



Scientific Committee on Consumer Safety

SCCS

OPINION ON

Cetylpyridinium chloride - Submission II

COLIPA n° P97

The SCCS adopted this opinion at its 9th Plenary meeting on 25 March 2015

About the Scientific Committees

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

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

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines 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.).

Scientific Committee members

Ulrike Bernauer, Qasim Chaudhry, Pieter Coenraads, Gisela Degen, Maria Dusinska, Werner Lilienblum, Elsa Nielsen, Thomas Platzek, Suresh Chandra Rastogi, Christophe Rousselle, Jan van Benthem.

Contact

European Commission

Health & Food safety

Directorate C: Public Health

Unit C2 – Health Information/ Secretariat of the Scientific Committee

Office: HTC 03/073 L-2920 Luxembourg

SANTE-C2-SCCS@ec.europa.eu

© European Union, 2015

ISSN 1831-4767

ISBN 978-92-79-56130-6

Doi:10.2875/962022

EW-AQ-16-007-EN-N

The opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The opinions are published by the European Commission in their original language only.

http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

ACKNOWLEDGMENTS

SCCS Members

Dr. U. Bernauer
Prof. P.J. Coenraads
Prof. G. Degen
Dr. M. Dusinska
Dr. W. Lilienblum
Dr. E. Nielsen (Rapporteur)
Prof. T. Platzek
Dr. S. Ch. Rastogi (chairperson)
Dr. Ch. Rousselle
Dr. J. van Benthem

Former SCCS Member

Prof. A. Luch

External experts

Prof. A. Bernard
Prof. A.M. Giménez-Arnau
Prof. T. Vanhaecke

For the revision

SCCS Members

Dr. U. Bernauer
Dr. Q. Chaudhry
Prof. P.J. Coenraads
Prof. G. H. Degen (Chairperson)
Dr. M. Dusinska
Dr. W. Lilienblum
Dr. E. Nielsen (Rapporteur)
Prof. T. Platzek
Dr. Ch. Rousselle
Dr. J. van Benthem

External experts

Prof. A. Bernard
Prof. J. Duus-Johansen
Dr. J. Ezendam
Prof. A. M. Giménez-Arnau
Dr. E. Mirkova
Dr. E. Panteri
Prof. T. Vanhaecke

This opinion has been subject to a commenting period of minimum 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

Revision of the Opinion on Cetylpyridinium chloride (P97)

maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

In this case, the overall SCCS comment to oral mucosal irritation on page 16 has been revised, the section 3.3.11 human data now includes the pharmacovigilance information provided by the applicant (pages 44-45), the section 3.3.13 oral and dermal application (including the tables) have been revised, the section 3.3.14 discussion was modified under section irritation/sensitisation on page 49, and finally the conclusion 1 on page 51 was revised too.

Keywords: SCCS, scientific opinion, cosmetic ingredient, preservative, P97, Cetylpyridinium chloride, Regulation 1223/2009, CAS 123-03-5 (anhydrous form) and 6004-24-6 (monohydrate form), EC 204-593-9

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on Cetylpyridinium chloride - submission II (P97), SCCS/1548/15, 25 March 2015, revision of 15 December 2015

TABLE OF CONTENTS

ACKNOWLEDGMENTS	3
1. BACKGROUND	6
2. TERMS OF REFERENCE.....	6
3. OPINION.....	7
4. CONCLUSION	52
5. MINORITY OPINION.....	52
6. REFERENCES.....	53

1. BACKGROUND

Submission I for Cetylpyridinium chloride (Colipa No P 97) was submitted in March 2005 by COLIPA¹.

The Scientific Committee on Consumer Products (SCCP) adopted at its 7th plenary meeting the 28 of March 2006 the opinion (SCCP/0985/06) with the following conclusion:

In view of the poor quality of the toxicological data presented in the current dossier, the SCCP requires a new dossier to be submitted in which data is provided to all relevant toxicological end-points and conforming to currently accepted standards.

Submission II for Cetylpyridinium chloride was submitted in July 2011 by Cosmetics Europe² supplemented by separately submitted company data. These submissions are intended to demonstrate the safety of the ingredient for use as preservative in various categories of cosmetic products. The submission applies for inclusion of Cetylpyridinium chloride in Annex VI of the Cosmetics Directive 76/768/ECC, soon in Annex V of the Cosmetic Regulation (EC) n.1223/2009.

2. TERMS OF REFERENCE

1. *On the basis of the data provided, does the SCCS consider that cetylpyridinium chloride is safe for consumers, when used as a preservative in cosmetic products in the following specified concentrations:*

- *mouthwashes cosmetic products up to a concentration of 0.1 %*
- *all other oral hygiene cosmetic products up to a concentration of 0.5 %*
- *skin lotions and creams up to a concentration of 0.2 %*
- *anti-perspirant deodorants up to a concentration of 2.0 %*

2. *Does the SCCS have any further scientific concerns with regard to the use of cetylpyridinium chloride in cosmetic products?*

¹ COLIPA – The European Cosmetics Association

² Ex-COLIPA – The European Cosmetics Association

3. OPINION

Submission I for cetylpyridinium chloride was submitted in March 2005. The SCCP concluded (SCCP/0985/06) that in view of the poor quality of the toxicological data presented in the submitted dossier a new dossier should be submitted with data on all relevant toxicological end-points and conforming to currently accepted standards.

Submission II for cetylpyridinium chloride was submitted in July 2011. Submission II is almost identical to Submission I, i.e. Submission II consists mainly of the same references as Submission I with many of the pdf files being of poor quality (including incomplete pages, handwritten notes) that makes reading very difficult.

Submission II is supplemented with a separate submission by a Company (called Company Submission in this Opinion) in 2011 to the European Chemicals Agency (ECHA) for use as a biocide.

