref: 21
	Rabbit	Effects on cornea, iris, and conjunctivae	0.1 mL barsoap (0.055% TCC)	Non irritating $MAS_{unrinsed}$ = 19.7 MAS_{rinsed} = 1.3	Procter and Gamble, 1981, Ref. 27
	Rabbit	Effects on cornea, iris, and conjunctivae	10 µL liquid handsoap (0.15% TCC)	Non irritating $MAS_{unrinsed}$ = 16.7 MAS_{rinsed} = 0.7	Procter and Gamble, 1987a, Ref.: 29
	Rabbit	Effects on cornea, iris, and conjunctivae	10 µL liquid handsoap (0.15% TCC)	Non irritating $MAS_{unrinsed}$ = 28 MAS_{rinsed} = 0	Procter and Gamble, 1987b, Ref.: 30

A single dose of 0.1 ml equalling approximately 42 mg Triclocarban (purity 98.8%) was placed in the conjunctival sac of one eye of each of three New Zealand white albino rabbits (Bayer AG, 1992b). Following application, the lids were gently held together for about 1 second to limit loss of test material. After 24h, treated eyes were rinsed with saline solution and evaluated at 1, 24, 48 and 72h and 7, 14 and 21 days for irritation according to the Draize eye irritation grading scale. No effects in cornea, iris, conjunctivae or aqueous humour were observed in any rabbit at any time.

Under the conditions of the test, Triclocarban was therefore considered to be not irritating to the rabbit eye. The study was conducted in compliance with GLP and according to OECD guideline 405.

Ref.: 3

In another study, 20 mg of finely ground powder from two different samples of Triclocarban containing varying levels of dichloro- and tetrachlorocarbanilide (i.e., sample 1 contained 6 - 8% 4,4'-dichlorocarbanilide and 6 - 8% 3,3',4,4'-tetrachlorocarbanilide; sample 2 contained 15 - 20% 4,4'-dichlorocarbanilide and 15 - 20% 3,3',4,4'-tetrachlorocarbanilide) were placed in the conjunctival sac of the right eye of each of three albino rabbits. Following application, the eyes were rinsed with warm isotonic saline solution after 24h and observed for irritation over a period of several days. The eye irritation index (i.e., EII), calculated according to the method of Draize, was 7.3 for the first sample and 6.6 for the second sample, indicating that the test products were only slightly irritating to rabbit eyes (Monsanto, 1979).

Ref.: 21

In a further study, the relative level of eye irritation of bar soap containing 0.55% Triclocarban in a 10% w/v aqueous solution was tested (i.e, Triclocarban in test-solution 0.055%) (Procter and Gamble, 1981). New Zealand rabbits were treated with 0.1 ml of the test solution which was placed into the conjunctival sac of one eye of each of six rabbits. Four seconds after application the eyes of three rabbits were flushed with lukewarm water. The rabbits' eyes were examined for corneal opacity, iritis and conjunctivitis according to the 'Draize method' (Draize, 1965). Eyes were scored after one, two, three, four, seven, fourteen, and twenty-one days following treatment. In both groups rinsed and un-rinsed it was noted if the rabbits exhibited any response indicative of discomfort upon instillation of test compound. No pain response was noted in any of the treated animals following application and corneal epithelial peeling was observed in the eyes of two animals in the non-flushed group whereas none of the animals exhibited any corneal or iridal involvement when the eyes were flushed. The maximum average score (i.e., MAS) was calculated for each test group which was the highest EII observed for any given observation period. Scoring of the rabbits in the un-rinsed group resulted in a MAS of 19.7. The symptoms in 2 rabbits were cleared in 7 days and in four days in the third rabbit. Rinsing the eye reduced the MAS to 1.3 and days to clear were 1, 2 and 3. The study protocol followed the principles of OECD guideline 405 and was found to be reliable from a scientific point of view.

Ref.: 27, 8

A liquid handsoap containing 0.15% Triclocarban was administered to the eyes of 6 albino New Zealand rabbits to determine the level of eye irritation of the undiluted formulation (Procter and Gamble, 1987a). In this study, 10 µL of the undiluted test substance was applied directly to the cornea of the right eye in six rabbits. The eyes of 3 rabbits were rinsed four seconds after application by spraying 20 mL of lukewarm water gently into the eye. In the un-rinsed group one day after treatment clear discharge, petechiae and corneal dulling over 40% of the eye were noted in all three rabbits. These symptoms were cleared after 3 days in two rabbits and by the fourth day in the third rabbit. In the rinsed group redness of the conjunctivae was noted in only one rabbit one day after application this had cleared by the second day. The other rabbits in the treated rinsed group were without irritation. The MAS in the un-rinsed group was 16.7 and in the rinsed group was 0.7. This indicates that the test product was only minimally irritating in the un-rinsed group with a median of 4 days to clear and non-irritating in the rinsed treatment group

with a median of 1 day to clear. The study protocol followed GLP guidelines and the principles of OECD guideline 405. It was found to be reliable from a scientific point of view.

Ref.: 29

Another liquid handsoap formulation containing 0.15% Triclocarban was evaluated for eye irritation (Procter and Gamble, 1987b) according to the low volume procedure in the fashion described above. Again, 10 µL of the undiluted test substance was placed directly onto the cornea of six New Zealand rabbits, following application the eyes of three rabbits were rinsed with water. The two treatment groups un-rinsed and rinsed were assigned a MAS of 28 and 0, respectively. Symptoms observed in the un-rinsed group at day 1 were a slightly obscured cornea, congestion and swelling of the iris and redness and discharge observed in the conjunctivae. In two rabbits these symptoms had completely cleared by day 3 and in the third rabbit by day 4. In conclusion, the test substance caused no discernable eye irritation in the rinsed group and was only mildly irritating with a median day of to clear of 4 in the un-rinsed group. This study was scientifically sound and performed according to the principles of GLP.

Ref.: 30

3.3.3. Skin sensitisation

Table 4: Summary of human experience data and information (sensitisation)

Study type	Duration	Endpoint	Exposure	Result	References
3-Patch Application Test	24 h, 3 x week	Skin Irritation (0-4 scale)	1%, 0.25%, 0.1% liquid hand soap (0.15% TCC)	1% (1.21 slightly) 0.25% (0.43, mild) 0.1% (0.29, very mild)	Procter and Gamble, 1991 Ref.: 32
3-Patch Application Test	24 h, 3 x week	Skin Irritation (0-4 scale)	2% bar soap (1.5% and 1.35% TCC)	1.5% (1.36 , slightly) 1.35% (1.32, slightly)	Procter and Gamble, 1986 Ref.: 28
Continuous patch daily renewal	21 days	Skin irritation (0-4 scale)	0, 1, 3, 9% TCC in petrolatum	No irritation	Maibach *et al.*, 1978 Ref..: 16
Single patch	48 hours	Skin irritation	1, 3, 9% TCC in petrolatum	No irritation	Maibach *et al.*, 1978, Ref. : 16
Phototoxicity	1h	Skin irritation	9% TCC in petrolatum	No irritation	Maibach *et al.*, 1978, Ref.: 16
Repeated Insult Patch test (Shelanski method)	24h Induction, 24h rest, 24h hour reapplication, 2 weeks rest, 24h challenge	Skin sensitization	Neat TCC	No primary irritation, no fatiguing and no skin sensitization	Monsanto, 1963 Ref.: 20
Human Repeat Insult Patch Test (HRIPT)	3 week induction phase, 2 week rest and 24h challenge application	Skin sensitization	5 % bar soap (1.2% TCC)	No skin sensitization	Procter and Gamble, 2000a Ref.: 36

Study type	Duration	Endpoint	Exposure	Result	References
HRIPT	3 week induction phase, 2 week rest and 24h challenge application	Skin sensitization	10% bar soap (1.2% TCC)	No skin sensitization	Procter and Gamble, 1999a Ref.: 34
HRIPT	3 week induction phase, 2 week rest and 24h challenge application	Skin sensitization	10% bar soap (1.2% TCC)	No skin sensitization	Procter and Gamble, 1999b Ref.: 35
HRIPT	3 week induction phase, 2 week rest and 24h challenge application	Skin sensitization	10 % bar soap (1.2% TCC)	No skin sensitization	Procter and Gamble, 2000b Ref.: 37
HRIPT	3 week induction phase, 2 week rest and 24h challenge application	Skin sensitization	5 % bar soap (0.95 % TCC)	Mild primary irritation, no skin sensitization	Procter and Gamble, 1997 Ref.: 33
Repeated Insult Patch Test (Draize method)	3 week induction phase, 2 week rest and 24h challenge application	Skin sensitization	1.5 % and 10% TCC in petrolatum	No skin sensitization	Marzulli and Maibach, 1973 Ref.: 18
Repeated Insult Patch Test (modified Draize method)	3 week induction phase, 2 week rest and 24h challenge application	Skin sensitization	9% TCC in petrolatum	No skin sensitization	Maibach *et al.*, 1978 Ref.: 16
Diagnostic patch testing in dermatitis patients	Routine patch testing (probably 48h)	Skin reaction	1% TCC in petrolatum,	1 positive of 2200 tested	Maibach *et al.*, 1978 Ref.: 16

