



Scientific Committee on Consumer Safety

SCCS

OPINION ON TRICLOSAN

COLIPA n° P32

**ADDENDUM to the SCCP Opinion on Triclosan
(SCCP/1192/08) from January 2009**



The SCCS adopted this opinion at its 10th plenary meeting
of 22 March 2011

About the Scientific Committees

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

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

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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This opinion has been subject to a commenting period of four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

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1. BACKGROUND

Triclosan (CAS 3380-34-5) with the chemical name 5-chloro-2-(2,4-dichlorophenoxy)phenol or 2,4,4'-trichloro-2'-hydroxy-diphenyl ether has been included in the annexes of the Cosmetic Directive since 1986. It is currently regulated as a preservative in Annex VI, entry 25 with a maximum concentration of 0.3%.

A dossier containing both toxicological data and information on the aspect of microbial resistance was submitted in 2007 by COLIPA.

In the resulting opinion (SCCP/1192/08) adopted on 21 January 2009, addressing the toxicological properties of Triclosan, the SCCP concluded:

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

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

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

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

Meanwhile, a preliminary opinion (SCCS/1251/09) on triclosan and antimicrobial resistance was adopted at the SCCS plenary meeting 23 March 2010 with the opinion:

"At present, several distinct hazards have been identified: (i) the effect of triclosan on the triggering/regulation of resistance genes in bacteria (ii) the existence of mechanisms which can promote resistance and cross-resistance to biocides and antibiotics in bacteria, (iii) high concentrations of triclosan have been measured in certain environmental compartments, (iv) the presence of resistance genes in soil bacteria, and, (v) bacterial biofilms are widespread in the environment and are able to survive exposure to adverse environmental factors. The first two of these hazards have been identified in vitro. However, the six in situ studies and the one meta-analysis quoted in this document have failed to demonstrate an increase in antibiotic resistance following triclosan use. While these results are at first sight reassuring, these in situ data are not sufficient to draw a conclusion on whether the continuous use of triclosan is involved in the development of resistance. Thus, additional in situ information is needed to provide an answer on the level of risk. This opinion concerns the safety of triclosan in terms of microbiology, i.e. generation of bacterial resistance harmful for human health. Based on the available scientific information, it is not possible to quantify the risk of development of antimicrobial resistance induced by triclosan applications, including its use in cosmetics. However, there are environmental concentrations in a number of geographically distinct areas high enough to suggest that triggering of bacterial resistance could also occur in the environment. The applications of triclosan which contribute to those high environmental concentrations cannot be properly identified nor quantified at present. This should be taken into account when considering the current and future uses of triclosan in all applications so as to ensure that the demonstrable benefits for human health in certain applications are not compromised.

The current supplementary submission contains the applicant's response to the opinion of January 2009 with the toxicological endpoints and provides arguments for an alternative interpretation of the available safety data on triclosan.

On the same issue a new exposure scenario was submitted by Colipa on e.g. mouthwashes, based on their new exposure study forwarded to the Commission services in September 2009. Colipa applies for an additional use of triclosan in mouthwash products at a maximum concentration up to 0.15%.

Furthermore, another applicant claims that the concentration in mouthwash product should be up to 0.2%. And finally, an applicant has asked for authorisation of an additional use of triclosan in nail cosmetics at a concentration up to 0.3%.

2. TERMS OF REFERENCE

1. *In the light of this supplementary submission and the preliminary opinion on antimicrobial resistance, does the SCCS consider it necessary to revise the toxicological evaluation made by the SCCP in its opinion SCCP/1192/08? If the answer to question 1 is yes, does the SCCS consider a continued use of Triclosan as a preservative in all cosmetic products as safe for the consumers at the current concentration limit of maximum 0.3%?*
2. *Taking into account the safe use of Triclosan at a maximum concentration of 0.3% in toothpaste, hand soaps, body soap/shower gels and deodorant sticks ("common use products"), does the SCCS consider an additional use of Triclosan in mouthwashes as safe for the consumer at a concentration limit of maximum 0.15%, alternative 0.2% taking into account the provided exposure data and the preliminary opinion on antimicrobial resistance?*
3. *Taking into account the safe use of Triclosan at a maximum concentration of 0.3% in toothpaste, hand soaps, body soap/shower gels and deodorant sticks ("common use products"), does the SCCS consider an additional use of Triclosan in nail products as safe for the consumer at a concentration limit of maximum 0.3% taking into account the provided exposure data and the preliminary opinion on antimicrobial resistance?*

3. OPINION

This opinion of the SCCS constitutes an ADDENDUM to the previous SCCP Opinion on Triclosan (SCCP/1192/08) adopted on January 2009.

The additional information forming the basis of this addendum consisted of the following documents:

- Statement from the applicant on uses and supplement I
- New exposure study provided by Colipa
- Exposure through finger - and foot nail cosmetic
- Spanish company's use of triclosan in mouthwashes with 3 articles:
 - Bhargava and Leonard (1996) Triclosan: Applications and safety. *Am J Infect Control* 24:209-218
 - Zuckerbraun *et al.* (1998) Triclosan: cytotoxicity, mode of action, and induction of apoptosis in human gingival cells *in vitro*. *European Journal of Oral Sciences* 106: 628-636
 - DeSalva *et al.* (1989) Triclosan: A safety profile. *Am J Dent.* 2:185-196

Considering the issues raised in this supplementary submission, the SCCS in its subsequent evaluation has focused on the major points, namely

- a) The human **exposure** to triclosan with consumer products, and
- b) The contribution of different product groups to the overall exposure;
- c) The choice of a **NOAEL** for the safety assessment.
- d) New studies on the toxicological or endocrine properties of triclosan

The applicant did not discuss new studies on the toxicity of triclosan in their supplementary submission. Yet, a literature search by SCCS retrieved additional recent studies discussed here with regard to their possible relevance for the safety assessment.

Since the majority of the opinion on triclosan (SCCP/1192/08) is not in question (and will remain the same), the SCCS decided to respond in an "Addendum" to this opinion. In the first part of this text ('Critical Issues'), the major points and issues raised by the applicant are discussed. The second part (following the typical format of opinions) provides an update of some sections or briefly states when sections of SCCP/1192/08 remain as before.

PART I: Critical Issues

Exposure

In a response to opinion SCCP/1192/08, the European Cosmetics Association (COLIPA) claimed (Ref. 1) that triclosan is no longer used in mouthwash at 0.3% by its members, rather only 1 company formulates at 0.15%, the majority at 0.03%.

This would reduce exposure from "common use products" + face powder and concealer + mouthwash and, according to the proposed calculation, result in a MOS of 105.5

However, this proposed calculation of a MOS does not take into account the following:

- a) Information received by the European Commission (Ref. 2) clearly states that one company commercializes two mouthwashes containing 0.2% triclosan in some countries in Europe. Therefore, the SCCP calculation for oral products, specifically an SED from mouthwash with 0.2% triclosan is still valid.
- b) A product group, namely body lotion, was omitted in the applicant's new MOS calculation. These leave-on products contain between 0.15 to 0.3% triclosan, accounting for either 0.0823 or 0.1646 mg/kg bw/d, respectively, according to the SCCP calculation for marginal use products, but SED values for body lotion were not considered in the applicant's calculation.

According to the recent revision of the Notes of Guidance (Ref. 3) some adjustments for the amount of certain products applied and/or frequency of use are indicated, and thus a complete update of the section 3.3.13. is included in part II of this opinion.

In addition, a calculation of *aggregate exposure* through use of various cosmetic product types is given. This table serves to illustrate the need for reducing the overall/total exposure of consumers to triclosan from cosmetic products. By providing values for product types which are major and minor contributors to the total exposure, the table can assist risk managers in choosing options for risk reduction measures.