In contrast to Submission II (and Submission I) the Company Submission includes studies on acute oral, dermal and inhalation toxicity, skin and eye irritation, and skin sensitisation, which have been performed according to currently accepted test guidelines.

Two genotoxicity studies (*in vitro* mouse lymphoma test and *in vitro* chromosome aberration test) performed according to currently accepted test guidelines are included in Submission II (and Submission I). The Company Submission also includes an *in vivo* micronucleus study.

The studies in the Company Submission on repeated dose toxicity, toxicity to reproduction and on ADME are identical to those in Submission II (and Submission I).

No studies have been included in the Company Submission regarding carcinogenicity and dermal absorption, but a justification for non-submission of data has been provided.

The EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) has recently published a scientific opinion on the evaluation of the safety and efficacy of Cecure[®] for the removal of microbial surface contamination of raw poultry products (Ref.: 24). The active ingredient in Cecure[®] is cetylpyridinium chloride. Repeated dose toxicity studies evaluated in the EFSA opinion and considered of relevance for the safety assessment of cetylpyridinium chloride as a cosmetic ingredient are included in this opinion.

3.1. Chemical and Physical Specifications

The information in this section has been taken from the Company Submission Document IIA and the relevant documents in III unless otherwise stated.

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Cetylpyridinium chloride (INCI name)

3.1.1.2. Chemical names

n-Hexadecylpyridinium chloride (IUPAC name)
Cetylpyridinium chloride;
Hexadecylpyridinium chloride;

Revision of the Opinion on Cetylpyridinium chloride (P97)

1-Hexadecylpyridinium chloride;
Pyridinium, 1-hexadecyl-, chloride

3.1.1.3. Trade names and abbreviations

Pyrisept, Ceepryn, Cetamium, Dobendan, Medilave, Cepacol, Merocet, Pristacin

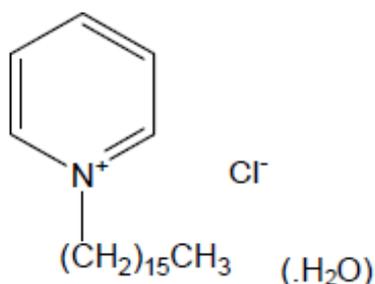
COLIPA n°: P97

3.1.1.4. CAS / EC number

CAS: 123-03-5 (anhydrous form)
6004-24-6 (monohydrate form)

EC: 204-593-9

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $\text{C}_{21}\text{H}_{38}\text{NCl}$ (anhydrous form)
 $\text{C}_{21}\text{H}_{38}\text{NCl} \cdot \text{H}_2\text{O}$ (monohydrate form)

3.1.2. Physical form

White powder

3.1.3. Molecular weight

Molecular weight: 339.99 g/mol (anhydrous form)
358.07 g/mol (monohydrate form)

3.1.4. Purity, composition and substance codes

Specification of the purity: $\geq 97\%$

Anhydrous cetylpyridinium chloride (CPC) was determined by titration of an aqueous-chloroform bi-layer system with sodium tetraphenyl boron using bromophenol blue as indicator. The method is the USP method for determination of anhydrous CPC.

SCCS comment

Chromatographic purity, for which tests are more reliable, was not determined.

3.1.5. Impurities / accompanying contaminants

Heavy metal 20 ppm max

No impurities of toxicological or ecotoxicological concern or impurities which exceed 1 g/kg are present in technical grade CPC.

SCCS comment

Qualitative and quantitative impurities, which may be present at <1 g/kg level, have not been reported.

3.1.6. Solubility

Water: >10 g/L (20 °C)

pH of the aqueous solution at 10 g/l: 6 -7

Water: 50 g/l (19.5 °C)

Freely soluble in water

Freely soluble in alcohol, chloroform; very slightly soluble in benzene and ether.

SCCS comment

The method(s) for the determination of the 3 different water solubilities of cetylpyridinium chloride is not reported.

3.1.7. Partition coefficient (Log P_{ow})

Log Pow= 1.71 (calculated)

Log Pow= 1.78 (measured value)

SCCS comment

Method for the measurement of Log Pow is not described.

3.1.8. Additional physical and chemical specifications

Melting point:	77-83 °C
Boiling point:	- not applicable, decomposition at 234 °C, the substance decomposes before boiling
Flash point:	/
Vapour pressure:	5.5x10 ⁻⁶ Pa (non-charged molecule at 25 °C) (calculated)
Density:	~ 0.370 g/ml (bulk density) Relative density not reported

Revision of the Opinion on Cetylpyridinium chloride (P97)

Viscosity:	/
pKa:	/
Refractive index:	/
pH:	/
UV_Vis spectrum (200-800 nm):	Acidic pH: absorbance is 0.4990 (at λ 259.28 nm) and 0.5391 (at λ 214.03 nm); corresponding molar absorption coefficients: 4322.1 and 4669.5 Neutral pH: absorbance is 0.5068 (at λ 259.20 nm) and 0.5437 (at λ 214.14 nm); corresponding molar absorption coefficients: 4389.7 and 4709.3 Basic pH: absorbance is 0.5068 (at λ 259.23 nm); corresponding molar absorption coefficient: 4389.7

3.1.9. Homogeneity and Stability

Thermal decomposition at 234 °C

CPC has been demonstrated to be stable under normal storage conditions.

It is known that quaternary ammonium compounds degrade at elevated temperatures by a Hoffman elimination. In the case of CPC, this degradation will form hexadecane and pyridine-HCl. The temperature for this degradation to occur is about 130 °C. The stability of CPC monohydrate has been demonstrated through a stability testing programme. The supplier tested for the presence of hexadecane and cetyl chloride in CPC samples over a 5-year period. Hexadecane and cetylchloride were <0.05% in the stability samples over this time.