Triclocarban (purity 98.8%), formulated as a suspension with Cremophor EL® (2% v/v) in physiological saline solution, was evaluated in the Magnusson-Kligman guinea pig maximisation test (Bayer AG, 1992d). The study included a treatment group with 20 male animals and 2 control groups of 10 animals each. In the induction phase, the treatment group was injected (0.1 ml) in a row on each side of the vertebral column on day zero in duplicate with: 1:1 mixture Freund's complete adjuvant (FCA) and physiological saline solution; 5% test substance formulated with Cremophor solution and 5% test substance in a 1:1 mixture FCA. The animals in the control groups were treated in the same way, except that the formulations for injections 2 and 3 contained a corresponding amount of Cremophor solution instead of the test substance. A week later, a patch containing 0.5 mL of a 50% solution of the test substance was placed over the injection area for 48 hours in the treatment group. The control groups were treated in the same manner but with 0.5 mL Cremophor solution instead of the test substance. Three weeks

after the induction phase, the back and the flanks of the treated and the control animals were shaved and an occlusive 'challenge' patch containing a 50% test substance formulation as a suspension with Cremophor EL® (2% v/v) in physiological saline solution (or Cremophor EL® (2% v/v) in physiological saline solution in case of the control group) was applied to the left flank of the animals for 24h. Forty-eight hours and 72h after challenge, any observed skin reactions were recorded according to the Magnusson-Kligman grading scale. Under the test conditions, Triclocarban did not cause skin sensitization in guinea pigs. The study was conducted in compliance with GLP and according to OECD guideline 406.

Ref.: 5

Unkovic *et al.* (1988) studied the photosensitisation potential of Triclocarban in guinea pigs. The test compound was applied to the left side of animals' (six to ten per group) backs, the right side receiving the solvent (control). Animals were then irradiated with either both UVB and UVA or UVA alone, the energy used being equal to the minimal erythema dose or fractions of it. Erythemateous reactions were read 24h after first treatment to determine phototoxicity. Induction of photoallergy consisted of six successive treatments and irradiations, followed by a 3-week recovery period after which a non-phototoxic challenge dose was administered to determine photoallergy. At a concentration of 4% Triclocarban did not exhibit a photoallergic activity in guinea pigs.

Ref.: 41

Vaginal irritation

The potential irritant effect of barsoap containing 0.95% Triclocarban and 0.05% Triclosan (i.e., TRS) was evaluated as a 10% aqueous solution in the vaginal tissue of New Zealand White rabbits and compared with the same barsoap formulation not containing Triclocarban or Triclosan (Procter and Gamble, 1998). Five female rabbits received a 1 ml dose of the diluted test material in the vaginal vault for 10 consecutive days. After the exposure period the test animals were euthanized and the vaginal tissue was removed for histopathological examination. Microscopic slides were evaluated for oedema, vascular congestion, leukocytic infiltration and the epithelium structure was graded. Focal erosion was observed in one rabbit whereas vascular congestion, oedema and leukocytic infiltration never exceeded the score of mild in any of the tested rabbits treated with the formulation containing Triclocarban and Triclosan. Similar severities of observations were recorded for the rabbits in the group given the formulation not containing Triclocarban and Triclosan. In summary, the composite group average score for the vaginal tissue treated with the test product containing Triclocarban and Triclosan was 6.3 whereas in the barsoap without these ingredients the score was 5.3. The composite group scores represented mild irritation to the acolumnar epithelial lined anterior vagina of the rabbit and were considered within the acceptable range for this test in both treatment groups. Similarity in response to the two products indicates that other ingredients in the formulation than Triclocarban could be responsible for eliciting a very mild irritation response. This study was conducted according to GLP and the protocol followed USEPA guidelines for this test.

Ref.: 31

Human data

The allergic contact sensitization potential of Triclocarban upon human skin has been tested according to the Shelanski Repeated Insult Patch Test Method (Monsanto, 1963). Fifty human volunteers were treated on the back with 15 semi-occlusive patches containing approximately 50 mg of undiluted Triclocarban for 24 hours. The patches were then removed and the sites examined for skin irritation reactions. After a 24 hours rest, the patches were reapplied for 24 hours and the sites examined on removal. After a two week rest period, a final 24 hour challenge application of 50 mg undiluted test substance was made. All applications were made to the same site on the skin of each subject. There were no observed reactions on any of the fifty human test subjects, to either of the 15 primary applications or the challenge applications. From the results obtained in the study it was concluded that Triclocarban was neither a primary irritant, nor a skin sensitizer to any of the fifty subjects tested. This may be due to the lack of solubility of the test substance.

Ref.: 20

Ninety-seven human volunteers were treated with patches of a 5% w/v aqueous solution of bar soap containing 1.2% Triclocarban, resulting in a concentration of 0.06% Triclocarban under the patches (Procter and Gamble, 2000a). The patches were applied under semi-occlusive conditions, for 24 hours, three times a week at the same skin site, for 3 weeks during the induction period. After a two week rest period, a final 24 hour challenge application of the test material was made and read after 48 and 96 hours. An acceptable level of irritation was observed throughout the study.
At challenge there was no evidence of skin sensitization in any of the 97 human subjects who completed the test.

Ref.: 36

Hundred and two human volunteers were treated with patches of a 10% w/v aqueous solution of bar soap containing 1.2% Triclocarban, resulting in a concentration of 0.12% Triclocarban under the patches (Procter and Gamble, 1999a). The patches were applied on the back under semi-occlusive conditions, for 24 hours, three times a week at the same skin site, for 3 weeks during the induction period. After a 10-14 days rest period, a final 24 hour challenge patch was applied on the same site on the skin of each subject, and read after 48 and 72 hours. At challenge there was no evidence of skin sensitization in any of the 102 human subjects who completed the test.

Ref.: 34

Hundred and five human volunteers were treated with patches of a 10% w/v aqueous solution of bar soap containing 1.2% Triclocarban, resulting in a concentration of 0.12% Triclocarban under the patches (Procter and Gamble, 1999b). The patches were applied on the back under semi-occlusive conditions, for 24 hours, three times a week at the same skin site, for 3 weeks during the induction period. After a 10-12 days rest period, a final 24 hour challenge application of the test material was made and read after 48 and 72 hours. At challenge there was no evidence of skin sensitization in any of the 105 human subjects who completed the test.

Ref.: 35

114 human volunteers were treated with patches of a 10% w/v aqueous solution of bar soap containing 1.2% Triclocarban, resulting in a concentration of 0.12% Triclocarban under the patches (Procter and Gamble, 2000b). The patches were applied on the back under semi-occlusive conditions, for 24 hours, three times a week at the same skin site, for 3 weeks during the induction period. After a 10-14 day rest period, a final 24 hour challenge application of the test material was made and read after 48 and 72 hours.
At challenge there was no evidence of skin sensitization in any of the 101 human subjects who completed the test.

Ref.: 37

Hundred and seven human volunteers were treated with patches of a 5% w/v aqueous solution of bar soap containing 0.95% Triclocarban, resulting in a concentration of 0.05% Triclocarban under the patches (Procter and Gamble, 1997). The patches were applied under semi-occlusive conditions, for 24 hours, three times a week at the same skin site, for 3 weeks during the induction period. After a 12-20 days rest period, a final 24 hour challenge patch was applied on the same and alternate site on the skin of each subject, and the skin was evaluated after 48 and 72 hours. Of the 107 subjects completing the study, four exhibited skin responses suggesting primary irritation during the induction period. One subject exhibited moderate erythema with oedema (grade 2) on skin grading day 6. The patch was moved and no further skin responses were noted. The three other subjects exhibited mild erythema (grade 1). During the challenge period one subject exhibited mild erythema at the 48 hour grading period on the alternate site and another subject exhibited primary irritation. At challenge there was no evidence of skin sensitization in any of the human test subjects.

Ref.: 33

In a Draize method test Triclocarban (1.5% and 10%) was applied to the upper portion of the arm of male test subjects (Marzullli and Maibach, 1973). The test was dived into the induction phase (3½ weeks of repeated chemical insults to the skin) then a 2 week incubation period or rest period followed by a challenge phase in form of contact with the test material at 1% Triclocarban for 72h to determine if sensitization had taken place. The skin was then graded according to the Draize criteria for oedema and erythema. There was no evidence of skin sensitization in the 88 volunteers tested and it was concluded that Triclocarban was not a skin sensitizer under the tested conditions.

Ref.: 18

In a modified Draize procedure a single concentration (9%) of Triclocarban was applied to the upper lateral portion of the arm of 185 male subjects (Maibach *et al.*, 1978). Material was applied to the same skin site three times weekly and remained in place either 48 hours (during the week) or 72 hours over the weekend. Each individual was given a total of 10 applications. The induction period (3½ weeks) was followed by a rest phase (2 weeks) and then a challenge patch of 9% Triclocarban was applied for a final 72h contact period and graded at 96 hours. No positive responses were observed in any of the test subjects. This study was not conducted under GLP or OECD protocol, however, the study methodology was reliable and the results were subjected to peer review.