Critical endpoints - Choice of NOAEL

For the initial safety evaluation of triclosan, the applicant proposed a NOAEL of 48 mg/kg/day, derived from a chronic rat study (based on body weight reduction and increased food consumption in males, changes in clinical chemistry (mostly over the initial 52 weeks of the study), and microscopic liver pathology noted at weeks 13, 52 and 78, although not at the end of the study). The SCCP considered the NOAEL for this study to be 12 mg/kg body weight/day, based on changes in haematological parameters and decreased absolute and relative spleen weights.

In the supplementary submission, the applicant has asked to reconsider the choice of NOAELs by the SCCP. Thus, the SCCS has reviewed the points of critique, and discusses here the most pertinent studies and endpoints for a safety evaluation of triclosan, with a focus on haematotoxicity as well as reproductive and developmental toxicity. In addition, a comment on species differences in the kinetics of triclosan is given, and on a recent proposal to use nephrotoxicity as critical endpoint and apply benchmark responses for further safety evaluations.

1. Haematotoxicity

In the opinion SCCP/1192/08 (page 34) the following rationale was given on the choice of NOAEL:

The derived NOAELs from subchronic and chronic studies in different species were compiled. EPA in its recent evaluation selected the NOAEL of the baboon study (30 mg/kg bw/d) for risk assessment based on clinical signs of toxicity which are presumably due to oral treatment. This might not be relevant for cosmetic uses. The applicant in its safety evaluation used the NOAEL of the 95-week study in hamsters as this species was judged to be the most relevant to humans based on pharmacokinetics (75 mg/kg bw/d). Alternatively as a more conservative value, the NOAEL of the 104-week rat study (\approx 48 mg/kg bw/d for both sexes) was used. SCCP considers the NOAEL of this long term toxicity study in rats as 12 - 17 mg/kg bw/d (\approx 14.5 mg/kg bw/d) due to haematotoxicity and decreased absolute and relative spleen weights. Haematotoxicity was also detected in the 13-week subchronic oral toxicity studies in mice and rats, in hamsters only at higher doses and in the 1-year toxicity study in baboons. This was further confirmed by changes in haematology parameters in the long term studies in mice and hamsters. Interestingly, also in the 13-week subchronic dermal toxicity study in rats changes in erythrocytes

parameters were observed. The SCCP will use the NOAEL of 12 mg/kg bw/d of the long term toxicity study in rats for risk assessment.

The applicant performed a further analysis of the relevant studies (Ref. 4). It was argued that the spleen and haematopoietic findings noted in the long term toxicity rat study do not carry sufficient weight or relevance to establish a NOAEL. Furthermore it was stated that "most importantly human exposure has indicated that the red blood cell system does not appear to be affected by triclosan exposure".

The latter argument is considered not convincing since a) only a limited number of subjects was investigated, b) exposure in the respective clinical studies is orders of magnitude lower than those given in the animal experiments, and c) in one human study indeed haematological changes were reported.

As further defending argument the publication of Muller *et al.* (2006; Ref. 5) was given by the applicant which deals with haematotoxicity. However, this paper specifically outlines criteria for legal classification in the EU (R48 'Danger of serious damage to the health by prolonged exposure') based on anaemia (as a sufficiently serious effect for classification). Yet, these criteria (for classification) cannot be simply transferred to or viewed as prerequisite for setting a NOAEL. Regarding haematotoxicity, the SCCS understands that not every single finding at certain dosages of any study can definitely be associated with triclosan treatment and might be interpreted as such as incidental. But taken together, there is evidence that the haematopoietic system could be a target of systemic toxicity in all species investigated. Furthermore, the SCCP/SCCS assessment is fully in line with the evaluation of EFSA/SCF where also haematotoxicity in the long-term toxicity study in rats was used for derivation of a migration limit for food contact materials.

2. Reproductive and developmental toxicity

The reproductive and developmental toxicology of triclosan has been investigated in teratology studies in the mouse, rat, and rabbit, and a two-generation reproductive toxicity study in the rat. From these studies SCCP in the opinion SCCP/1192/08 derived a NOAEL of 65 mg/kg bw/d based on decreases in foetal body weights and the mean number of live pups.

Since then, a paper was published dealing with testicular steroidogenesis and histopathology of reproductive system in male rats treated with triclosan (Kumar *et al.* 2009, Ref. 6). Wistar rats were orally treated by gavage with 0, 5, 10 and 20 mg/kg bw/d triclosan (about 98% purity) suspended in phosphate buffered saline for 60 days, the group size was 8 animals per group. The weights of testes and accessory sex tissues were significantly reduced at 10 and 20 mg/kg bw/d. At the dose 20 mg/kg bw/d daily sperm production was decreased and histopathological changes were noted in the vas deferens and in the prostate. The underlying mechanism of these changes was discussed as being antiandrogenic based on changes in the expression of the androgen receptor and the testicular steroidogenic acute regulatory protein (StAR) both at transcription and protein level, the activity of testicular steroidogenic enzymes 3 β -HSD and 17 β -HSD, and serum hormone levels. A reduced level of StAR was also indicated by immunohistochemistry in Leydig cells (see also new section 3.3.12.5 in the updated opinion).

Based exclusively on this study, a NOAEL of reproductive toxicity of 5 mg/kg bw/d could be derived. However, some doubts arise on the reliability of the study due to the non-careful writing (e.g. Fig. 4 legend 'increase' instead of decrease) and uncertainty with regard to impurities in the test compound. Furthermore, the strong decrease in testes weights observed at 10 and 20 mg/kg bw/d in this study was not seen in a 90 day study in rats following doses up to 600 mg/kg. In addition, reproduction parameters (fertility) were not influenced in the two generation reproduction toxicity study in rats at doses up to 176 mg/kg bw/d in F0-males (see 3.3.8.1. in SCCP/1192/08). The latter study of 1988 (Morseth

et al.) is consistent with old OECD guidelines; however, parameters such as sperm counts and viability of sperm which are considered nowadays to be useful in such settings (Ref. 7) were not examined at that time. On the other hand, effects on weight and histopathology of gonads were not reported in studies of several species with triclosan of high purity.

Additional studies published since adoption of the SCCP opinion in 2009 have now been included in section 3.3.12.4 of this updated opinion.

In a recently published study by Paul *et al.* (2010, Ref. 8) weanling female Long-Evans rats received triclosan (98.2% purity, at 10, 30, 100, 300, 1000 mg/kg/day) by gavage for 4 days. In line with previous reports, triclosan produced dose-dependent decreases in thyroid hormones, more pronounced for serum T4 than for T3. Total T4 decreased to 43% of control at 1000 mg/kg/day, and total T3 decreased to 89 and 75% of control at 300 and 1000 mg/kg/day. TSH did not change. The triclosan-induced hypothyroxinemia in rats can be due to the observed upregulation of hepatic enzymes, *i.e.* induction of CYP2B1/2 and PROD activity, and increased glucuronidation and sulfation of thyroid hormone. On the other hand, the lack of CYP1A1 (EROD) induction demonstrated that the minor dioxin contaminants found in the triclosan sample used in this study, 2,8-dichloro-dibenzodioxin and 2,4,8-trichlorodibenzodioxin, did not induce AhR-mediated effects on phase I and phase II hepatic enzymes. As discussed by the authors, the effective dose that reduced T4 by 20% in this study (BMD 99.4 mg/kg bw/d) was higher than doses reported in other studies, which is likely due to differences in exposure duration as well as sex, age and strain of rats.