The stability of CPC (D1470.01) was determined at concentrations of 2 and 50 mg/ml (Ref.: 4) and of 5 mg/ml (Ref.: 6). The results showed that the formulations were stable for 10 days at room temperature. In the rat teratogenicity study, the test formulations stored for 10 days under ambient conditions contained 99-108% of the initial day 0 levels confirming stability under the conditions of storage.

Overall SCCS comment to physico-chemical characterisation

The impurities in commercial cetylpyridinium chloride have not been described. The applicant has declared that "*No impurities of toxicological or ecotoxicological concern or impurities which exceed 1 g/kg are present in technical grade CPC.*" However, qualitative and quantitative impurities, which may be present at <1 g/kg level, have not been reported.

3.2. Function and uses

Cetylpyridinium chloride is used as a private area and public health area disinfectant and in other biocidal products used for the disinfection of air, surfaces, materials, equipment and furniture that are not used for direct food or feed contact in private, public and industrial areas, including hospitals, as well as in products used as algacides. Usage areas include, inter alia, swimming pools, aquariums, bathing and other waters; air-conditioning systems; walls and floors in health and other institutions; chemical toilets, waste water, hospital waste, soil or other substrates (in playgrounds).

According to COMMISSION REGULATION (EC) No 1451/2007 (of 4 December 2007 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal

products on the market) cetylpyridinium chloride is used as biocide in product categories 1-7, 9 and 20 of the biocide directive.

The present submission applies for inclusion of Cetylpyridinium chloride in Annex VI of the Cosmetics Directive 76/768/ECC, soon in Annex V of the Cosmetic Regulation (EC) n.1223/2009.

Request for use in:

- *mouthwashes cosmetic products up to a concentration of 0.1 %*
- *all other oral hygiene cosmetic products up to a concentration of 0.5 %*
- *skin lotions and creams up to a concentration of 0.2 %*
- *anti-perspirant deodorants up to a concentration of 2.0 %*

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	OECD TG 425 (2001)
Species/strain:	Rat, Sprague-Dawley derived, albino
Group size:	7 females
Test substance:	Cetylpyridinium chloride
Batch:	Lot #00217966
Purity:	100%
Vehicle:	water
Dose levels:	95, 300, 950 mg/kg bw, 40% concentration
Administration:	gavage
GLP:	in compliance
Study period:	9 February – 27 May 2005

A default starting dose level of 95 mg/kg bw was administered to one healthy female rat by oral gavage. Following the 'Up and Down Procedure', six additional female rats were dosed at levels of 300 or 950 mg/kg bw, 3 animals at each dose level. The test substance was administered as a 40% w/w mixture in distilled water. All animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for up to 14 days after dosing. Body weights were recorded prior to administration and again on days 7 and 14 (termination) after dosing or after death. Necropsies were performed on all animals.

Results

The animal dosed with 95 mg/kg bw survived, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse pharmacological effects, abnormal behaviour, or gross abnormalities when the animal was necropsied at the conclusion of the 14-day observation period.

All animals dosed with 300 mg/kg bw survived and gained body weight during the study. Two animals exhibited soft faeces, reduced faecal volume, or piloerection following administration of the test substance, but recovered by day 2 and appeared active and healthy for the remainder of the 14-day observation period. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

All animals dosed with 950 mg/kg bw died within two days of test substance administration. Toxic signs noted prior to death included diarrhoea, ano-genital staining, piloerection, hypoactivity, hunched posture, and reduced faecal volume. Gross necropsy of the decedents

Revision of the Opinion on Cetylpyridinium chloride (P97)

revealed discoloration of the intestines and/or lungs and/or gaseous distention of the intestines.

Conclusion

Under the conditions of this study, the acute oral LD₅₀ value of cetylpyridinium chloride was estimated to be 560.3 mg/kg bw in female rats with an approximate 95% confidence interval of 950 mg/kg bw (upper) and 300 mg/kg bw (lower).

Ref.: 17

3.3.1.2. Acute dermal toxicity

Guideline: OECD TG 402 (1987)
 Species/strain: Rat, Sprague-Dawley derived, albino
 Group size: 5 males, 5 females
 Test substance: Cetylpyridinium chloride
 Batch: Lot #00217966
 Purity: 100%
 Vehicle: water
 Dose levels: 5000 mg/kg bw, 65% concentration (dry paste)
 Administration: dermal
 GLP: in compliance
 Study period: 24 January – 27 May 2005

The test substance was moistened with distilled water to achieve a dry paste by preparing a 65% w/w mixture; 5000 mg/kg bw of the test substance was then applied to the skin of ten healthy rats for 24 hours. The animals were observed for mortality, signs of gross toxicity, and behavioural changes at least once daily for 14 days. Body weights were recorded prior to application and again on days 7 and 14 (termination). Necropsies were performed on all animals.

Results

All animals survived and gained body weight during the study. Following application, two females exhibited irregular respiration, but recovered by day 2 and appeared active and healthy for the remainder of the 14-day observation period. Dermal irritation (erythema, oedema, hyperkeratosis, and eschar) was noted at the dose site of all animals throughout the study. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

Conclusion

Under the conditions of this study, the single dose acute dermal LD₅₀ value of cetylpyridinium chloride was greater than 5000 mg/kg bw in male and female rats.

Ref.: 18

3.3.1.3. Acute inhalation toxicity

Guideline: OECD TG 403 (1981)
 Species/strain: Rat, Sprague-Dawley derived, albino
 Group size: 20 (5 per sex and per dose)
 Test substance: Cetylpyridinium chloride
 Batch: Lot #00217966
 Purity: 100%
 Vehicle: /

Revision of the Opinion on Cetylpyridinium chloride (P97)

Dose levels: 0.05 and 0.5 mg/l
 Administration: inhalation, nose only
 GLP: in compliance
 Study period: 24 January – 27 May 2005

Twenty healthy rats (5/sex/group) were selected for testing and equally distributed into two exposure groups of 0.05 and 0.5 mg/l. Each group of animals was exposed to the test atmosphere for 4 hours. Chamber concentration and particle size distributions of the test atmosphere were determined periodically during each exposure period. The animals were observed for mortality, signs of gross toxicity, and behavioural changes at least once daily for up to 14 days following exposure. Body weights were recorded prior to exposure and again on days 7 and 14 or after death. Necropsies were performed on all animals.