Ref.: 16

In a challenge patch test for further definition of irritancy potential, concentrations of 1, 3 and 9% Triclocarban in petrolatum were applied to the paraspinal skin of 213 white male human subjects (Maibach *et al.*, 1978). Solution (0.1 ml) was applied to patches, patches were left for 48 h and tests subjects were graded 48 hours after removal of the patch. No positive responses were observed in any of the test subjects following treatment with Triclocarban, indicating that the concentrations are not likely to be irritating in diagnostic patch testing.

Ref.: 16

In-use studies

The response of sensitive subgroups to Triclocarban was evaluated in a screening programme with dermatitis patients (Maibach *et al.*, 1978). Members of the International Contact Dermatitis Research group included 1% Triclocarban in petrolatum in their routine test battery for 6 months. Such routine series are used in defining the etiology of suspected allergic contact dermatitis cases. All positive reactions were to be retested with a second patch to verify the results of the initial patch. Patients reacting in the repeat patch test, if any, were to be instructed to apply a Triclocarban use-dilution sample (1.5% Triclocarban in bar soap) twice daily to their cubital fossa for a week. These patients were to be re-examined at that time. Over 2200 dermatitis patients were patch tested with 1% Triclocarban when tested with the standard series. A single patient reacted, and the reaction was reproducible on repeat patch testing. Product-use testing of this individual was negative after 21 days of use.
The overall frequency of response was so low that its further testing in a routine battery was deemed unjustified.

Ref.: 16

3.3.4. Dermal / percutaneous absorption

In vitro

The dermal absorption of ^{14}C-labeled Triclocarban was investigated in static and flow-through *in vitro* skin cell systems using full thickness human newborn and adult as well as a monkey skin. Triclocarban (4 µL) in acetone was applied onto the skin models at a concentration of 27 µg/cm^2. The static cells had a volume of 3.77 ml and an epidermal surface area of 0.126 cm^2. The flow cells had the same surface area and a flow rate of 15 ml/h. At 37 °C, 2.5% of the applied dose was absorbed in human newborn foreskin, 0.60 % in human adult foreskin, 0.29 % in human infant abdominal skin, 0.26 % in human newborn abdominal skin and 0.23 % in human adult abdominal skin. In the monkey adult abdominal skin model 0.25 % of the applied dose was absorbed. In the continuous flow system at 23°C, 6% ± 2.0 % of the applied dose was reported to be absorbed in the human adult abdominal skin model. The latter findings were in good correlation with a human *in vivo* investigation. In this study, ^{14}C-labeled Triclocarban was applied topically to a skin surface area of 500cm² at a concentration of 4 µg/cm². Measuring, urinary excretion of ^{14}C-labeled Triclocarban species over a period of 10 days, it was calculated that about 7.0% ± 2.8% of the administered dose penetrated through the skin (Wester *et al.*, 1985).

Ref.: 43

The dermal absorption of Triclocarban was investigated *in vitro* from a polyethylene glycol 400 solution at concentration of 20 mg/g and from liquid soap at a concentration 10 mg/g using intact and artificially compromised human skin models. A quantity of 0.00035 to 0.0017 % was absorbed through normal skin while 0.00164 to 0.0032 % was absorbed through compromised skin. This corresponds to permeability constants of 1.48 x 10^{-8} to 7.08 x 10^{-8} cm x h^{-1}. Hence, on the basis of these *in vitro* experiments, artificially compromised skin appeared to be twice as permeable as intact human skin (Marty and Wepierre, 1979).

Ref.: 17

Animals

The *in vivo* dermal absorption, distribution and excretion of ^{14}C-labeled Triclocarban were evaluated in rats. A 10% soap solution containing ^{14}C-labeled Triclocarban was administered to the clipped skin area of 10 cm^2 in a single dermal dose at a concentration of 141 nmol/cm². After administration of the single radioactive dose, urine faeces and bile were collected separately during three 24h periods and analysed for radioactivity. The skin of the dosing site and the non-dosed skin were also taken as separate samples. Skin samples were extracted by boiling with acetone and the extract was analysed. Absorption occurred at a constant rate over 72h and the flux was calculated to be 0.15 nmol/cm²/h (Hiles, 1977).

Ref.: 10

Howes and Black (1976) conducted a series of experiments aiming at determining the dermal penetration of Triclocarban in rats and humans (human studies discussed in section 4.1.3). The route and rate of excretion of Triclocarban given by parenteral injection was investigated and compared to that after topical application of Triclocarban in acetone in the rat. In a second part of the study the investigators studied the dermal absorption of Triclocarban in rats and humans under occluded and non-occluded conditions following dermal application of ^{14}C-labeled Triclocarban in soap or dimethyl formamide (i.e., DMF). The data obtained demonstrate that elimination by the rat was rapid and complete principally via faeces. The blood levels after parenteral injection in the rat were low and comparison of radioactivity and chemical determinations suggested rapid metabolism of the Triclocarban. The dermal absorption studies revealed that the absorption of Triclocarban through occluded rat skin was about 6 times greater from DMF carrier (i.e., about 6.2 µg/cm^2) than from soaps (i.e., about 1.0 µg/cm^2). Under non-occluded conditions, the dermal absorption was less and dependent on the concentration applied but independent of duration of contact. No measurable Triclocarban was present in blood and urine samples of volunteers during or shortly after a 28d intensive bathing regimen. The investigators estimated on the basis of their data that about 23% of Triclocarban deposited on rat skin was absorbed through the skin after 48h. The authors further estimated that the rat skin is between four and seven times more permeable to Triclocarban than human skin.

Ref.: 14

Humans

In humans, absorption of Triclocarban through skin after bathing with a Triclocarban-containing soap for 28 days has been investigated by Howes and Black (1976). The soap used in this study contained 10% sodium alkoyl isothionate and 2% Triclocarban. The bathing regime was to immerse the body up to the neck when lying in a domestic bath for 5 minutes in water at 40ºC. A

thorough lathering by applying the soap bar directly to the body for 2-3 minutes to produce a stiff foam over the entire body while standing in the bath was followed by a 5 minute immersion in the bath. The regimen was followed for 28 days and blood samples were taken on the morning of day 0, 14, and 28. Urine was collected on days 0, 7, 14, 21, 28. A total of 12 volunteers participated in this trial. At a detection limit of 25 ppb, no Triclocarban was detected in blood and urine in any of the samples.

Ref.: 14

The dermal absorption and metabolic disposition of Triclocarban was studied in a well designed and conducted body showering study by Scharpf *et al*. (1975). Prior to the showering experiment, a single intravenous doses of trace amounts of ^{14}C-labeled Triclocarban in propylene glycol was given to six healthy male human panellists to determine the pharmacokinetic properties of Triclocarban. The radioactivity was rapidly cleared from blood with a blood clearance half life time of 8.6h. About 54% of the dose was excreted in the faeces and 21% of the dose in the urine with a urinary half life time of 10h. In the main part of the study, six adult male volunteers took a single shower employing a whole body lather with approximately 7g of soap containing 2% ^{14}C-labeled Triclocarban for a total lathering time of 2.5 minutes prior to rinsing off the soap. Urine and faecal samples from all panellists were collected prior to the study to establish the baseline and over a 20-day period after the experiment. Care was taken to prevent contamination with radioactivity. About 0.23% of the applied radioactive dose was recovered in the faeces after six days and 0.16% of the dose in the urine after two days. Hence, the maximum amount of radioactivity absorbed was determined to be 0.39% of the totally applied dose. At all sampling times blood levels of radioactivity were below the detection limit of the method (10 ppb).

Ref.: 39

In another study, 37 kilobecquerel of ^{14}C-labeled Triclocarban dissolved in acetone was applied topically to a skin surface area of 500 cm² of five human subjects at a concentration of 4 µg/cm². Urine was collected for 10 days and the dermal absorption was determined by the ratio of urinary excretion of radiolabeled Triclocarban following topical and intravenous administration. On the basis of urinary excretion of ^{14}C-labeled Triclocarban species, it was calculated that about 7.0% ± 2.8% of the totally administered dose penetrated through the skin (Wester *et al.*, 1985).

Ref.: 43

3.3.5. Repeated dose toxicity

Table 5: Summary of repeated dose toxicity

Study type	Species	Endpoint	Exposure	Result	Reference
Subchronic (30 d) oral gavage	Rat	Subchronic toxicity	0, 500, 1000 mg/kg bw/d	NOAEL > 1000 mg/kg bw/d	Monsanto, 1960, Ref.: 19
Subchronic (8 w) dietary feeding	Rat	Subchronic toxicity	25, 75, 250 mg/kg bw/d	NOAEL = 75 mg/kg bw/d LOAEL = 250 mg/kg bw/d	Monsanto, 1985, Ref.: 24
Chronic (2 years) dietary feeding	Rat	Carcinogenicity and chronic toxicity	0, 25, 75, 250 mg/kg bw/d	NOEL = 25 mg/kg bw/d LOEL = 75 mg/kg bw/d Not carcinogenic	Monsanto, 1981 Ref.: 22

Study type	Species	Endpoint	Exposure	Result	Reference
Chronic (2 year) dietary feeding	Rat	Chronic toxicity	1000, 3000, 10000 ppm	Insufficient study information to establish a NOEL	Wright *et al*., 1975 Ref.: 44

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

Sprague-Dawley rats (10 animals/sex /group) were dosed with a 25% aqueous solution of Triclocarban at 0 (controls), 500 or 1000 mg/kg bw by intubation 5 days a week during 30 days (Monsanto, 1960). Food consumption and body weights were recorded weekly and observations were made for signs of toxicity. After 30 days, representative animals from each group were sacrificed for necropsy. The viscera of the 1000 mg/kg bw and control groups were examined microscopically. The viscera of the 500 mg/kg bw group were held for potential further examination. Based on food consumption, growth data and tissue examination, the NOAEL was determined to be > 1000 mg/kg bw. The study was not conducted in compliance with GLP regulations, but met generally accepted scientific standards.