A recent study conducted by Stoker *et al.* (2010, Ref. 9) evaluated the effects of triclosan in a 20-day female pubertal assay and an immature rat uterotrophic assay (3-day exposure) with Wistar rats. The test compound was 99.8% pure as determined by HPLC analysis. Rats were dosed orally after weaning with triclosan doses up to 300 mg/kg bw/day (PND 22-42 in the prepubertal assay; for 3 days in the uterotrophic assay, either alone or co-treated with 3 mg/kg bw ethinylestradiol in the latter). In the pubertal study, triclosan advanced the age of onset of vaginal opening and increased uterine weight at 150 mg/kg, indicative of an estrogenic effect. Uterine weight was increased in the EE group (positive control, uterotrophic assay) as compared with the untreated rats but was not affected by triclosan alone. Yet, there was a clear dose-dependent increase in the group co-treated with EE and triclosan (≥ 4.7 mg/kg) as compared with EE alone which may be due to decreased catabolism of steroid hormone. Moreover, triclosan decreased thyroid hormone levels in a dose-dependent manner in both assays: the NOEL for total serum T4 was 9.4 mg/kg bw/day, the LOEL was 18.75 mg/kg bw/day in this study. The study shows that triclosan can suppress thyroid hormones at lower doses than reported previously (Crofton *et al.* 2007; Zorilla *et al.* 2009; Paul *et al.* 2010).

Another study (Rodriguez and Sanchez, 2010, Ref. 10), retrieved from the open literature by SCCS, was published after the current submission. This publication describes results from studies where triclosan (purity 99.6%) was given by drinking water (at 1, 10 or 50 mg/kg bw/d) from 8 days before mating to lactation day 21 to female Wistar rats and/or to their offspring after weaning. Dams (n=12 per dose group) showed no apparent external signs of toxicity; their thyroid hormone blood levels examined during pregnancy and lactation showed dose-related decreases, more notable for serum thyroxine (T4) than for triiodo-thyronine (T3) levels. There were no treatment-related effects on gestation length, litter size number of implantation sites or weaning index, but, live birth index and 6-day survival index were significantly decreased in the highest (50 mg/kg bw/d) dose group; the sex ratio was decreased (less males) in all dose groups. Female offspring were reared and sexual development was followed in n=9 animals per dose group until puberty, in animals treated *in utero* and during lactation and also in animals exposed additionally to triclosan up to puberty. Female pup mean body weights before weaning were lower in all groups, with no dose-related effect. Rats exposed to triclosan showed a delay in sexual development, *i.e.* day of vaginal opening and day of first estrus, and had a higher body weight at these points in time. The effect was similar in all dose groups. The results of this study (which did not

intend to identify specific mechanisms of action) are consistent with an effect of triclosan on thyroid homeostasis and a possible effect on the hypothalamic-pituitary-ovarian axis. Triclosan had an impact on thyroid hormone (T4) levels at 30 mg/kg bw/day (NOEL 3 mg/kg bw/day) in this study.

Remarks and Conclusions

Overall, the new studies confirm previous results that triclosan can affect thyroid hormone homeostasis in the rat. The effective doses vary between studies, which are probably related to differences in exposure duration as well as sex, age and strain of rats. Nonetheless, a NOEL of 9.4 mg/kg bw/day, and a LOEL of 18.75 mg/kg bw/day for decreases in total serum T4 (from Stoker *et al.* 2010) can be considered as valid estimates. The likely mode of action for this effect is increased clearance of T4 hormone. It is worth noting that the rat is a rather sensitive model for chemical induced changes in thyroid hormones compared to humans, due to a lack of T4 binding protein which results in a shorter T4 serum half-life. Thus, the NOEL/LOEL for effects on thyroid hormones in rats is not used in the risk assessment.

With regard to reproductive toxicity, a new study (Rodriguez & Sanchez 2010, Ref. 17) reports significant decreases in live birth index and 6-day survival index in the highest (50 mg/kg bw/d) dose group, but not at the lower triclosan doses (1 or 10 mg/kg bw/d). The SCCP in the opinion SCCP/1192/08 derived a NOAEL of 65 mg/kg bw/d based on decreases in foetal body weights and the mean number of live pups reported in previous reproductive toxicity studies in the rat.

3. Kinetics

In the supplementary submission, it is claimed that hamster is the most appropriate species for extrapolation to humans, noting that the lowest NOAEL in the hamster studies was 75 mg/kg body weight/day based on changes in kidney function parameters (Ref. 11). Since triclosan, in the form of conjugates, undergoes extensive enterohepatic recirculation in rats and in mice, but not in hamsters (mostly eliminated with urine) and not in humans, the applicant has asked SCCS to further consider differences in kinetics between species.

In the opinion SCCP/1192/08 (page 72) a comment was made on this aspect with regard to chronic studies at relatively high doses: "These data indicate that in chronic dosing studies, plasma levels in mice were slightly higher or comparable to plasma levels in rats (based on dose-normalized data) and much higher than plasma levels in hamsters (greater than 4- to 5-fold)". However, from single and repeated dose kinetic studies at lower, more relevant doses (Tab. 23, 24 in SCCP/1192/08) it appears that plasma toxicokinetics in rats and hamster are not as different as one may expect due to enterohepatic recirculation in one but not in the other species. The SCCS thus sees no reason to favour hamster and disregard rat data in its evaluation of triclosan.

4. Recent evaluations

Recently, Rodricks *et al.* (2010; Ref. 12) reviewed the available data on triclosan toxicity, kinetics, mode of action, and exposure for a safety evaluation of this compound. The authors considered in their dose-response assessment several non-cancer endpoints, and then selected data of nephrotoxicity in the hamster (and some other endpoints) for application of benchmark models (quantal models in the US EPA software). For the selected endpoints, the BMDs (benchmark dose) and BMDLs (benchmark dose lower bound; equals the lower 95% or 90% confidence limit of the BMD) were defined, based on a BMR (benchmark response) of 10% extra risk. The endpoint resulting in the lowest estimated

BMDL was the incidence of kidney nephropathy in the male hamster (17.30 to 51.89 mg/kg bw/d, by different models) with adequate or best fit of data for BMDLs of 30 and 47 mg/kg bw/d (Table 17 in Rodricks *et al.* 2010).

The SCCS in principle supports the use of a benchmark dose (BMD) approach. However, given the severity and high incidences of the nephrotoxic effects, the question arises why Rodricks *et al.* (2010) have chosen a BMR of 0.1 and not 0.05 (extra risk of 10% instead of 5%). The BMD approach undertaken to model the dose response-relationship for hamster nephropathy is not convincing. A BMD calculation has not been conducted for the critical endpoint haematotoxicity chosen by SCCS.

PART II: Update of opinion

3.1. Chemical and Physical Specifications

same as in SCCP/1192/08 opinion

3.2. Function and uses

same as in SCCP/1192/08 opinion

3.3. Toxicological Evaluation

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|------------------------------|
| 3.3.1. Acute toxicity |
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same as in SCCP/1192/08 opinion

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| 3.3.2 Irritation and corrosivity |
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same as in SCCP/1192/08 opinion

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| 3.3.3. Skin sensitisation |
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same as in SCCP/1192/08 opinion

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| 3.3.4. Dermal / percutaneous absorption |
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same as in SCCP/1192/08 opinion

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| 3.3.5. Repeated dose toxicity |
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same as in SCCP/1192/08 opinion

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| 3.3.6. Mutagenicity / Genotoxicity |
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same as in SCCP/1192/08 opinion

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| 3.3.7. Carcinogenicity |
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same as in SCCP/1192/08 opinion

3.3.8. Reproductive toxicity

same as in SCCP/1192/08 opinion

Additional Comment

A new study (Rodriguez & Sanchez 2010, Ref. 10), described in more detail in section 3.3.12, reports significant decreases in live birth index and 6-day survival index in the highest (50 mg/kg bw/d) dose group, but not at the lower triclosan doses (1 or 10 mg/kg bw/d). The SCCP in the opinion SCCP/1192/08 derived a NOAEL of 65 mg/kg bw/d based on decreases in foetal body weights and the mean number of live pups reported in previous reproductive toxicity studies in the rat.