Results

All animals exposed to the test atmosphere of 0.05 mg/l survived and gained body weight over the 14-day observation period. Following exposure, clinical observations observed for four animals included reduced faecal volume and hypoactivity, but recovered by day 3 and appeared active and healthy for the remainder of the 14-day observation period. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period. The gravimetric and nominal chamber concentrations were 0.054 and 0.14 mg/l, respectively, and the mass median aerodynamic diameter was estimated to be 2.8 µm.

All five males and four females died within three days of exposure to the test atmosphere of 0.5 mg/l. Toxic signs noted prior to death included abnormal respiration, abnormal posture, and hypoactivity. The surviving female exhibited similar clinical signs as well as reduced faecal volume, but recovered by day 7 and appeared active and healthy for the remainder of the 14-day observation period. Although this animal lost weight through day 7, it gained body weight over the 14-day observation period. Gross necropsy of the decedents revealed discoloration and oedema of the lungs and mucous-filled tracheas. No gross abnormalities were noted for the euthanised animal when necropsied at the conclusion of the 14-day observation period. The gravimetric and nominal chamber concentrations were 0.51 and 1.24 mg/l, respectively, and the mass median aerodynamic diameter was estimated to be 2.9 µm.

Conclusion

Under the conditions of this study, the acute inhalation LC₅₀ of cetylpyridinium chloride was between 0.054 and 0.51 mg/l in male and female rats.

Ref.: 19

Overall SCCS comment to acute toxicity studies

The studies on acute oral, dermal and inhalation toxicity described above and performed according to currently accepted test guidelines are the studies included in the Company Submission but not in Submission II. Submission II includes several studies of older date and generally not performed according to currently accepted test guidelines; these studies have not been evaluated in this opinion as the more recent studies performed according to currently accepted test guidelines are considered to be more reliable.

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline: OECD TG 404 (2002)
 Species/strain: Rabbit, New Zealand, albino

Revision of the Opinion on Cetylpyridinium chloride (P97)

Group size: 1 male, 2 females
Test substance: Cetylpyridinium chloride
Batch: Lot #00217966
Purity: 100%
Vehicle: water
Dose level: 0.77 g of a dry paste by preparing a 65% w/w mixture
Dose volume: /
GLP: in compliance
Study period: 24 January – 27 May 2005

Prior to application, the test substance was moistened with distilled water to achieve a dry paste (65% w/w mixture) in order to ensure adequate contact with the skin (70-80% mixtures were too dry to assure adequate skin contact). 0.4 g of the test substance (0.77 g of the test mixture) was applied to the skin of three healthy rabbits under occlusive patches. After 4 hours of exposure, the occlusive patches were removed and the test sites were gently cleansed of any residual test substance. Individual dose sites were scored according to the Draize scoring system at approximately 1, 24, 48, and 72 hours, and at 7, 10, and 14 days after patch removal.

Results

All animals appeared active and healthy. Apart from the dermal irritation noted below, there were no other signs of gross toxicity, adverse pharmacological effects, or abnormal behaviour.

Within 24, 48 and 72 hours of patch removal, all three treated sites exhibited moderate to severe erythema (mean score: 3.0) and slight oedema (mean score: 2.0). Although the overall severity of irritation gradually decreased, dermal irritation (erythema, oedema, desquamation, and/or superficial eschar) persisted for all animals throughout the 14-day observation period (mean score at day 14 for erythema of 1.7 and for oedema of 1.3).

Conclusion

Under the conditions of this study, the test substance was classified as moderately irritating to the skin.

Ref.: 20

SCCS comment

The data indicate that the test substance has a moderate to severe irritant potential to the skin of the rabbit under the conditions of the experiment. The skin irritation potential is considered as severe because the mean score was 3.0 for erythema and irritation was observed throughout the 14-day observation period, i.e. not reversible within the observation period.

Overall SCCS comment to skin irritation studies

The study on skin irritation described above and performed according to a currently accepted test guideline is the study included in the Company Submission but not in Submission II. Submission II includes several studies of older date that were in general not performed according to currently accepted test guidelines, and which were only reported in secondary literature; these studies have not been evaluated in this Opinion because the more recent study performed according to a currently accepted test guideline is considered to be more reliable.

3.3.2.2. Mucous membrane / Eye irritation

Guideline: OECD TG 405 (2002)
 Species/strain: Rabbit, New Zealand, albino
 Group size: 3 males
 Test substance: Cetylpyridinium chloride
 Batch: Lot #00217966
 Purity: 100%
 Vehicle: water
 Dose level: 0.04 g
 Dose volume: /
 GLP: in compliance
 Study period: 24 January – 27 May 2005

Prior to instillation of the test substance, two drops of ocular anesthetic (tetracaine hydrochloride ophthalmic solution, 0.5%) were placed into both the treated and control eye of each animal. 0.1 ml (0.04 g) of the test substance was then instilled into the conjunctival sac of the right eye of each rabbit. The other eye of each rabbit remained untreated with the test substance and served as a control. Ocular irritation was evaluated according to the Draize scoring system at 1, 24, 48, and 72 hours and at 4 days post-instillation.

Results

One animal exhibited reduced faecal volume on day 5; the other two animals appeared active and healthy during the entire observation period.