Ref.: 19

Triclocarban (purity 98.6%) was administered to three groups of 35 Sprague-Dawley rats in their diet at concentrations equivalent to 25, 75 and 250 mg/kg bw/d for 8 weeks (Monsanto, 1985). No control group was included in the study. Animals were observed twice daily for morbidity and mortality and once daily for clinical signs. Body weight, food consumption and detailed clinical signs were recorded weekly. Blood samples were taken from 5 animals per group every two weeks for evaluation of blood levels of Triclocarban. No necropsy was performed at the end of the study. There were no signs of toxicity or treatment related mortalities throughout the study. Mean bodyweight and food consumption were lower in the highest dose group, however the statistical significance of this difference could not be evaluated due to the absence of a control group. No compound-related pathological or histopathological findings were noted. The NOAEL and LOAEL were determined to be 75 and 250 mg/kg bw/d, respectively. The absence of a control group, histology of tissues and blood chemistry is a critical weakness in this study.

Ref.: 24

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

No data submitted

3.3.5.3. Chronic (> 12 months) toxicity

Triclocarban was tested in groups of 80 Sprague Dawley rats per sex in a two year chronic feeding study at dose levels of 0, 25, 75 and 250mg/kg bw/d (Monsanto, 1981). Clinical signs, body weight and food consumption were monitored throughout the study. Ophthalmoscopic examinations were conducted at regular intervals. Clinical evaluations (haematology, clinical chemistry and urinalysis) were carried out at 6, 12, 20, 23 (males) and 25 (females) months. At study termination, all animals were subject to complete necropsy and pathological examination. Gross lesions were examined microscopically for possible neoplastic changes. There were no treatment-related clinical signs or mortality throughout the study. Signs of laboured breathing, emaciation and rales as well as increased mortality were observed in particular in control and

treated males in weeks 64 – 86 and 70 – 83, respectively. These were attributed to a respiratory infection present predominantly in males during this time period. No differences were observed between any of the groups with regard to ophthalmic observations or food consumption. Mean body weight of males at 250 mg/kg bw/d and females at 75 and 250 mg/kg bw/d were slightly reduced compared to controls during most of the study. Anaemia was seen in males at 75 and 250 mg/kg bw/d and females at 250 mg/kg bw/d. Blood chemistry analysis showed a slight increase in alkaline phosphatase, blood urea nitrogen, glucose and total bilirubin at various time points for the high-dose males. Urinalysis demonstrated no differences between control and test animals throughout the study. Statistically significant changes were seen in certain organ weights compared to controls. These included increased liver weights in both sexes at 75 and 250 mg/kg bw/d, increased spleen weights at 75 (males) and 250 mg/kg bw/d (males and females), and increased testes and heart weights in males at 250 mg/kg bw/d. No microscopic changes were noted in any of the organs to account for these increased organ weights, therefore the changes may not have been biologically significant. An increase in incidence of small and flaccid testes was observed in males at 250 mg/kg bw/d that died spontaneously or were killed moribund between 12 and 23 months. A similar treatment-related increase was not apparent at terminal sacrifice. There was no evidence for dose-related increases in tumour incidence at any site. Based on these results, the NOEL and LOEL for the study were considered to be 25 and 75 mg/kg bw/d, respectively. The study was performed based on a protocol approved by Food and Drug Administration (i.e., FDA).

Ref.: 22

Wright *et al.* (1975) reported a chronic toxicity study in which rats were fed with a diet containing doses of Triclocarban of 3000 and 10000 ppm resulting in a degeneration of the germinal epithelium lining of the seminiferous tubules, atrophy of the tubules, and oligospermia after 6 months of exposure. No testicular lesions were present in rats fed 1000 ppm (approximately 100mg/kg/day). No other gross, biochemical, haematological, central nervous system or histopathological effects related to Triclocarban were observed in the course of this study. It was concluded that Triclocarban was not carcinogenic in rats that were fed with a diet containing 10000 ppm Triclocarban for 24 months. There was not enough information available to evaluate the quality of the study, nor to establish a NOEL.

Ref.: 44

3.3.6. Mutagenicity / Genotoxicity

Table 6: Summary of available genotoxicity data

Study type	Test system	Endpoint	Result	References
Ames test	*S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537	Mutagenicity	Not mutagenic with or without metabolic activation	Bayer AG, 1992c Ref.: 4
Ames test	*S. typhimurium* strains TA 98, TA 100, TA 1537 and TA 1538	Mutagenicity	Not mutagenic with or without metabolic activation	Bonin *et al.*, 1982 Ref.: 7
Chromosomal aberration test	Chinese hamster ovary cells (CHO)	Clastogenicity	Negative for the induction of chromosomal aberrations with or without metabolic activation	Soap and Detergent Association, 2002 Ref.: 40

In vitro

Triclocarban (purity 98.8%) was evaluated in an Ames test for its potential to induce reverse mutations in the presence and absence of a metabolic activation system (Bayer AG, 1992c). Mutagenicity was evaluated in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA 1537 exposed *in vitro* to 0 (control), 8, 40, 200, 1000 and 5000 µg Triclocarban /plate in test 1, and 0 (control), 125, 250, 500, 1000, 2000 and 4000 µg Triclocarban /plate in test 2. The solvent and negative control was dimethyl sulfoxide. Four plates per strain and dose, both with and without the activation of a metabolizing system (Aroclor 1254 induced rat liver S9 mix) were used. Sodium azide (10 µg/plate), nitrofurantoin (0.2 µg/plate), 4-nitro-1,2-phenylene diamine (10 µg/plate) and 4-nitro-1,2-phenylene diamine (0.5 µg/plate) were used as positive controls for the *Salmonella* strains TA1535, TA100, TA1537 and TA98, respectively. Aminoanthracene (3 µg/plate) was a control for the metabolic activation system. Due to substance precipitation as of 2000 µg/plate, doses of 4000 and 5000 µg were not used for assessment. Doses up to and including 2000 µg/plate did not cause any bacteriotoxic effects. A biologically relevant increase of the mutant count over control levels was not observed. In spite of the low doses used, the positive controls increased mutant counts significantly over the negative control levels, demonstrating the sensitivity of the test system. Therefore, Triclocarban was considered not to be mutagenic with and without S9 mix in the Ames test. The study was conducted in compliance with GLP regulations and according to OECD guideline 471.

Ref.: 4

A further Ames test assessed the mutagenicity of Triclocarban (purity not noted) in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 (Bonin *et al*., 1982). Triclocarban was dissolved in DMSO and a minimum of 2 plates per dose point were tested, with and without metabolic activation (Aroclor 1254 induced rat liver S9 mix). The study concluded that Triclocarban was not mutagenic with and without S9 mix in the Ames test. The study was conducted according to the method described by Ames *et al.* (1975).

Ref.: 7

The ability of Triclocarban (purity 100%) to induce chromosome aberrations was studied in Chinese hamster ovary (i.e., CHO) cells, both in the presence and absence of an Aroclor 1254-induced rat liver S9 activation system (Soap and Detergent Association, 2002). Triclocarban dissolved in DMSO was tested at dose levels of 31.3, 62.5, 125, 250, 500, 1000, 1500 and 2000 µg/mL. Mitomycin C was used as the positive control in the non-activated study and cyclophosphamid as the positive control in the activated study. In the assay, CHO cells were treated for 4 and 20h in the non-activated cells and for 4h in the S9 activated test system. All cells were harvested 20 h after treatment initiation. The cytotoxic concentration was found to be > 3160 µg/mL in both the 4h activated and non-activated test systems and equal to 3160 µg/mL in the 20h non activated system. The percentage of cells with structural or numerical aberrations in the test groups was not significantly increased compared to the solvent control at any dose level in any of the test groups. Based on these findings, it was concluded that Triclocarban was negative for the induction of structural and numerical chromosome aberrations in CHO cells. The study was conducted in compliance with GLP and generally accepted testing guidelines.

Ref.: 40

3.3.7. Carcinogenicity

Table 7: Summary of carcinogenicity data

Study type	Species	Endpoint	Exposure	Result	References
Chronic (2 years) dietary feeding	Rat	Carcinogenicity and chronic toxicity	0, 25, 75, 250 mg/kg bw/d	Not carcinogenic	Monsanto, 1981 Ref.: 22
Chronic (2 years) dietary feeding	Rat	Carcinogenicity and chronic toxicity	3000, 10000 ppm	Not carcinogenic	Wright *et al.*, 1975, Ref.: 44

Two year dietary toxicity study

Triclocarban was tested in groups of 80 Sprague-Dawley rats per sex in a two year chronic feeding study at dose levels of 0, 25, 75 and 250 mg/kg bw/d. The exact details of the study design are reported in 3.3.5.3. In this study, there was no evidence of a dose-related increase in tumour incidence at any site (Monsanto, 1981).