3.3.9. Toxicokinetics

same as in SCCP/1192/08 opinion

3.3.10. Photo-induced toxicity

same as in SCCP/1192/08 opinion

3.3.11. Human data

same as in SCCP/1192/08 opinion; section 3.3.11.7 has been expanded

A recent study (Allmyr et al. 2009; Ref. 29) reports data on triclosan blood levels in 12 humans exposed via toothpaste (0.3% triclosan in the product, used twice a day) for two weeks. Their plasma triclosan concentrations increased from 0.009–0.81 ng/g on day 0 to 26–296 ng/g (ranges) upon exposure for 14 days. Despite this, there were no significant changes in plasma levels of either plasma 4 β -hydroxycholesterol or thyroid hormones during the exposure. This indicated that the normal use of triclosan-containing toothpaste is not likely to alter metabolism of drugs via CYP3A4 induction or cause adverse events because of thyroid disturbances in humans. After exposure, the median triclosan concentration in this study was 54 ng/g plasma. This is three times higher than the median triclosan concentration of 16 ng/g plasma previously measured in 36 exposed women from the Swedish population (Allmyr et al. 2006, Ref. 25). One individual had a high plasma triclosan concentration of 296 ng/g after exposure with toothpaste (Allmyr et al. 2009; Ref. 29), a level which is in the range found after an oral dose of 4 mg triclosan in a previous study (Sandborgh-Englund et al, 2006; Ref. 30).

3.3.12. Special investigations

sections 3.3.12.1 to 3.3.12.3 remain the same as in SCCP/1192/08 opinion; the previous section 3.3.12.4 has been expanded, and section 3.3.12.5 has been added to account for more recent publications.

3.3.12.4. Effects of Triclosan on Rat Thyroid

Effects of triclosan on thyroid hormone levels in rats have been investigated in two recent studies, one with 4-day oral gavage exposure (10, 30, 100, 300, 1000 mg/kg bw/d) in weanling female Long-Evans rats (Crofton *et al.*, 2007, Ref. 13), the other with oral administration (3, 30, 100, 200, 300 mg/kg bw/d) from postnatal day (PND) 23 to 53 in male Wistar rats (Zorilla *et al.*, 2009, Ref. 14).

Short term oral exposure in female rats resulted in dose dependent decreases in serum thyroxine levels: serum T4 was decreased 28, 34 and 53% following treatment with 100, 300 and 1000 mg/kg bw/day triclosan, respectively. No significant changes were seen at 10 and 30 mg/kg bw/day triclosan in female weanling rats. The authors of this study (Crofton

et al. 2007, Ref. 13) suggest that decreases in T4 may result from increases in the sulfation or glucuronidation via PXR-linked genes. This view is consistent with triclosan-induced up-regulation of liver enzymes documented in other studies that have been described in opinion SCCP/1192/08 (section 3.3.12.3. and Tab. 3.3.12.3.2-1 and Tab. 3.3.12.3.2-2).

The purpose of the second study was to determine effects of triclosan on pubertal development and thyroid hormone levels in the male rat. After 31 days of exposure, triclosan significantly decreased serum thyroxine (T4) in a dose-dependent manner at 30 mg/kg bw/day and higher (Zorilla *et al.*, 2009, Ref. 14). The active thyroid hormone triiodothyronine (T3) was decreased significantly only at 200 mg/kg bw/day, and thyroid stimulating hormone (TSH) was not statistically different from controls at any dose. Liver weights were increased at 100 mg/kg bw/day triclosan and above suggesting that induction of hepatic enzymes have contributed to the altered T4 and T3 levels. The authors did not consider the levels of change in glucuronidation (UDPGT) activity at 30 mg/kg as sufficient to explain the observed decrease in T4 levels; however, sulfation activity was not assessed. Triclosan did not alter the age at onset of puberty (assessed by preputial separation) or the development of androgen-dependent tissues, even though there was a 60% decrease in androgen serum levels in the 200 mg/kg dose group.

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

Weanling female Long-Evans rats received triclosan (98,2% purity, 10, 30, 100, 300, 1000 mg/kg/day) by gavage for 4 days (Paul *et al.*, 2010; Ref. 15). Triclosan was found to produce dose-dependent decreases in thyroid hormones, more pronounced for serum T4 than for T3. Total T4 decreased to 43% of control at 1000 mg/kg/day, and total T3 decreased to 89 and 75% of control at 300 and 1000 mg/kg/day. TSH did not change. The triclosan-induced hypothyroxinemia in rats can be related to the observed upregulation of hepatic enzymes, i.e. induction of CYP2B1/2 and PROD activity, and increased glucuronidation and sulfation of thyroid hormone. Importantly, the lack of CYP1A1 (EROD) induction demonstrated that the minor dioxin contaminants found in the triclosan sample used in this study, 2,8-dichloro-dibenzodioxin and 2,4,8-trichloro-dibenzodioxin, did not induce AhR-mediated effects on phase I and phase II hepatic enzymes. The effective dose that reduced T4 by 20% in this study (BMD 99.4 mg/kg bw/d) was higher than doses reported in other studies (Crofton, *et al.* 2007, Ref. 13; Zorilla *et al.* 2009, Ref. 14). As discussed by the authors, this is likely due to differences in exposure duration as well as sex, age and strain of rats.

Recently the effects of triclosan were evaluated in a 20-day female pubertal assay and an immature rat uterotrophic assay (3-day exposure) with Wistar rats (Stoker *et al.*, 2010; Ref. 16). The test compound was 99.8% pure as determined by HPLC analysis, and devoid of CYP1A1 inducing activity. Rats were dosed orally after weaning with triclosan doses up to 300 mg/kg bw/day (PND 22-42 in the prepubertal assay; for 3 days in the uterotrophic assay, either alone or co-treated with 3 mg/kg bw ethinylestradiol in the latter). In the pubertal study, triclosan advanced the age of onset of vaginal opening and increased uterine weight at 150 mg/kg, indicative of an estrogenic effect. Uterine weight was increased in the EE group (positive control, uterotrophic assay) as compared to the untreated rats but was not affected by triclosan alone. Yet, there was a clear dose-dependent increase in the group co-treated with EE and triclosan (≥ 4.7 mg/kg) as compared with EE alone which may be due to decreased catabolism of steroid hormone. Moreover, triclosan decreased thyroid hormone levels in a dose-dependent manner in both assays: the NOEL for total serum T4 was 9.4 mg/kg bw/day; the LOEL was 18.75 mg/kg bw/day in this study. The study shows that triclosan can suppress thyroid hormones at lower doses than reported previously (Crofton *et al.* 2007, Ref. 13; Zorilla *et al.* 2009, Ref. 14; Paul *et al.* 2010, Ref. 15).

A recent publication (Rodriguez & Sanchez, 2010, Ref. 17) describes results from studies where triclosan (purity 99.6%) was given by drinking water (at 1, 10 or 50 mg/kg bw/d) from 8 days before mating to lactation day 21 to female Wistar rats and/or to their offspring after weaning. Dams (n=12 per dose group) showed no apparent external signs of toxicity; their thyroid hormone blood levels examined during pregnancy and lactation showed dose-related decreases, more notable for serum thyroxine (T4) than for triiodothyronine (T3) levels. There were no treatment-related effects on gestation length, litter size number of implantation sites or weaning index, but, live birth index and 6-day survival index were significantly decreased in the highest (50 mg/kg bw/d) dose group; the sex ratio was decreased (less males) in all dose groups. Female offspring were reared and sexual development was followed in n=9 animals per dose group until puberty, in animals treated in utero and during lactation and also in animals exposed additionally to triclosan up to puberty. Female pup mean body weights before weaning were lower in all groups, with no dose-related effect. Rats exposed to triclosan showed a delay in sexual development, i.e. day of vaginal opening and day of first estrus, and had a higher body weight at these points in time. The effect was similar in all dose groups. The results of this study are consistent with an effect of triclosan on thyroid homeostasis and a possible effect on the hypothalamic-pituitary-ovarian axis. Triclosan had an impact on thyroid hormone (T4) levels at 30 mg/kg bw/day (NOEL 3 mg/kg bw/day) in this study.