Within 24 hours of test substance instillation, all three treated eyes exhibited corneal opacity (mean score: 1.0), iritis (mean score: 3.0), and conjunctivitis (redness: mean score of 2.0; chemosis: mean score of 4.0; discharge: mean score of 3.0). The overall severity of irritation increased by 48 hours and remained extreme through day 4 (corneal opacity: mean score of 3.0; iritis: mean score of 2.0; conjunctival redness: mean score of 2.0; conjunctival chemosis: mean score of 4.0; conjunctival discharge: mean score of 2.3). On day 5, the study was terminated and all animals were euthanised for humane reasons.

Conclusion

Based on the results of this study, the irritation was deemed irreversible by the study report author.

Ref.: 21

SCCS comment

The data indicate that the test substance has an extreme irritant potential to the eyes of the rabbit under the conditions of the experiment.

Overall SCCS comment to eye irritation studies

The study on eye irritation described above and performed according to a currently accepted test guideline is the study included in the Company Submission but not in Submission II. Submission II includes several studies of older date that were in general not performed according to currently accepted test guidelines, and/or only reported in secondary literature or available as poorly readable pdf-files; these studies have not been evaluated in this Opinion because the more recent study performed according to a currently accepted test guideline is considered to be more reliable.

3.3.2.3. Oral mucosal irritation

The following text is reproduced from applicant's summary and conclusion:

CPC-containing mouthrinses have been tested in numerous oral mucosal irritation tests in Beagle dogs in concentrations ranging from 0.01 to 0.45% and generally found to have either little or no effect on the oral mucosa.

Generally, these CPC containing formulations were applied with saturated dental plugs either 3 or 5 times a day, with or without water rinsing for 4 days. The dental plugs were typically applied to the oral mucosa for 15 seconds. If a well defined area of mucosal irritation was noted 18 hours after the last daily treatment, the animal was removed from the study. These studies utilised a fluorescein rinse to detect very minor changes or "rough spots" on oral mucosal surfaces, so this method is very sensitive (could detect changes not evident to the naked eye). Each group normally consisted of three dogs.

Overall, twenty-six oral mucosal irritation tests have been performed from 1969 - 1990. Twelve studies were on CPC concentrations of 0.045% which found it to be generally non-irritating with rinsing conditions, and occasionally mildly irritating in no-rinse groups.

One study is representative of the 0.045% CPC studies. This study also included a high concentration of 0.45% CPC mouthrinse treatment. In this study, all animals completed both treatments following either 5x or 3x a day application with no rinse conditions resulting in no evidence of oral mucosal irritation or staining.

Four formulations containing 0.045% CPC applied 5x or 3x a day under no rinse conditions were not irritating to the oral muscosal tissue, while three of the 0.045% CPC formulations applied 5x or 3x a day under rinse and no rinse conditions showed only slight sloughing in some of the no rinse groups.

Eleven studies of mouthrinse containing between 0.075 and 0.25% CPC have been conducted.

A concentration of 0.25% showed no evidence of oral mucosal irritation when applied 5x or 3x a day with no rinse conditions as well as 3x a day with rinse.

A study with 0.1% CPC and 12% ethanol formulation showed no irritation in 3/6 dogs and 1 each of slight, moderate or severe in the remaining dogs when applied 5x a day with no rinse.

Other studies with a formulation containing 0.1% CPC and 5.5% ethanol produced either only slight sloughing in 3 dogs when applied 5x a day with no rinse or only slight staining and slight sloughing when applied 5x or 3x a day in both rinse and no rinse conditions.

In those instances where irritation was observed it is likely that alcohol, increased alcohol concentration, or other ingredients may have been responsible.

One study involving 0.086% CPC formulation applied 5x a day under no rinse conditions resulted in slight sloughing in one dog after 5 treatments and only slight staining in two dogs after 20 treatments.

Three studies utilising 0.075% CPC formulations showed no evidence of irritation when applied 5x or 3x a day under no rinse conditions.

A study with treatments containing 0.075% and 0.1% CPC (mentioned above) applied 5x or 3x a day with both rinse and no rinse conditions showed only slight staining and slight sloughing.

Conclusion:

Various mouthrinse formulations containing CPC between 0.045% and 0.45% were generally found to have either little or no effect on the oral mucosa of Beagle dogs. These

studies utilised a sensitive method (fluorescein rinse) to detect very minor changes on oral mucosal surfaces not evident to the naked eye.

In one study, 0.1% CPC solutions were found to be severely irritating to 1 dog but only slightly to moderately irritating to two other dogs and entirely non-irritating to three dogs in the same study, while in another study 0.1% CPC was only slightly irritating to the three dogs in the study.

Concentrations of 0.25% and 0.45% CPC formulations did not cause irritation.

In instances where irritation was observed, it is likely that other ingredients such as alcohol in the mouthrinse formulation may have been responsible.

Overall SCCS comment to oral mucosal irritation

Based on the submitted studies, the SCCS considers that cetylpyridinium chloride may be slightly irritating to the oral mucosa when used as a preservative in mouthwashes, cosmetic products up to a concentration of 0.1%, or in all other oral hygiene cosmetic products up to a concentration of 0.5%, cf. Terms of Reference.

3.3.3. Skin sensitisation

Guideline:	OECD TG 406 (1992)
Species/strain:	Guinea pigs, Hartley, albino
Group size:	36, males and females
Test substance:	Cetylpyridinium chloride
Batch:	Lot #00217966
Purity:	100%
Vehicle:	water
Concentration:	5%
Positive control:	alpha-Hexylcinnamaldehyde (75% w/w mixture in mineral oil)
GLP:	in compliance
Study period:	24 January – 27 May 2005

The study was conducted according to the Buehler Method.

Induction phase: 0.4 ml of a 5% w/w mixture of the test substance in distilled water was topically applied to the left side of each test animal (20 guinea pigs) using an occlusive 25 mm Hill Top Chamber once a week for three weeks. After the 6-hour exposure period, the chambers were removed and the test sites were gently cleansed of any residual test substance. Approximately 24 and 48 hours after each induction application, local reactions (erythema) were scored.