Ref.: 22

Wright *et al.* (1975) reported that there was no evidence of carcinogenicity in a chronic toxicity study in which rats were fed with a diet containing doses of Triclocarban of 3000 and 10000 ppm. There was not enough information available on this study to evaluate the quality and reliability of the reported study results.

Ref.: 44

3.3.8. Reproductive toxicity

Table 8: summary of reproductive and developmental toxicity

Study type	Species	Endpoint	Exposure	Result	Reference
Three generation dietary feeding	Rat	Reproductive and developmental toxicity	0, 25, 500, 1000, 3000 ppm	$NOAEL_p$ = 3000ppm (280 mg/kg/day) NOAEL F_1 = 1000ppm (95 mg/kg/day) NOAEL F_2 = 3000ppm (300 mg/kg/day)	Monsanto, 1983 Ref.: 23
Reproductive toxicity and teratogenicity	Rat and rabbits	Endpoints indicative of reproductive toxicity and teratogenicity	Mixture TCC:TCF (2:1) Oral: 0.1%, 0.2%, 0.25% Dermal: 250, 500, 1000 mg/kg bw/d	LOEL = 0.25% (about 125 mg/kg bw/d) NOEL < 0.2% (about 100 mg/kg bw/d) NOEL> 1000 mg/kg bw/d	Nolen and Dierckman , 1979 Ref.: 26

Three generation reproductive toxicity

The long-term effect to rats of Triclocarban was tested in a three-generation reproduction study. At least 60 days before mating and continuously thereafter, Triclocarban was administered in the diet of groups of 12 male and 24 female Charles River CD rats at dietary levels of 0, 250, 500, 1000 and 3000 ppm. The highest mean test substance uptake were observed in 6 week old females during the growth phase (8 weeks) and the uptake was similar across different generations (250 ppm – 23 mg/kg/d, 500 ppm – 50 mg/kg/d, 1000 – 95 mg/kg/d, 3000 – 280 mg/kg/d). Each parent generation was mated to produce two litters. Additionally, a few F2 animals were mated to produce a third litter. Offspring from the second litters of the F0 and F1 parents were selected to be parents for the subsequent generations. The second (F2) and third (F3) generations received the test substance for an 80-day growth period before mating, then continuously thereafter. Observations for adverse effects and mortality were carried out twice weekly during the study. Detailed physical examinations, body weight and food consumption were recorded weekly. All animals dying spontaneously in the course of the study or terminated due to moribund condition were examined and tissues preserved in 10 % formalin. Dead or stillborn pups were given a gross post-mortem examination and preserved in 70% ethanol. Offspring not selected to be parents for future generations and offspring from the first litter of each generation were given a gross external and internal evaluation; tissues were then taken and saved from randomly selected pups (10/sex/group) for each interval. Also, randomly selected first litter pups from the third generation (F3) were given a gross post-mortem examination and tissues were evaluated microscopically from the control and 3000 ppm dose groups.

At sacrifice, all adult generations (F0, F1 and F2) were given a gross post-mortem examination and selected tissues were preserved. Subsequently, the tissues from the adults (10/sex) in the control and 3000 ppm dose groups were evaluated microscopically. Throughout the study, there were no treatment-related clinical observations or mortality in the adult generations and no adverse effects on body weight or food consumption during the growth and between mating rest periods. No consistent trend suggestive of an adverse treatment effect was seen in body weight during the several gestation/lactation phases of the study.

Mating indices and male fertility indices were not adversely affected by treatment in any of the generations. Pregnancy rates in the 250 and 1000 ppm dose groups were comparable to controls for all litter intervals. The pregnancy rate was unusually low at 3000 ppm during the second litter interval of the F1 generation. At all other intervals, pregnancy rates were comparable between the high dose group and controls. To further evaluate possible effects on fertility in the 3000 ppm dose group, a special mating study was conducted in which control and 3000 ppm dose level animals (males and females) that did not demonstrate fertility during the second litter interval were retained on study and co-housed with untreated females or in-house breeder animals. During this special mating study, two of three high-dose males and seven of ten high-dose females demonstrated fertility.

The evaluation of reproductive toxicity included observations like the length of gestation, pup viability, litter size at birth, litter survival indices, pup growth, and survival to weaning. The data retrieved for the groups treated at 250 and 1000 ppm were comparable to controls. At the highest dose level, the mean number of live pups at birth was lower than controls for both litter intervals of the F_0 generation. A similar trend was not observed in the F_1 and F_2 generation. At day 21, reduced mean pup weight was observed for both litter intervals F_0 generation. A similar, but statistically not significant observation was made for the litters of F_1 and F_2 generation.

Gross post-mortem of weanlings did not reveal treatment-related effects. Spleen and liver weights of second litter F3 weanlings as of 1000 ppm and the kidney/bodyweight ratio at 3000 ppm were lower than controls. Histological examination of kidney sections from first litter F1 weanlings showed varying degrees of effects as of 500 ppm. The only compound-related histopathological change in second litter F3 weanlings was splenic congestion in females at 3000 ppm. Organ weights for the adult generation were similar to controls at 250 ppm. As of 500 ppm, differences were seen in absolute and relative spleen, kidney, liver, adrenal, heart and/or pituitary weights. Histopathological evaluation of selected tissues from adult animals at 3000 ppm revealed effects in the spleen, liver, kidneys and bone marrow. Histopathology examinations of target organs were not conducted at dose levels below 3000 ppm as these were evaluated in an earlier 2-year chronic feeding study (Monsanto, 1981).

Ref.: 22

In conclusion, signs of systemic toxicity such as changes in absolute and relative organ levels were observed in the adult generations at dose levels above 500 ppm equalling a daily exposure of about 50 mg/kg bw. The no observed adverse effects level (i.e., NOAEL) for reproductive and developmental toxicity were determined to be 3000 ppm (i.e., approximately 280 mg/kg/d) for the F_0 generation, 1000 ppm (i.e., approximately 95 mg/kg/d) for the F_1 generation and 3000 ppm (i.e., approximately 300 mg/kg/d) for the F_2 generation. Although the study did not follow GLP or OECD guidelines, it was described in sufficient detail and met generally accepted scientific methods (Monsanto, 1983).

Ref.: 23

Other studies

Nolen and Dierckman (1979) studied the reproductive toxicity of a 2:1 mixture of Triclocarban and 3-Trifluoromethyl-4,4'-dichlorocarbanilide (TCF) in rats and the teratogenicity of the same mixture in rabbits. Charles River CD rats, 21-23 days old were fed with a diet containing 0.25% of the 2:1 mixture of Triclocarban and TCF from weaning until each was bred three times. There were significant reductions, compared to controls in the number of animals that conceived, in the numbers of pubs born to those that did conceive, in the numbers of pups that survived until weaning, and in their body weights at weaning. Dietary concentrations of 0.2% and less of the mixture did not cause these effects. None of the treatments, including feeding the mixture as 0.25% of the diet only during days 6-15 of gestation were teratogenic. In another experiment in this series of studies, pregnant New Zealand rabbits were given a 2:1 mixture of Triclocarban and TFC as topical doses of 250, 500, or 1000 mg/kg bw/d or as oral doses of 50, 100, or 250 mg/kg/d on day 7 – 18 through gestation. The topical treatments elicited no adverse response beyond very mild skin irritation at the application site. The oral doses caused dose-related evidence of maternal toxicity, including weight losses, abortions, and deaths.

Ref.: 26

Wright *et al.* (1975) reported a chronic toxicity study in which rats were fed with a diet containing doses of Triclocarban of 3000 and 10000 ppm which resulted in a degeneration of the germinal epithelium lining of the seminiferous tubules, atrophy of the tubules, and oligospermia after 6 months of exposure. No testicular lesions were present in rats fed 1000 ppm (approximately 100 mg/kg/day). No testicular lesions were present in monkeys given 300

mg/kg/day orally for 90 days, in rabbits treated dermally with 40 mg/kg/day for 90 days, or in mice treated dermally with 600 mg/kg on alternate days for 18 months. Triclocarban showed no evidence of teratogenic or feticidal activity. There was no evidence of teratogenicity. There was not enough information available to evaluate the quality of the study, nor to establish a NOEL.

Ref.: 44

3.3.9. Toxicokinetics

Animals

The absorption, distribution and excretion of Triclocarban was evaluated in rats given a single oral, intravenous or dermal dose of ^{14}C-labeled Triclocarban. With each route of administration more than 65% of the absorbed radioactivity was eliminated in the bile during the first 72h after dosing. The amount of Triclocarban in the organs was distributed as follows at 72h: highest content in the liver then kidneys and then lungs and testes. The investigators concluded that there were enough similarities between intravenous, oral and dermal exposure with respect to elimination routes and tissue distribution to justify the conclusion that oral exposure could be used in toxicity evaluations and still be relevant to dermal exposure. Between 30 and 60% of the material eliminated in into the gastro-intestinal tract was reabsorbed. Triclocarban was suggested to be metabolised extensively, but there was evidence that cleavage of the C-N bond was not involved to a detectable extent (Hiles, 1977).