Comment

Several studies show that triclosan can affect thyroid hormone homeostasis in the rat. The effective doses vary between studies, which are probably related to differences in exposure duration as well as sex, age and strain of rats. Overall, a NOEL of 9.4 mg/kg bw/day, and a LOEL of 18.75 mg/kg bw/day for decreases in total serum T4 can be considered as valid estimates. The likely mode of action for this effect is increased clearance of T4 hormone. But, the rat is a rather sensitive model for chemical induced changes in thyroid hormones compared to humans, due to a lack of T4 binding protein which results in a shorter T4 serum half-life. It is important to acknowledge major differences in the thyroid hormone physiology and regulation between rats and humans (SCCNPf 2004, Ref. 18). Thus, the NOEL/LOEL for effects on thyroid hormones in rats is not used in the risk assessment of triclosan. There are conflicting data on potential effects of triclosan with regard to onset of puberty in female rats, with one study reporting a delay at doses up to 50 mg/kg bw/day (Ref. 10), another an earlier onset of vaginal opening at 150 mg/kg bw/day (Ref. 9).

3.3.12.5 Effects of Triclosan on Steroidogenic Enzymes in the Male Rat

Effects of triclosan on steroidogenic enzymes are reported in some *in vitro* studies and in a recently published *in vivo* study in male rats. The observed effects indicate an anti-androgenic activity of triclosan.

The *in vitro* study by Chen *et al.* (2007, Ref. 19) applied an androgen receptor-mediated transcriptional activity assay to evaluate the androgenic/anti-androgenic activity of parabens and selected other antimicrobials. Triclosan inhibited the transcriptional activity induced by testosterone (0.125 nM) by more than 92% at a concentration of 10 µM, and 38.8% at a concentration of 1.0 µM. Triclosan showed no androgenic activity in the assay without testosterone, and was not cytotoxic at the concentrations tested. Triclosan displaced [³H]-testosterone from binding to the ligand binding domain of rat androgen receptor *in vitro*. It was also found to inhibit the induction of an androgen responsive reporter gene in S115 mouse mammary tumour cells and T47D human breast cancer cells by testosterone (Gee *et al.* 2008, Ref. 20). The results are in line with an anti-androgenic activity of triclosan.

Kumar *et al.* (2008, Ref. 21) studied *in vitro* the action of triclosan-induced anti-androgenicity in isolated rat Leydig cells. Treatment of cells with several concentrations of triclosan (0.001, 0.01, 0.1, 1 and 10 µM) resulted in a decreased activity of adenylyl cyclase enzyme and a decreased synthesis of cAMP. This decreased cAMP level resulted in

disruption of the entire steroidogenic cascade, and caused a dose dependent reduction in the synthesis of testosterone, significant at 0.01 μ M triclosan and more.

Alterations of testicular steroidogenesis were also apparent in a rat study by Kumar *et al.* (2009, Ref. 6). Male rats received different doses of triclosan (0, 5, 10 and 20 mg/kg bw/day; daily by gavage) for 60 days. RT-PCR analysis showed a significant decrease in mRNA levels for testicular steroidogenic acute regulatory (StAR) protein, cytochrome P450(SCC), cytochrome P450(C17), 3beta-hydroxy-steroid dehydrogenase (3beta-HSD), 17beta-hydroxysteroid dehydrogenase (17beta-HSD) and androgen receptor (AR) in treated rats. Western blot analysis showed also reduced levels of testicular StAR, and AR proteins. Furthermore, there was a significant decrease ($p < 0.05$) in the level of serum luteinizing hormone (LH), follicle stimulating hormone (FSH), cholesterol, pregnenolone, and testosterone in triclosan treated rats (at 20 mg/kg bw/day). Moreover, in the absence of notable effects on body weight triclosan treatment reduced the weights of testis and accessory sex tissues, significant at the two highest dose levels (10 and 20 mg/kg bw/day). The histopathological examination revealed malformations in the testis and sex accessory tissues. Testicular sperm content and daily sperm production (DSP/g testis), determined from the freshly removed testis of the rats on completion of the treatment, were clearly reduced in the high dose group.

Overall this study showed that triclosan decreased the synthesis of androgens followed by reduced sperm production in treated male rats which could be mediated by a decreased synthesis of LH and FSH thus involving hypothalamo-pituitary-gonadal axis.

Comment

The authors of this study did not derive a NOAEL for triclosan, although based on weight changes in testis and accessory sex tissue, along with changes in reproductive hormone levels, a NOAEL of 5 mg/kg bw/day could be derived. But, there is uncertainty with regard to impurities in the test compound (purity about 98 %), and bolus application (gavage) in the Kumar *et al.* 2009 (Ref. 6) study may have aggravated the effects. Such decreases in testis weight were not observed in a 90 day study in rats following doses up to 600 mg/kg bw with diet.

Also, in a two generation study, triclosan exposure with food (\approx 17, 56, and 176 mg/kg bw/d) had no measurable effects on fertility of male rats; sporadic changes were observed in testis of some animals, but were apparently not dose-related (Morseth 1988, Ref. 22). This study of 1988 is consistent with old OECD guidelines; yet, parameters such as sperm counts and viability of sperm were not examined at that time. On the other hand, effects on weight and histopathology of gonads were not reported in studies of several species with triclosan of high purity.

3.3.13. Safety evaluation (including calculation of the MoS)

Based on the SCCP/1192/08 opinion, with some adjustments for the amount of certain products applied and/or the frequency of use, now according to the current Notes of Guidance (Ref. 3)

3.3.13.1 Consumer Exposure Assessment

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

Calculations were made for individual products and for product groups in which triclosan is used according to the industry submission, i.e. most prevalently in toothpaste, deodorant, hand and body soaps (referred to as common-use products) and for products in which triclosan is used less frequently, such as facial cosmetic products, body lotion, and mouthwash (referred to as marginal-use products).

For the purpose of SED calculations for oral formulations (toothpaste, mouthwash) it was assumed that triclosan is 100% bioavailable (see Table 1).

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

Table 1: SED Calculation for Oral Products (replaces table 48 of opinion SCCP/1192/08)

| Product | Assumed bioavailability (%) ¹ | Amount applied (mg) ² | Retention ³ | Calculated daily exposure | Triclosan content (%) ⁵ | BW (kg) | SED (mg/kg bw/d) ⁴ |
|------------|--|----------------------------------|------------------------|---------------------------|------------------------------------|---------|-------------------------------|
| Toothpaste | 100 | 2750 mg per day | 0.05 | 138 mg | 0.3 | 60 | 0.0069 |
| Mouthwash | 100 | 21620 mg per day | 0.1 | 2160 mg | 0.15 | 60 | 0.054 |
| Mouthwash | 100 | 21620 mg per day | 0.1 | 2160 mg | 0.2 | 60 | 0.072 |
| Mouthwash | 100 | 21620 mg per day | 0.1 | 2160 mg | 0.3 ⁶ | 60 | 0.108 |

Abbreviations: BW, body weight; d, day; NA, not applicable; SED, systemic exposure dose

¹ For the purposes of these calculations (i.e., for oral products), it was assumed that the bioavailability of triclosan was 100%

² Amount of application value is taken from Table 3, Section 4-2 in SCCS, 2011 (Ref. 3)

³ Retention value was taken from Table 3, Section 4-2 in SCCS, 2011 (Ref. 3)

⁴ Formula: $SED = (\text{Bioavailability in \%} \times \text{amount of product applied (mg)} \times \text{Frequency} \times \text{Retention factor} \times \text{amount of triclosan in product}) / BW$

⁵ Conflicting information on current maximal use levels in marketed mouthwash products has been received, accordingly, alternative calculations for different use concentration are included

⁶ maximally authorised concentration

For dermal formulations, SED calculations were based on percutaneous absorption data from in vitro human studies (see SCCP/1192/08, Table 37). SED calculations for individual personal-care products containing triclosan were carried out based on dermal absorption values ($\mu\text{g}/\text{cm}^2$) from in vitro percutaneous absorption studies conducted with deodorant and w/o formulations containing 0.2% triclosan and dilute soap solution formulation containing 0.02% triclosan.