Challenge phase: Twenty-seven days after the first induction dose, 0.4 ml of a 0.1% w/w mixture of the test substance in distilled water (highest non-irritating concentration) was applied to a naive site on the right side of each animal as a challenge dose, using the same procedure as for the induction phase. These sites were evaluated for a sensitisation response (erythema) approximately 24 and 48 hours after the challenge application.

Naive control group: Ten guinea pigs were maintained under the same environmental conditions and treated with the test substance at challenge only.

Historical positive control group: The procedures used in this study were validated using alpha-Hexylcinnamaldehyde Technical (HCA) as a positive control substance. The most recent validation was completed on March 4, 2005.

Two indices were used for the evaluation of the sensitisation response at challenge, one for incidence and one for severity. The incidence index (the number of erythema scores greater than 0.5 divided by the number of animals evaluated) and the severity index (the sum of erythema scores divided by the number of animals evaluated) are presented for both the 24- and 48-hour intervals after challenge evaluation.

The following criteria were used to classify the test substance as a potential contact sensitiser: At the 24-hour and/or 48-hour scoring interval, 15% or more of the test animals

Revision of the Opinion on Cetylpyridinium chloride (P97)

exhibit a positive response (scores > 0.5) in the absence of similar results in the vehicle control group. The positive reaction at the 24-hour interval must persist to 48 hours in at least one test animal.

The test substance was expected to be stable for the duration of testing.

Results

Induction phase: Very faint to faint erythema (scores: 0.5-1) was noted for all test sites in both test animals and in the historical positive control animals at 24 and 48 hours.

Challenge phase: Very faint erythema (score: 0.5) was noted for 7/20 test sites at 24 and 48 hours following the challenge application. Very faint erythema (score: 0.5) was noted for 2/10 naive control test sites at 24 hours and persisted at one site through 48 hours. Faint erythema (score: 1) was noted for 4/9 and very faint erythema (score: 0.5) was noted for 4/9 historical positive control animals at 24 and 48 hours following the challenge application. Very faint erythema (score: 0.5) was noted for 3/5 positive control naive control test sites at 24 hours and persisted at one site through 48 hours.

Conclusion

Based on the findings and on the evaluation system used, cetylpyridinium chloride is not considered to be a contact sensitizer. The positive response observed in the historical positive control validation study with alpha-hexylcinnamaldehyde validates the test system used in this study.

Ref.: 22

SCCS comment

Information of stability of the test substance was provided by the sponsor. It is not mentioned in the study report whether the test solutions were prepared each time prior to application.

Based on the findings and on the evaluation system used, cetylpyridinium chloride did not show contact sensitising potential.

It is noted that a concurrent positive control group was not included in the study. The SCCS, however, agrees that the historical positive control validation study with alpha-hexylcinnamaldehyde conducted at the same time as the test guideline study can be used as a validation for the test system used in the test guideline study.

The concentration of the positive control used in the historical positive control validation study is not clear, as '75% w/w mixture in mineral oil' is stated at several places in the text whereas 'undiluted' is stated in the table presenting the reaction scores for the induction phase.

The study on skin sensitisation described above and performed according to a currently accepted test guideline is the study included in the Company Submission, but not in Submission II.

Guideline:	not stated
Species/strain:	Guinea pigs, Hartley, albino
Group size:	30 (20 treated, 10 controls)
Test substance:	Cetylpyridinium chloride
Batch:	Lot #15629
Purity:	99%
Vehicle:	water
Concentration:	induction 5%, challenge 0.5%
Positive control:	none
GLP:	/
Study period:	1977

Revision of the Opinion on Cetylpyridinium chloride (P97)

The study was conducted according to the Buehler Method.

Induction phase: Patches were soaked with 0.5 ml of a 5% solution and applied on the clipped upper quadrant of the backs of 20 animals. Patches were removed after 6 hours. Re-application to the same site was performed once per week, for a total of three applications. The 10 control animals were not exposed until challenge.

Challenge phase: Two weeks after the last induction the lower quadrants of the backs of all animals were clipped, and on the following day a 0.5% solution of CPC was applied on treated animals and controls. Scoring of skin reactions was done the next day (24 hrs after the challenge) and again 48 hrs after challenge.

Results:

None of the treated animals showed a skin reaction at 24 hrs and 48 hrs. Of the 10 control animals, only one showed slight patchy erythema.

Conclusion:

The test did not show evidence of sensitisation.

Ref.: 1

A report on the toxicity profile of CPC refers to an intradermal study in ten guinea pigs, performed in 1970, showing inconclusive results (TNO/BIBRA 1988).

A Buehler test has been performed with a shampoo that contained, besides other ingredients such as a fluoropolymer, CPC 0.31% (Ritz 1975). The tests appeared to be intended for the evaluation of the fluoropolymer. Therefore this report will not be evaluated here.

In one report, the results of two Buehler tests are described (Winrow 1979). The tests were performed with shampoos that contained, besides CPC, other ingredients such as a fluoropolymer and a fluorocarbon surfactant. Because of the difficulties regarding a correct interpretation of the test results pertaining to CPC, this report will not be further evaluated here.

Overall SCCS comment to skin sensitisation

The skin sensitisation studies described above were based on the Buehler test method. This test is regarded as less sensitive than the GPMT adjuvant test method and may underestimate the sensitising potential. Reports of studies on CPC with LLNA could not be identified. In humans contact sensitisation to CPC has been reported (see 3.3.11 Human data).