Ref.: 10

Warren *et al*. (1978) investigated the metabolism and excretion of ^{14}C-labeled Triclocarban in the rat after oral administration and subsequent oral intubation. Male Charles River rats were fed a diet containing 2000 ppm Triclocarban for ten days. After fasting the animals for about 24h, a solution containing ^{14}C-labeled Triclocarban in propylene glycol was administered orally by intubation. Urine and faeces were collected for 5 days. The majority of the radioactivity recovered was found in the faeces (i.e., 77% of administered dose). Less than 6% of the administered dose was detected in the urine. The major faecal metabolite was a result of ortho-hydroxylation on the monochlorophenyl ring and was found at a level three times that found for the meta-hydroxylated metabolite. The same quantitative relationship was found for dihydroxylated metabolites. Despite the fact that the total metabolite fraction varied significantly between the test animals, the relative amounts of one metabolite to another were consistent. The pattern of metabolite excretion differed in the urine and faeces. However, because <6% of the dose was excreted in the urine, it has only little relevance to the overall metabolism. The majority of the metabolites found in the urine were conjugated, but the exact amounts were not quantified. The majority of the faecal metabolites were, however, not conjugated. The latter was demonstrated by incubating Triclocarban with rat faecal homogenates. In this experiment, no alterations were detected.

Ref.: 42

Rats fed with ^{14}C-labeled Triclocarban containing diet at doses between 2 and 318 µmol/kg bw/d showed disproportionately greater plasma levels of Triclocarban-derived materials at intake levels greater than 60 µmol/kg bw/d than would have been predicted if a linear relationship exists between them (Hiles and Birch, 1978). This non-linear response was primarily due to disproportionate increases in the 2'-Hydroxy-triclocarban-sulfates, in 2',6-Dihydroxy-

Triclocarban-sulfate and in a metabolite that was suspected to be strongly bound to protein. Glucuronide conjugates increased proportionately with dose. No free, unmetabolized Triclocarban was found. Despite the disproportionalities in plasma response, the concentration of all metabolites in the bile, which was the major excretory route, increased proportionately with dose. Tissue concentrations of Triclocarban derived materials increased in a nonlinear manner, but not to the same extent as did the plasma concentrations. This nonlinear step suggested saturation in the metabolism. The kinetics of elimination of Triclocarban metabolites from the plasma were mono-phasic first order ($t_{1/2}$ = 50-60hr) at levels < 60 µmol/kg/day and biphasic first order ($t_{1/2}$ = 5-12hr and $t_{1/2}$ = 50-60hr) at higher intake levels. The fraction of Triclocarban metabolites lost by the rapid phase increased with increasing dietary intake levels above 60 µmol/kg/day and was due to the sulphate conjugate. The slower phase resulted from the removal of the metabolite which was strongly bound to protein. No saturation process was observed which could explain the nonlinear relationship between dietary level and steady-state plasma concentration.

The metabolism and disposition of intravenously administered radioactive labelled Triclocarban was investigated in adult and newborn rhesus monkeys. In the adult animals, the major metabolic reactions were the formation of the N-glucuronide or hydroxylation of phenyl ring followed by conjugation with glucuronic acid or sulfuric acid. Major urinary metabolites were the N-glucuronides of Triclocarban. Tissue residues of ^{14}C were low and limited to liver, kidneys and lungs. The bile was the major route of elimination with glucuronide conjugates as the major component. Enterohepatic circulation was extensive. The newborn monkey also metabolized Triclocarban by N-glucuronidation or hydroxylation. Plasma kinetics and tissue distribution were similar to adults. It was concluded that the infant monkey could readily metabolize and eliminate Triclocarban (Hiles *et al*., 1978).

Ref.: 13

Humans

Investigations by Birch *et al*. (1978) were aimed at identifying and comparing the metabolites of Triclocarban after oral exposure in rats, monkeys and humans. Adult male rats were fed with a diet containing ^{14}C-labeled Triclocarban at a dose of 100 mg/kg/d for 5 consecutive days. On the 5th day, urine was collected from the bladders of the rats. Following anaesthetisation of the animals, bile was collected from their common bile ducts for 30 minutes and plasma was collected by venipuncture. Urine was collected from an adult Rhesus monkey that had received 10 consecutive daily oral doses of 25.5 mg Triclocarban per kilogram body weight suspended in methyl cellulose. Plasma and bile were obtained from adult male rhesus monkeys that received ^{14}C-labeled Triclocarban by intravenous infusion over 10 hours at a rate of 0.18 mg Triclocarban/kg/h. Plasma was collected by venipuncture near the end of the infusion period and bile was collected from the gall bladder at necropsy immediately after the infusion period. Each of six men weighing about 80kg was given a single oral dose of 0.7 mg ^{14}C-labeled Triclocarban per kilogram body weight dissolved in corn oil. Urine was collected over a period of 4 hours and plasma was sampled by venipuncture, 3 hours after dosing. Radioactive materials in the plasma and urine of all three species and in the bile of rats and monkeys were separated by high performance liquid chromatography. Table 1 summarizes the results of this comparative investigation. From the studies by Birch *et al.* (1978), it can be concluded that Triclocarban is extensively metabolised to compounds that are more water soluble and hence more readily excreted than the parent compound. Both direct conjugation and hydroxylation followed by conjugation are reactions that are generally associated with detoxification. It was observed that

human and rhesus monkey urine and plasma contained the same major metabolites. The rat, unlike the human and monkey, did not produce significant amounts of the N-glucuronides of Triclocarban. In as much as these glucuronides were the major urinary metabolites in the human and monkey, their absence in rat urine indicates that there were differences in rats' metabolism of Triclocarban to that of humans' and monkeys'.

Ref.: 6

Table 9: Metabolites identified by Birch *et al.* (1978), Ref.:6

Sample	Identified conjugate	Chromatographed radioactivity (%)[1)]	Hydrolyzed by Glucuronidase (%)	Hydrolyzed by Sulfatase (%)
Human urine	- TCC	46	100	
	- TCC	48	100	
Monkey urine	- TCC	44	82	
	- TCC	34	82	
Rat urine	Not examined	Not examined	Not examined	Not examined
Human plasma	- TCC	4	74	
	- TCC	3	74	
	- 3'-OH-TCC	3	0	77
	- 2'-OH-TCC; 6-OH-TCC[2)]	62	0	72[2,3)]
	- TCC[3)]	5		
Monkey plasma	- Not identified	0-10[5)]	0	0
	- Not identified	0-3[5)]		
	- Not identified	7	0	0
	- 3'-OH-TCC	10	0	96
	- 2'-OH-TCC; 6-OH-TCC	35	0	89
	- TCC[4)]	11		
Rat plasma	- 2',6-diOH-TCC	25	0	26
	- 2'-OH-TCC	51	0	76
Human bile	Not examined	Not examined	Not examined	Not examined
Monkey bile	- Not identified		3	4
	- TCC	6	78	
	- TCC	7	78	
	- 3'-OH-TCC	15	86	
	- 2'-OH-TCC; 6-OH-TCC	37	98	
	- 3'-OH-TCC	13	1	89
	- Not identified	2	3	9
Rat bile	- 3'-OH-TCC	9	92	
	- 2',6-diOH-TCC	12	87	
	- 2'-OH-TCC; 6-OH-TCC	49	95	
	- 3'-OH-TCC			
	- 2'-OH-TCC; 6-OH-TCC	7 4	0 0	60 70

[1)] Percentage of radioactivity applied to column that is contained in specific peak.
[2)] With addition of α-amylase containing arylsulfatase; both 2'-OH-TCC (46% of hydrolysate) and 6-OH-TCC (3% of hydrolysate) were identified as metabolite conjugates.
[3)] Upon repeated treatment with Glusulase. Only 2'-OH-TCC (99% of hydrolysate by RID) was found.
[4)] Present in unconjugated form.
[5)] The amounts of these peaks varied from sample to sample.

Hiles and Birch (1978a) conducted further studies in humans.
In their investigation, male volunteers received an oral dose of ^{14}C-labeled Triclocarban and Triclocarban in corn oil at a total dose of 2.2 μmol Triclocarban per kilogram body weight. Urine, blood and faeces were sampled over a period of 10 days and analysed for the radioactivity and metabolites. In summary, faecal elimination (i.e., 70% of dose) was completed after 120h and the urinary excretion (i.e., 27% of dose) of Triclocarban and its metabolites was completed in 80h after dosing. Biotransformation of Triclocarban was rapid, but did not appear to involve splitting of the basic structure. The major plasma metabolites in humans were the N and N'-glucuronides which were eliminated with a half-life time of about 2h to the urine. 2'hydroxy-Triclocarban sulphate and 6-hydroxy-Triclocarban sulphate were removed with a half-life of about 20h presumably into the bile. From the elimination data one could conclude that oral absorption of Triclocarban was low. However, it appeared that the bile was just an important route of Triclocarban metabolic elimination as could be confirmed by observations that after an intravenous dose of ^{14}C-labeled Triclocarban in humans only 21% of the radioactivity was found in the urine, whereas 54% was in the faeces.
Thus, the authors concluded that on the basis of their oral exposure studies, absorption must have been greater that 27%.