In each calculation for dermal products, an extrapolated value for flux ($\mu\text{g}/\text{cm}^2$ absorption) was used, based on the assumption that skin penetration is by passive diffusion, such that flux would be proportional to the concentration of triclosan applied to the skin. For hand soap and body soap, the conversion of the $\mu\text{g}/\text{cm}^2$ dermal absorption value to a current-use value for amount of triclosan in the product type assumed a 10-fold dilution of 0.3% triclosan. For both soaps, a retention factor was not used due to the inclusion of a rinse-off step in the relevant in vitro percutaneous absorption study. The results of these calculations are provided in Tables 2 and 3 below, for leave-on and rinse-off products, respectively.

Table 2: SED Calculation for Leave-On Products (replaces table 49 of opinion SCCP/1192/08)

| Product | 24-h dermal absorption based on 0.2% triclosan ($\mu\text{g}/\text{cm}^2$) ¹ | Triclosan content (%) | Calculated 24-h dermal absorption based on triclosan content ($\mu\text{g}/\text{cm}^2$) ² | SA (cm^2) ³ | F (per d) | Conversion (mg/ μg) | R | BW (kg) | SED (mg/kg bw/d) ⁵ |
|---------|---|-----------------------|---|-----------------------------------|-----------|---------------------------------|---|---------|-------------------------------|
|---------|---|-----------------------|---|-----------------------------------|-----------|---------------------------------|---|---------|-------------------------------|

Opinion on triclosan, addendum to the SCCP Opinion on Triclosan (SCCP/1192/08) from January 2009

| | | | | | | | | | |
|-------------------|-------|-------------------|-------|-------|------|--------------------|---|----|--------|
| Deodorant stick | 0.303 | 0.3 | 0.455 | 200 | 2 | 1x10 ⁻³ | 1 | 60 | 0.003 |
| Body Lotion | 0.420 | 0.15 ⁶ | 0.315 | 15670 | 2.28 | 1x10 ⁻³ | 1 | 60 | 0.1876 |
| Body Lotion | 0.420 | 0.3 ⁷ | 0.630 | 15670 | 2.28 | 1x10 ⁻³ | 1 | 60 | 0.3753 |
| Face powder | 0.420 | 0.2 ⁶ | 0.420 | 565 | 1 | 1x10 ⁻³ | 1 | 60 | 0.0040 |
| Face powder | 0.420 | 0.3 ⁷ | 0.630 | 565 | 1 | 1x10 ⁻³ | 1 | 60 | 0.0060 |
| Blemish Concealer | 0.420 | 0.15 ⁶ | 0.315 | 57 | 1 | 1x10 ⁻³ | 1 | 60 | 0.0003 |
| Blemish Concealer | 0.420 | 0.3 ⁷ | 0.630 | 57 | 1 | 1x10 ⁻³ | 1 | 60 | 0.0006 |

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

- ¹ Dermal absorption values based on *in vitro* data using 0.2% in deodorant formulation (for deodorant stick) and 0.2% water/oil emulsion (for body lotion, face powder, and stick concealer)
- ² Calculation: (Absorption from 0.2% triclosan applied in the relevant *in vitro* study) x (triclosan content for the product/0.2%) = Absorption from 0.3% triclosan. This assumes that skin penetration is by passive diffusion, such that flux would be proportional to the concentration of triclosan applied to the skin
- ³ Area of application values are taken from Table 2, Section 4-2 in SCCS, 2011 (Ref. 3).. The skin area for blemish concealer was assumed to be 1/10th of the face
- ⁴ Frequency of application values for use per day are taken from Table 2, Section 4-2 in SCCS, 2011 (Ref. 3). Frequency of application values for face powder and stick concealer were assumed to be once per day
- ⁵ Formula: SED = (24-h dermal absorption in µg/cm² based on use levels of triclosan in product x surface area of application in cm² x frequency of application per day x retention x conversion factor) / BW
- ⁶ Current use concentration according to Industry information
- ⁷ Maximally authorised concentration

Table 3: SED Calculation for Rinse-Off Products (replaces table 50 of opinion SCCP/1192/08)

| Product | 24-h dermal absorption based on 0.02% triclosan (µg/cm ²) ¹ | Calculated 24-h dermal absorption based on 0.03% triclosan (µg/cm ²) ² | SA (cm ²) ³ | F (times/d) ⁴ | Conversion (mg/µg) | BW (kg) | SED (mg/kg bw/d) ⁵ |
|----------------------|--|---|------------------------------------|--------------------------|--------------------|---------|-------------------------------|
| Hand soap | 0.0306 | 0.046 | 860 | 10 | 1x10 ⁻³ | 60 | 0.0066 |
| Shower gel/body soap | 0.0306 | 0.046 | 17500 | 1.43 | 1x10 ⁻³ | 60 | 0.0192 |

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

- ¹ Dermal absorption value based on *in vitro* data using 0.02% soap solution
- ² Calculation: (Absorption from 0.02% triclosan applied in the relevant *in vitro* study) x (0.03%/0.02%) = Absorption from 0.03% triclosan solution. This assumes that 1) a 10X dilute solution of 0.3% triclosan is applied and 2) skin penetration is by passive diffusion, such that flux would be proportional to the concentration of triclosan applied to the skin
- ³ Area of application values are taken from Table 2, Section 4-2 in SCCS, 2011 (Ref. 3)
- ⁴ Frequency of application values for use per day are taken from Table 2, Section 4-2 in SCCS, 2011 (Ref. 3)⁵
Formula: SED = (24-h dermal absorption in µg/cm² based on 0.03% triclosan x surface area of application in cm² x frequency of application per day x conversion factor) / BW

According to the current submission it is intended to use triclosan at 0.3% in professional products for the cleaning of hand and foot nails (as antifungal agent) (Ref. 23). Such products are applied in amounts of 10 mg per nail, every 3 to 4 weeks or every 2 weeks as worst-case. In light of this use frequency and the poor (if any) penetration of triclosan through nails, SCCS considers exposure of consumers from this source as negligible.

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

Table 4: SED Calculation for Various Product Types

| Summary of Triclosan SED Values from the Use of Personal Care Products | | |
|---|-----------------------|--------|
| Type of Product(s) | Triclosan content (%) | SED |
| Toothpaste (Adults) | 0.3 | 0.0069 |
| Hand Soap | 0.3 | 0.0066 |
| Body Soap/shower gel | 0.3 | 0.0192 |
| Deodorant (Stick) | 0.3 | 0.0030 |
| Mouthwash | 0.15 | 0.0540 |
| Mouthwash | 0.2 | 0.0720 |
| Mouthwash | 0.3 | 0.1080 |
| Face powder | 0.2 | 0.0040 |
| Face powder | 0.3 | 0.0060 |
| Body lotion | 0.15 | 0.1876 |
| Body lotion | 0.3 | 0.3753 |
| Stick-type concealer | 0.15 | 0.0003 |
| Stick-type concealer | 0.3 | 0.0006 |
| All Products at max. authorised concentration | 0.3 | 0.5256 |
| All Products at current use concentration (toothpaste, hand soap, body soap/shower gel, deodorant stick, mouthwash, body lotion, face powder, stick concealer) | 0.15-0.3 | 0.2996 |
| Common-Use Products (toothpaste, hand soap, body soap/shower gel, deodorant stick) | 0.3 | 0.0357 |
| Common-Use Products + mouthwash 0.15% | var. | 0.0897 |
| Common-Use Products + mouthwash 0.2% | var. | 0.1077 |

Abbreviations: SED, systemic exposure dose

Internal exposure and absorption of triclosan under simulated use conditions by humans can be approximated also from in vivo studies with volunteers that applied triclosan-containing personal care products for a prolonged time period (see SCCP/1192/08, Table 36). Precise information on the use-pattern and the level of triclosan content of the formulations was available in the study by Beiswanger and Tuohy (1990, Ref. 24), in which 182 subjects used a toothpaste (0.28 % triclosan), a bar soap (0.75% triclosan) and a deodorant (0.39% triclosan) for 13 weeks. The results of this study indicated that all subjects reached a stable plateau plasma level after 3 weeks of use of the toothpaste, deodorant, and soap. The results showed plasma levels of 19-23 ppb (exposure to toothpaste only), and 29-31 ppb (exposure to toothpaste, deodorant, and soap). A more recent study with 12 adults exposed to triclosan for two weeks via toothpaste (0.3%) found median plasma levels of 54 ppb, and a very high value of 296 ppb in one individual (Allmyr *et al.* 2009, Ref. 29).