3.3.4. Dermal / percutaneous absorption

***In vitro* dermal absorption**

Guideline:	OECD 428 (2002) Species/strain: Frozen human post-mortem epidermis
Membrane integrity:	Assessed by measurement of their electrical resistance across the skin membrane. Membranes with a measured resistance < 10kΩ were excluded
Group size:	6 intact membranes from 4 different subjects
Test substance:	Cetylpyridinium Chloride monohydrate

Revision of the Opinion on Cetylpyridinium chloride (P97)

Test batch:	00216056
Purity:	99.8% (w/w)
Test item:	0.2% (w/w) Cetylpyridinium chloride oil-in-water formulation
Radiolabel:	¹⁴ C-Cetylpyridinium Chloride, [pyridine-2,6- ¹⁴ C] (specific activity 1.998GBq/mmol, radiochemical purity 99.1% as confirmed by thin layer chromatography)
Exposed membrane area:	2.54cm ²
Dose applied:	2 mg/cm ² (i.e. 5.08 mg formulation per cell ≈ 4 µg Cetylpyridinium chloride/cm ²)
Sample volume:	100 µl
Sampling period:	24 hours
Receptor fluid:	Physiological saline
Mass balance analysis:	Provided
Tape stripping:	Yes (5)
Carbon filter:	No
Method of Analysis:	Liquid Scintillation Counting
GLP:	In compliance
Study period:	28 August - 16 September 2003

The absorption and distribution of Cetylpyridinium chloride from a nominal 0.2% w/w oil-in-water emulsion formulation has been measured *in vitro* through human epidermis. The formulation was applied undiluted (2 mg/cm²= 4µg Cetylpyridinium chloride/cm², total amount applied = 5.08 mg/cell) and left unoccluded at 32°C for an exposure period of 24 hours. Six diffusion cells containing intact skin membranes from 4 subjects were used. A fraction of the Cetylpyridinium chloride included ¹⁴C-cetylpyridinium chloride, [pyridine-2,6-¹⁴C]. At regular intervals (1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24h), the receptor fluid in the cell was sampled (100µl) and analysed by liquid scintillation counting. Upon completion of the exposures, the mass balance of the applied dose was determined. Successive layers of the stratum corneum were removed by tape stripping (5 strips) and the Cetylpyridinium chloride content was determined.

Results

The recovery for Cetylpyridinium chloride in the test system was very good, with a mean recovery of 96.5% ± 19.4%.

The vast majority (mean 93.7 ± 18.2%) of the applied Cetylpyridinium chloride was removed from the surface of the skin during the washing procedure at the end of the 24 hour exposure period. The total mean amount recovered from tape strips (representing the *stratum corneum*) was 0.046 ± 0.023 µg/cm² (1.14 ± 0.57% of dose). The mean amounts in the individual strips indicated that Cetylpyridinium chloride concentrations in the *stratum corneum* decreased approximately 5-fold between the top-most strip (0.022 µg/cm²; 0.556% of dose) and the lowest (0.004 µg/cm²; 0.090% of dose).

The mean amount of Cetylpyridinium chloride penetrating through the epidermis into the receptor fluid was below the limit of quantification (i.e. <0.003 µg/cm² or <0.07% of dose), and a mean of 0.052 µg/cm² (1.30% of dose) was extracted from the epidermis remaining after the tape-stripping procedure. The mean absorbed proportion of the dose was therefore regarded as 0.054 µg/cm² (1.3% of dose).

Conclusion

The results obtained in this study indicate that the systemic absorption of Cetylpyridinium chloride following application of this oil-in-water emulsion formulation to human skin will be very low. In normal use of the formulation, the vast majority of the applied dose would be removed from the surface of the skin by normal washing procedures.

SCCS comment

According to the Notes of Guidance, at least 8 skin samples obtained from 4 different donors should be used. Here, 4 different donors were used and replicates were only done for 2 of the 4 donor skin samples making 6 skin samples in total. The thickness of the skin is not given and there is no indication of the freezing period of the epidermal membranes before use. No information on the pH of the test formulation is provided. Individual mass balances range between 77.9% and 121%; only 1 of the 6 mass balances is within the limit of $100 \pm 15\%$. For liquid formulations, it is recommended to use up to 10 μl instead of 2 mg/cm^2 . In addition, as pure epidermis instead of skin samples were used, the material available in the 5 tape strips should have been considered as systemically available.

The SCCS notes that the pages 12 and 13 are missing in the study report.

Because of all these major deviations, the dermal absorption of body lotion formulations containing 0.2% (w/w) cetylpyridinium chloride cannot be estimated based on the presented data.

Guideline:	OECD 428 (2002)
Species/strain:	Frozen human epidermis taken post-mortem and/or from surgery
Membrane integrity:	Assessed by measurement of their electrical resistance across the skin membrane. Membranes with a measured resistance < 10k Ω were excluded
Group size:	6 intact membranes from 6 different subjects
Test substance:	Cetylpyridinium Chloride monohydrate
Test batch:	00216056
Purity:	99.8% (w/w)
Test item:	2% (w/w) Cetylpyridinium chloride glycol/water formulation
Radiolabel:	^{14}C -Cetylpyridinium Chloride, [pyridine-2,6- ^{14}C] (specific activity 1.998GBq/mmol, radiochemical purity 99.1% as confirmed by thin layer chromatography)
Exposed membrane area:	2.54 cm^2
Dose applied:	5 mg/cm^2 (i.e. $\approx 100 \mu\text{g}$ Cetylpyridinium chloride/ cm^2)
Sample volume:	100 μl
Sampling period:	24 hours
Receptor fluid:	Physiological saline
Mass balance analysis:	Provided
Tape stripping:	Yes (5)
Carbon filter:	No
Method of Analysis:	Liquid Scintillation Counting
GLP:	In compliance
Study period:	28 August - 16 September 2003

The absorption and distribution of Cetylpyridinium chloride from a nominal 2% w/w glycol/water formulation has been measured *in vitro* through human epidermis. The formulation was applied undiluted (5 $\text{mg}/\text{cm}^2 = 100\mu\text{g}$ Cetylpyridinium chloride/ cm^2) and left unoccluded at 32°C for an exposure period of 24 hours. 6 diffusion cells containing intact skin membranes from 4 subjects were used. A fraction of the Cetylpyridinium chloride included ^{14}C -cetylpyridinium chloride, [pyridine-2,6- ^{14}C]. At regular intervals (1, 2, 3, 4, 6, 8, 10, 12, 20 and 24h), the receptor fluid in the cell was sampled (100 μl) and analyzed by liquid scintillation counting. Upon completion of the exposures, the mass balance of the applied dose was determined. Successive layers of the stratum corneum were removed by tape stripping (5 strips) and the Cetylpyridinium chloride content was determined. Although for one of the 6 cells, tape stripping was stopped prematurely since the underlying epidermis started to disintegrate. The mean amounts in the individual strips were used.