Ref.: 11

Plasma and urine samples of human subjects using Triclocarban-containing bar soap were analyzed. The major metabolites found in the urine were the glucuronides of Triclocarban, with typical levels averaging 30 ng/ml. The major plasma metabolite was found to be the sulfate of 2'-OH-TCC with levels ranging from 0-20 ng/ml. No evidence of the presence of 3'-OH-Triclocarban or 6-OH-Triclocarban was observed (Gruenke *et al*., 1987).

Ref.: 9

Conclusion

Triclocarban was moderately absorbed via the oral route and, depending on the vehicle and exposure conditions, poorly to moderately absorbed by dermal application. On the basis of a number of ADME investigations in various species, it was estimated that the absorption following *oral* exposure in humans must be greater than *27 %.* The dermal exposure of Triclocarban contained in various vehicles such as acetone, DMF or cleanser was studied and quantified for humans or rats. Generally, it was suggested that the rat skin is between 4 to 7 times more permeable to Triclocarban than human skin. In *humans*, it was estimated that without a rinsing step about *7 %* of topically applied Triclocarban dissolved in acetone was absorbed and systemically available. In a typical rinse-off scenario mimicked by a showering study with human subjects using Triclocarban containing bar soap, about 0.39% Triclocarban of the total applied dose was systemically available. *In vitro* studies with intact and artificially compromised human skin models suggested that compromised human skin was about 2 times more permeable than intact skin.

In all species investigated, Triclocarban was extensively metabolized to compounds that were more water-soluble and hence, more readily excreted than the parent compound. Both, direct conjugation and hydroxylation followed by conjugation were observed. Humans and monkeys show great similarities in their metabolic profile. The principal metabolites common to all species were the sulphate and glucuronide conjugates of 2', 3'- and 6-Hydroxy-Triclocarban. The rat also produced the glucuronide and sulphate of 2',6-Dihydroxy-Triclocarban. In none of the species examined, the C-N bond in Triclocarban was cleaved as a result of metabolism.

While the major plasma and urinary metabolites in humans and monkeys were the N and N'-glucuronides of Triclocarban, rats did not produce a significant amount of N-glucuronides. In humans, the plasma N- and N'-glucuronides of Triclocarban were eliminated with a half-life time of 2h into urine, while the ortho-hydroxy sulfates of Triclocarban were removed with a half life time of 20h, presumably into the bile. The faeces were the major route of excretion of radioactivity in humans after intravenous and dermal absorption of Triclocarban. The ratio of amounts of faecal to urinary excretion of radioactivity was about 2:1. In rats, faecal excretion was also the major route. Depending on the exposure route, about 60 – 90% of Triclocarban was excreted in the faeces.

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

/

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

/

3.3.11. Human data

See elsewhere in the opinion.

3.3.12. Special investigations

/

3.3.13. Safety evaluation (including calculation of the MoS)

Triclocarban containing products are utilized in "rinse-off" scenarios and dermal exposure occurring to the face, hands and whole body during the cleansing process was the major route of exposure. The aggregate exposure to Triclocarban due to the potential use of Triclocarban-containing cosmetic products was calculated to be 0.032 mg/kg bw/d for a 60 kg adult. This exposure estimate considers exposures stemming from uses of Triclocarban in bar and liquid soaps and in body wash products at a maximum concentration of 1.5%. At these levels, the Margin of Safety (i.e., MOS) was calculated to be 778 for a 60 kg adult.

Exposure estimations and calculations of MOS considering individual exposures and aggregate exposures to Triclocarban-containing bar and liquid soap and shower gels are described below. The exposure estimates were based on the highest concentration level per product type in the context of the typical product use. Consumer exposure data from the applicant as quoted by the SCCNFP Notes of Guidance (2000) were utilised.

Aggregate Exposure Assessment

The study conducted by Scharpf *et al*. (1975) was considered to reflect most realistically the in-use exposure (i.e., rinse-off scenario) to Triclocarban resulting from the use of Triclocarban-containing bar and liquid soaps and shower gels. Scharpf *et al*. (1975) determined the intake of Triclocarban via the use of Triclocarban-containing bar soap in a showering experiment with human volunteers. The study was conservatively designed in the sense that prior to conducting the actual experiment, the panellists were asked to lather with a bar soap to remove lipid and sebaceous materials from the skin. On entering the study, each subject applied the ^{14}C-labeled Triclocarban soap solution on the whole body with a total lathering time of 2.5 minutes prior to rinsing off the soap. The investigators estimated that under showering conditions approximately 0.39% of an applied dose of Triclocarban (i.e., 7g bar soap containing 2% Triclocarban) was absorbed and systemically available. Studies conducted by Wester *et al*. (1985) and Maibach *et al*. (1978) determined a human dermal absorption of topically applied Triclocarban of about 7%. However, the exposure conditions chosen (e.g., Triclocarban in acetone, no rinse, 24h exposure time) do not mimic the actual exposure resulting from the use of Triclocarban-containing soaps and shower gels.

Ref.: 39, 43, 16

For the purpose of calculating the dermal exposure to Triclocarban under soap and body gel use conditions, the findings of Scharpf *et al*. (1975) will be used in combination with the consumer exposure data from COLIPA as quoted by the SCCNFP Notes of Guidance. Table A1 presents the default values used for calculating exposures to Triclocarban via its uses in bar and liquid soaps and body wash (COLIPA).

Ref.: 39

Table A1: Default values and assumptions used in dermal exposure calculations

Product	Triclocarban in product[1] %	Amount of product used[2] g/use	Product use Frequency/day[2]	Total product used[2] g/day	Dermal Absorption[3]
Bar Soap	1.5%				
Face		0.3	1	0.3	0.39%
Hand		0.4	10	4	0.39%
Body		3.3	1	3.3	0.39%
Liquid Soap	1.5%	2	10	20	0.39%
Body Wash/ Shower Gel	1.5%	5	1	5.35	0.39%

[1] Maximum use concentration
[2] Use data gathered by COLIPA
[3] Scharpf et al. (1975)

Ref.: 39

The systemic exposure to Triclocarban (TCC) from its use in bar and liquid soap and body wash is calculated according to the following ***Equation I***:

$$\frac{(\%\ \text{TCC in product}) \times (\text{Product uses/day}) \times (\text{Grams product used/use}) \times (\%\ \text{TCC absorbed}) \times CF}{BW}$$

Where: CF = Conversion factor (1000mg/g)
BW = Body weight

A summary of the exposure estimates calculated by *Equation I*, using COLIPA product use scenarios described in Table A1 is provided in Table A2. Exposures were calculated an adult with an assumed body weight of 60kg.

Table A2: Exposure estimates for dermal exposure to bar and liquid soaps and body wash containing Triclocarban

Product	Adult exposure (mg/kg bw/d)
Bar Soap	0.0074
Face	0.0003
Hands	0.0039
Body	0.0032
Liquid Soap	0.0195
Body Wash/ Shower Gel	0.0052
Worst Case: Total aggregate exposure	0.0321

The total aggregate consumer exposure to Triclocarban was calculated from combined use of Triclocarban-containing bar and liquid soaps and body wash. For a 60kg adult, the total aggregate exposure was estimated to amount 0.03 mg/kg bw/d. More than 60% of the total aggregate exposure from Triclocarban stems from the daily use of Triclocarban-containing liquid soaps.

CALCULATION OF MARGIN OF SAFETY

In accordance with the SCCP Notes of Guidance and in the absence of chronic dermal toxicity data, the results of an oral study have been used for the calculation of the Margin of Safety (MOS). The lowest no observed effect level (NOEL) for Triclocarban was determined to be 25 mg/kg bw/d on the basis of a 2 year chronic oral feeding study in Sprague Dawley rats (see section 3.3.5).

To calculate a MOS for dermal exposure, a route-to-route extrapolation was made. The information on absorption after oral administration, i.e. 27%, was used to calculate an internal NOEL. This internal NOEL (25 x 0.27 = 6.75 mg/kg bw/d) is subsequently divided by the estimated dermal maximum systemic exposure dose, as presented in Table A2.

The results of the MOS calculations for the individual as well as the aggregate exposure scenarios are presented in Table A3.

Table A3: Calculated Margins of Safety for uses of Triclocarban in liquid and bar soaps and shower gels

Product	Adults
Bar Soap	
Exposure	0.0074 mg/kg bw/d
MOS	**912**
Liquid Soap	
Exposure	0.0195 mg/kg bw/d
MOS	**346**
Body Wash	
Exposure	0.0052 mg/kg bw/d
MOS	**1298**
Aggregate Scenario	
Exposure	0.0321 mg/kg bw/d
MOS	**210**

Based on the internal NOEL derived from the oral chronic feeding study and with respect to the oral absorption rate of 27%, the Margin of Safety in the aggregate scenario will be 210 for a 60 kg adult.

As recent reports in literature point to a possible persistence of Triclocarban in the environment and despite of the low solubility in water, consideration should be given to the actual concentrations in these respects, followed by an appropriate risk assessment.