3.3.13.2 Safety Assessment

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

Tab. 27 in SCCP/1192/08) to derive plasma level based MoS. However, this approach is only possible for a few product types (toothpaste, deodorant and hand soap). For several other triclosan containing products (and aggregate exposure), the conventional MOS calculation has to be applied (Table 5).

Table 5: Margin of Safety (MoS) calculations for different exposure scenarios

| Types of Products Used | SED (mg/kg bw/d) | MoS Based on Rat NOAEL of 12 mg/kg bw/d | MoS Based on Plasma Levels |
|--|---------------------------------|--|---|
| Toothpaste | 0.0069 | 1739 | 1408 |
| Toothpaste, deodorant stick, and hand soap | 0.0165 | 727 | 939 |
| <u>Common-Use Products 0.3% triclosan</u> (toothpaste, hand soap, body soap/shower gel, deodorant stick) | 0.0357 | 336 | no human plasma data available |
| <u>Common-Use Products + mouthwash 0.15%</u> | 0.0897 | 134 | no human plasma data available |
| <u>Common-Use Products + mouthwash 0.2%</u> | 0.1077 | 111 | no human plasma data available |
| <i>"Acceptable" exposure from some products</i> | <i>0.1200</i> | <i>100</i> | |
| <u>All Products 0.15 – 0.3% triclosan</u> (toothpaste, hand soap, body soap/shower gel, deodorant stick, mouthwash, body lotion, face powder, blemish concealer) | 0.2996 | 40 | no human plasma data available |
| <u>All Products 0.3% triclosan</u> (toothpaste, hand soap, body soap/shower gel, deodorant stick, mouthwash, body lotion, face powder, blemish concealer) | 0.5256 | 23 | no human plasma data available |

From the calculations above it can be deduced that the use of triclosan in all products cannot be considered safe because of the magnitude of the aggregate exposure. This conclusion is reached regardless whether current use levels as given by the applicant or the maximally authorised use level is considered. However, the use in the common-use products (toothpaste, hand soap, body soap/shower gel and deodorant stick) is considered safe. The exposure to triclosan from face powder and blemish concealer (up to 0.3% triclosan) is low and not considered to be of concern in addition to exposure from common-use products. However, the use of triclosan in body lotions (at 0.15 or 0.3 %) results in high exposures. The use of mouthwashes containing more than 0.2% triclosan together with common-use products would also result in MOS below 100.

As can be derived from table 5, a total dose / SED total of 0.12 mg/day would correspond to a MOS of 100. An aggregate exposure from different products types which results in a higher SED is not considered safe for the consumer. Should other product type/concentration combinations different to the ones calculated above be considered in the

regulation, a recalculation of aggregate exposure can be made on the basis of the individual SEDs given in table 4. SEDs that would result from other use concentrations of the same product types can be extrapolated from the given information.

Safety of Triclosan in Children and Neonates

The very low levels of exposure as measured in breast milk indicate that maternal use of triclosan immediately post-partum is unlikely to be a safety concern for neonates (see SCCP/1192/08, section 3.3.11.2.6). Infant exposure to triclosan from breast milk has been shown to be significantly lower than triclosan exposure of the mother, based on a comparison of triclosan concentrations in breast milk and plasma (Allmyr et al., 2006, Ref. 25).

No measured exposure data for babies and young children following use of consumer products containing triclosan was identified in the literature, except spot urine measurements in the age group 6-11 years from the NHANES study (Calafat et al. 2008, Ref. 26). Based on the conversion of spot urine concentrations to estimated dose, this subpopulation had a lower aggregate exposure to triclosan than children of 11-19 years and adults (US EPA 2008, Ref. 27). The rapid increase in maturation of glucuronidation ability within the first year and the maturity of the sulfation pathway, indicate that capabilities of children to metabolise triclosan through glucuronidation or sulfation are likely comparable to those of adults. Also, glomerular filtration rates normalised to body weight approach adult values by around 6 months of age and renal tubular function matures to near-adult values by around 1 year of age (Alcorn and McNamara, 2002, ref. 28). Accordingly, studies have shown that elimination is comparable in adults and children (see SCCP/1192/08, section 3.3.11.2.1).

3.3.14. Discussion

Physico-chemical properties

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

Irritation, sensitisation

The irritation/corrosivity data from either irritation studies in the hamster, guinea pig, and rabbit, or skin toxicity studies conducted in the mouse, rat, monkey, and dog suggest that triclosan may cause slight reversible skin irritation at concentrations of 0.5 to 5% under experimental conditions. Triclosan at concentrations of 1 to 10% produced only slight, reversible irritation in the rabbit eye. Data from human use evaluating the skin and oral mucosa irritation effects of triclosan alone, or in combination with SLS, indicate that triclosan at 0.3% is not a skin or oral mucosal irritant.

In the guinea pig no sensitisation with triclosan in various formulations and concentrations (up to 10% in petrolatum) was found. However, clinical experience has shown that triclosan does have a low sensitisation potential in humans. In over 14,000 patients patch tested with triclosan (typically tested at a concentration of 2% in petrolatum), the range of positive results was 0.1 to 0.3% of the tested groups. When tested in patients with known or suspected cosmetic allergy or intolerance positive reaction rates ranged from 0.06 to 0.8% of a total of 11,887 tests conducted. Possible photocontact allergy has been rarely reported.

Dermal and oral absorption

Data from percutaneous absorption studies indicate that triclosan is well absorbed through the skin in all species tested with the extent of absorption being dependent on the formulation in which it was delivered. In the rat, percutaneous absorption was approximately 23 to 28% of the applied dose of triclosan in ethanol, ethanol/ water, soap suspension, or a cream formulation.

Triclosan is almost completely absorbed following oral administration, with no species-related differences, and in humans this is up to 98% of the dose. However, under normal conditions of toothpaste use (*i.e.*, expectoration and rinsing) or following percutaneous application of several different personal care products, absorption is more limited (approximately 5 to 10% of the dose *via* either of these routes of administration). See also brief summary of *in vivo* data under "Kinetics" in opinion SCCP/1192/08.

General toxicity

Triclosan is not acutely toxic *via* the oral route of administration, with high oral intubation LD₅₀ values in the range of 3,750 to 5,000 mg/kg body weight in mice and rats, and an oral capsule LD₅₀ value of greater than 5,000 mg/kg body weight in dogs. SCCP considers the NOAEL as 12 mg/kg bw/d due to haematotoxicity and decreased absolute and relative spleen weights (Mid Dose Females) in the long term toxicity study in rats.

Mutagenicity, genotoxicity

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

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

Carcinogenicity

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

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

Reproductive/developmental Toxicity

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

NOAEL (NOEL) values from the definitive GLP studies were summarized in Table 21 of opinion SCCP/1192/08. It is important to note the determination of the foetal NOAEL value for each study was based on foetal variation effects that were most likely secondary to general maternal toxicity, and not direct effects of triclosan *per se*. It is also worth noting that the low NOAEL value for foetal effects in the mouse study (25 mg/kg body weight/day)

is likely attributable to the sensitivity of the maternal mice to the liver effects of triclosan, also observed in the repeated dose and carcinogenicity studies in mice.

There are conflicting data on potential effects of triclosan with regard to onset of puberty in female rats, with one study reporting a delay at doses up to 50 mg/kg bw/day (Ref. 10), another an earlier onset of vaginal opening at 150 mg/kg bw/day (Ref. 9). Another recent study (Ref. 6) reported decreases in testis weight and accessory sex tissue, along with changes in reproductive hormone levels at daily doses of 10 and 20 mg/kg bw/day. But, there is uncertainty with regard to impurities in the test compound (purity about 98 %) in this study (Ref. 6), and effects on weight and histopathology of gonads were not reported in previous studies of several species with triclosan of high purity and at higher doses.

Several recent studies (Refs. 13-17) show that triclosan can affect thyroid hormone homeostasis in the rat. The effective doses vary between studies, which are probably related to differences in exposure duration as well as sex, age and strain of rats. Overall, a NOEL of 9.4 mg/kg bw/day, and a LOEL of 18.75 mg/kg bw/day for decreases in total serum T4 can be considered as valid estimates. The likely mode of action for this effect is increased clearance of T4 hormone (Ref. 8). But, the rat is a rather sensitive model for chemical induced changes in thyroid hormones compared to humans, due to a lack of T4 binding protein which results in a shorter T4 serum half-life. It is important to acknowledge major differences in the thyroid hormone physiology and regulation between rats and humans (SCCNPF 2004, Ref 18). Thus, the NOEL/LOEL for effects on thyroid hormones in rats was not used in the risk assessment of triclosan.

Kinetics

Numerous human and animal studies are available on the toxicokinetics of triclosan following oral and dermal exposure to single and repeated doses. The studies cover all important aspects, i.e. absorption, distribution, metabolism and excretion. Upon oral administration absorption of triclosan from the gastrointestinal tract is rapid and extensive in both humans and animals. But, limited buccal absorption was seen in humans following normal toothpaste use (up to 14% of the amount that would be absorbed upon ingestion of an equivalent dose). Upon dermal application in humans, absorption was at least 3% to 7%, and at least 14% in one volunteer.

Triclosan is rapidly distributed in the organism following oral or dermal exposure. The main metabolic pathways in humans and animals involve glucuronidation and sulfation by phase-2 enzymes. The half-life of elimination for orally administered triclosan ranged from 13 to 29 h in humans compared to 10 to 15 h in rats, 8-12 in mice and 25 to 32 h in hamsters. The major route of excretion in humans, hamsters, rabbits and primates is via urine, with excretion via faeces being of secondary importance in these species. The reverse situation is observed in rats, mice and dogs where biliary excretion is more important than renal excretion. The human oral and dermal data provide no evidence for a bioaccumulation potential. Likewise, the kinetic data in rats and hamsters provide no evidence for a bioaccumulation in these species, whilst in mice retention of triclosan (and/or metabolites) appears to occur in liver.

In conclusion, kinetics of triclosan are qualitatively similar in humans and animal species investigated, but the observed quantitative differences between humans and several animal species make human data the first choice for the safety evaluation of triclosan-containing consumer products.

Other aspects

Recently, the US EPA (2008, Ref. 27) utilized population-based biological monitoring data for triclosan (available from the NHANES study) to assess the co-occurrence of uses to develop an aggregate exposure assessment. Because of some uncertainties in converting spot urine concentrations to estimated dose, three conversion methods were used. Calculated exposure was then compared to the selected oral NOAEL of 30 mg/kg/day (from the chronic toxicity study in baboons). Based on the results at the mean and 99th percentile,

the aggregate risks to triclosan from all (personal care and other consumer products) uses did not trigger a risk of concern. The mean MOEs ranged from 4,700 to 19,000. The MOEs at the 99th percentile ranged from 260 to 1,500.

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

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

- USA-EPA chose a NOAEL of 30 mg/kg/d whereas SCCP selected a NOAEL of 12 mg/kg/d (based on haemotoxicity) as the critical effect level against which human exposure to triclosan is compared (for subsequent MOS calculations). The SCCP approach is in line with the evaluation of triclosan by EFSA for its use in food contact materials.
- US-EPA has estimated triclosan exposure in the US population on the basis of biomonitoring data from spot urine samples. Although this approach probably reflects exposure from current use concentrations in various products on the US market, it cannot be applied directly to the evaluation regarding the safe use of triclosan in cosmetic products by SCCP, since:
 - The current use concentrations in the USA may have been lower than the maximal triclosan concentration limit of 0.3% as preservative in cosmetic products in the EU, the safety of which SCCP was asked to evaluate according to this mandate (Question 1)
 - In estimating human exposure, the SCCP followed its Notes of Guidance to calculate systemic exposure doses (SED) from triclosan-containing products (at 0.3%) applied orally and dermally. This may be viewed as a worst-case scenario. The alternative approach, i.e. MOS calculations that are based on plasma levels (measured under simulated use-conditions) were only available for certain products, not for all triclosan-containing products. Representative biomonitoring data are not available for the European population.

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

The SCCS has critically re-evaluated the safety of triclosan, considering new information on effects and exposure. In accord with the recently revised Notes of Guidance (Ref. 3), some adjustments for the amount of certain products applied and/or their frequency of use have been made. However, this did not change the overall conclusions reached by the SCCP.

4. CONCLUSION

In response to the terms of reference, the SCCS concludes the following:

- 1. In the light of this supplementary submission, does the SCCS consider it necessary to revise the toxicological evaluation made by the SCCP in its opinion SCCP/1192/08? If the answer to question 1 is yes, does the SCCS consider a continued use of Triclosan as a preservative in all cosmetic products as safe for the consumers at the current concentration limit of maximum 0.3% ?*

Taking into account the information provided in the supplementary submission and additional publications on triclosan from the open literature, the SCCS considers the toxicological evaluation made by SCCP in its opinion SCCP/1192/08 still as valid. Thus, the continued use of triclosan as a preservative at the current concentration limit of maximum

0.3% in all cosmetic products is not safe for the consumer because of the magnitude of the aggregate exposure.

Based on the recent revision of the Notes of Guidance (7TH Revision) some adjustments for the amount of certain products applied and/or frequency of use were indicated, and resulted in some changes in the consumer exposure assessment and the related safety evaluation. The use of triclosan at a maximum concentration of 0.3% in toothpastes, hand soaps, body soaps/shower gels and deodorant sticks ("common-use products" as defined by the applicant) is considered safe. Additional use of triclosan in face powders and blemish concealers at this concentration is also considered safe. The use of Triclosan in other leave-on products (e.g. body lotions) is not considered safe for the consumer due to the resulting high exposures.

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

2. Taking into account the safe use of Triclosan at a maximum concentration of 0.3% in toothpaste, hand soaps, body soap/shower gels and deodorant sticks ("common use products"), does the SCCS consider an additional use of Triclosan in mouthwashes as safe for the consumer at a concentration limit of maximum 0.15%, alternative 0.2% taking into account the provided exposure data and the preliminary opinion on antimicrobial resistance?

An additional use of triclosan in mouthwashes at a concentration limit of 0.15 or 0.2 % is considered as safe for the consumer from a toxicological perspective whereas higher concentrations (0.3%) are not. The aspect of microbial resistance is not covered here and was discussed in the separate opinion (SCCP/1251/09, ref. 31).

3. Taking into account the safe use of Triclosan at a maximum concentration of 0.3% in toothpaste, hand soaps, body soap/shower gels and deodorant sticks ("common use products"), does the SCCS consider an additional use of Triclosan in nail products as safe for the consumer at a concentration limit of maximum 0.3% taking into account the provided exposure data and the preliminary opinion on antimicrobial resistance?

Consumer exposure from an additional use of triclosan in nail products at a concentration of 0.3 % is considered negligible (safe) under the provisions of the intended use (in products for the cleaning of finger and toenails; Ref. 23) and frequency (every 3 to 4 weeks or every 2 weeks as worst-case). The aspect of microbial resistance is not covered here and was discussed in the separate opinion (SCCP/1251/09, ref. 31).

5. MINORITY OPINION

Not applicable

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