3.3.14. Discussion

Acute toxicity

Triclocarban was found to be low acute oral, dermal and intraperitoneal toxicity. The acute oral LD_{50} for Triclocarban was greater than 2000 mg/kg body weight in rats and mice. Triclocarban applied dermally to rabbit skin resulted in an LD_{50} which was greater than 10000 mg/kg body weight. The intraperitoneal application of Triclocarban resulted in a LD_{50} greater than 2100 mg/kg body weight.

Systemic toxicity

The subacute, subchronic and chronic toxicity of Triclocarban was evaluated in a rat oral gavage (i.e., for subacute toxicity) and two rat feeding studies (i.e., for subchronic and chronic toxicity).
No effects were observed in the oral gavage study at the highest dose level of 1000 mg/kg bw/d. In the 8-week study, there were no mortality or signs of toxicity other than a reduced food consumption and a decrease in the mean body weight of the rats of the highest exposure group (i.e., 250 mg/kg bw/d). There were a number of critical weaknesses relative to the study design such as the absence of a control group, histology of tissues and blood chemistry and hence the proposed no observed effect level (i.e., NOEL) of 75 mg/kg bw/d should be treated in light of these study limitations.
In the 2-year chronic feeding study, the rats were fed with a diet containing Triclocarban at doses of 25, 75, and 250 mg/kg bw/d. At the highest administered dose, the investigators observed a reduction in food consumption, mean body weight and some changes in organ weights and the blood chemistry (i.e., increases in alkaline phosphatase, blood urea nitrogen, glucose, and bilirubin at various time points in the male animals). Decrease in food consumption, body weights, and organ weights (e.g., liver, spleen) were also observed in the animals in the mid dose group of 75 mg/kg bw/d. The NOEL was established at 25 mg/kg bw/d and the lowest observed effect level (i.e., LOEL) at 75 mg/kg bw/d.

Reproductive and developmental toxicity

The reproductive and developmental toxicity of Triclocarban was evaluated in a three generation study in rats. At least 60 days before mating and continuously thereafter, Triclocarban was administered to male and female rats at dietary levels of 0, 250, 500, 1000, and 3000 ppm which reflect a maximum dose-range of 20 mg/kg bw/d to 280 mg/kg/d. Signs of systemic toxicity such as changes in absolute and relative organ levels were observed in the adult generations at dose levels above 500 ppm equalling a daily exposure of about 50 mg/kg bw. Under the conditions of the study, the mating indices and the male fertility indices were not adversely affected by treatment in any of the generations. Only the pregnancy rate was observed to be unusually low at 3000 ppm during the second litter interval of the F_1 generation. The no observed adverse effect level (i.e., NOAEL) for reproductive and developmental toxicity were determined to be 3000 ppm (i.e., approximately 280 mg/kg/day) for the F_0 generation, 1000 ppm (i.e., approximately 95 mg/kg/day) for the F_1 generation and 3000 ppm (i.e., approximately 300 mg/kg/day) for the F_2 generation.

Absorption, distribution, metabolism and excretion (ADME)

Pharmacokinetic studies have been carried out with ^{14}C-labeled Triclocarban in various *in vitro* skin cell systems, and *in vivo* in rats and in humans. Triclocarban was moderately absorbed via the oral route and it was estimated that absorption following oral exposure in humans was greater than 27%. After topical application without rinsing, about 7% of the applied dose of

Triclocarban dissolved in acetone was absorbed and systemically available. In a typical rinse-off use scenario with Triclocarban containing bar soap, it was estimated that approximately 0.4% of the topically applied Triclocarban penetrated the skin and was systemically available. On the basis of *in vitro* studies artificially compromised human skin was found to be twice as permeable to Triclocarban as intact skin.
Triclocarban was readily metabolised to more water soluble products by both direct conjugation and hydroxylation followed by conjugation. In humans and monkeys, the principal metabolites were the N and N'-glucuronide of Triclocarban and sulphates of 2',6-dihydroxy-TCC and 6-hydroxy-TCC. The major plasma and urinary metabolites were the glucuronides of Triclocarban which were eliminated in with a half life of 2 hours into the urine, while the hydroxy- and dihydroxy-sulphates were removed with a half life time of 20 hours, presumably into the bile. Most of the orally and dermally absorbed Triclocarban was excreted via the faeces in humans, with the ratio of urinary versus faecal excretion being 1:2.

Skin irritation
The skin irritation potential of Triclocarban has been evaluated in rabbits and guinea pigs under occluded and non-occluded exposure conditions at concentrations from 0.5% to 100% for 4 and/or 24 hours. Observations were made over a period of several days after the exposure and the exposed skin sites were graded for skin irritation according to the Draize scoring scale. Under fully occluded conditions, neither 24h exposure to a 25% corn oil suspension of Triclocarban nor 4h exposure to undiluted Triclocarban resulted in any signs of skin irritation in rabbits. According to the Draize scoring system, Triclocarban was classified by all investigators as non-irritating under the conditions tested.
The potential irritant effect of a 10% aqueous solution of a Triclocarban-containing bar soap was further investigated in the vaginal tissue of New Zealand rabbits. Under the study conditions chosen, the investigators determined a maximum composite group average score of 6.3 which indicated only a mild irritation of the vaginal tissue of rabbits.
The cumulative skin irritation effects of aqueous solutions of Triclocarban-containing bar (i.e., 1.5% Triclocarban) and liquid soaps (i.e., 0.15%) were evaluated under fully occlusive conditions in several human 3-Patch application tests. Under the test conditions chosen, the Triclocarban containing products were only slightly irritating to human skin. In a 21-day cumulative patch test, 9% Triclocarban in petrolatum applied to human skin elicited only a mild skin irritation response.

Eye irritation
The application of Triclocarban as neat product and in liquid soap and bar soap was virtually non-irritating to the rabbit eye, especially if the eyes were rinsed shortly after application of the product. Following exposure, any observed reactions to the eye cleared quickly (longest 7 days after initial application) even when the product was not rinsed from the eye. The neat product was less irritating then soap formulations containing 0.15% Triclocarban to the rabbit eye which leads to the conclusion that the eye irritation potential of soap formulations is not related to its content of Triclocarban. Symptoms observed were restricted to swelling of the iris as well as redness and discharge from the conjunctivae.

Sensitisation
The skin sensitization potential of Triclocarban was evaluated in a Magnusson-Kligman guinea pig maximisation test. In this study, a 2% formulated suspension of Triclocarban was found to be non-sensitizing. The photosensitisation potential of Triclocarban was evaluated in 4% Triclocarban solution and found to be non-photoallergenic to guinea pig skin.

The absence of skin sensitising properties of Triclocarban was further confirmed in a series of human repeat insult patch tests (i.e., HRIPT). Bar soap formulations containing up to 1.5% Triclocarban were tested for skin sensitization in HRIPTs. In none of the studies, there was any evidence for Triclocarban or any another ingredient in the tested bar soap formulations for causing skin sensitisation in humans.

Genotoxicity and carcinogenicity
Triclocarban was not found to be mutagenic in the Ames test or clastogenic in the chromosomal aberration test with and without metabolic activation. The potential for carcinogenicity was further evaluated in a two year rat chronic feeding study in which rats were fed with a diet containing Triclocarban at doses of 25, 75, and 250 mg/kg bw/d. There was no evidence for a dose-related increase in tumour incidence at any site and it was therefore concluded that Triclocarban was not carcinogenic under the conditions of the study.

Establishment of a NOEL for human health risk assessment
For assessing the risk associated with human exposure to Triclocarban in context of its use in antibacterial soaps and deodorant body wash, it was suggested to take the no observed effects level of 25 mg/kg bw/d. This value was the lowest NOEL from all repeated dose toxicity studies and derived from the results of a well conducted 2-year feeding study in rats.

SUMMARY OF THE RISK ASSESSMENT

Triclocarban-containing bar and liquid soaps and shower gels were utilised in “rinse-off” scenarios and dermal exposure occurring to the face, hands and the whole body during the cleansing process was the major route of exposure.
The toxicological profile of Triclocarban indicates that the material has a low order of toxicity, based on a variety of acute, subchronic and chronic toxicity studies. It was neither genotoxic in examined *in vitro* systems, nor did it cause carcinogenic or reproductive/ developmental effects in *in vivo* animal studies. The risk to human health was characterised by dividing the lowest NOEL derived from animal toxicity studies by the estimated maximum systemic exposure. The quotient is generally referred to as the Margin of Safety (i.e., MOS). The worst-case scenario for dermal exposure to Triclocarban from the combined use in bar and liquid soaps and shower gels at levels up to 1.5% led to an estimated systemic exposure dose of 0.032 mg/kg bw/d. Dividing the internal NOEL of Triclocarban of 6.75 mg/kg bw/d (25 mg/kg bw/d derived from a 2-year chronic feeding study and corrected for an oral bioavailability of 27%) by this exposure results in a MOS of 210 for a for 60kg adult.

4. CONCLUSION

In response to the questions asked, the SCCP is of the opinion that that the use of Triclocarban for non-preservative purposes in cosmetic rinse-off hand and body care products up to a maximum concentration of 1.5% does not pose a direct risk to the health of the consumer.

However, the SCCP would like to draw the Commission’s attention to the possible effects of triclocarban to the environment and, subsequently, on human health from such environmental contaminations.

5. MINORITY OPINION

Not applicable

6. REFERENCES

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