Cetylpyridinium chloride recovered from the remaining epidermis at the end of the exposure is also considered as being dermally absorbed, as it is recognised that a proportion of this material may become systematically available *in vivo* beyond the duration of the exposure investigated in this study.

Results

The recovery for Cetylpyridinium chloride in the test system was good, with a mean recovery of $91.0\% \pm 2.99\%$.

The vast majority (mean $87.2 \pm 4.0\%$) of the applied Cetylpyridinium chloride was removed from the surface of the skin during the washing procedure at the end of the 24-hour exposure period. The total mean amount recovered from tape strips (representing the *stratum corneum*) was $0.336 \pm 0.194 \mu\text{g}/\text{cm}^2$ ($0.336 \pm 0.194\%$ of dose). The mean amounts in the individual strips indicated that Cetylpyridinium chloride concentrations in the *stratum corneum* decreased approximately 4-fold between the top-most strip ($0.126 \mu\text{g}/\text{cm}^2$; 0.126% of dose) and the lowest ($0.035 \mu\text{g}/\text{cm}^2$; 0.035% of dose).

The mean amount of Cetylpyridinium chloride penetrating through the epidermis into the receptor fluid was below the limit of quantification (i.e. $<0.03 \mu\text{g}/\text{cm}^2$ or $<0.03\%$ of dose), and a mean of $0.567 \mu\text{g}/\text{cm}^2$ (0.567% of dose) was extracted from the epidermis remaining after the tape-stripping procedure. The mean absorbed proportion of the dose was therefore regarded as $0.597 \mu\text{g}/\text{cm}^2$ (0.597% of dose).

Conclusion

The results obtained in this study indicate that the systemic absorption of Cetylpyridinium chloride following application of this glycol/water formulation to human skin will be very low. In normal use of the formulation, the vast majority of the applied dose would be removed from the surface of the skin by normal washing procedures.

Ref.: 3

SCCS comment

According to the Notes of Guidance, at least 8 skin samples obtained from 4 different donors should be used. Here, four different donors were used, but triplicates were done for 1 out of the 4 donor skin samples whereas no replicates were done for the remaining 3 donors, making 6 skin samples in total. The thickness of the skin is not given and there is no indication of the freezing period of the epidermal membranes before use. No information on the pH of the test formulation is provided. The SCCS notes that the formulation tended to flow under the donor chambers as it was removed from the skin and therefore the donor chambers were washed and assumed as applied dose. For liquid formulations, it is recommended to use up to $10\mu\text{l}$ instead of 5mg per cm^2 . In addition, as pure epidermis instead of skin samples were used, the material available in the 5 tape strips should have been considered as systemically available.

The SCCS notes that the pages 14 and 15 are missing in the study report.

Because of all these major deviations, the dermal absorption of deodorant formulations containing 2% (w/w) cetylpyridinium chloride cannot be estimated based on the data presented.

Overall SCCS conclusion on dermal absorption

The SCCS notes that the two studies on dermal absorption in Submission II were also included in Submission I. The SCCP concluded that the quality of the dossier was such that an adequate assessment of Cetylpyridinium chloride (P97) was not possible. The SCCS agrees with the conclusion of the SCCP.

Because of all the major deviations as noted by the SCCS for the two studies on dermal absorption, the dermal absorption of cetylpyridinium chloride from body lotion formulations containing 0.2% (w/w) cetylpyridinium chloride or from deodorant formulations containing 2% (w/w) cetylpyridinium chloride cannot be estimated.

According to the Notes of Guidance (SCCS/1501/12), 100% dermal absorption is used in case data on dermal absorption are inadequate or unavailable. However, as the submitted *in vitro* studies on dermal absorption indicate a low dermal absorption and because cetylpyridinium chloride is a charged molecule, a value of 10% is used in the safety assessment for the calculation of the Margin of Safety (MoS).

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral toxicity

Guideline:	/
Species/strain:	Sprague Dawley Crl:CD rats
Group size:	8/sex/group
Test substance:	Cetylpyridinium chloride (CPC) (D1470.01)
Batch:	/
Purity:	Assumed to be 100%
Vehicle:	Deionised water
Dose levels:	0, 25, 50, 100, 200, 400 mg/kg bw
Dose volume:	10 ml/kg bw
Route:	Oral
Administration:	Gavage
GLP:	In compliance
Study period:	February 1993 to August 1994

CPC was administered once daily by gavage (vehicle: deionised water) to Sprague Dawley rats (8 animals/sex/group) at dose levels of 0, 25, 50, 100, 200, 400 mg/kg bw for 28 days. Rats were observed for mortality and moribundity twice daily. A thorough physical examination was conducted at each weighing interval. Food consumption and body weight were measured weekly. An ophthalmoscopic examination was performed on each animal prior to treatment and at termination. Necropsies were performed on all animals that died or were sacrificed in moribund condition during the study. Prior to scheduled sacrifice, blood and urine samples were collected for haematology, clinical chemistry, and urine analyses. After at least 28 days, necropsies were performed on all surviving animals. Organ weights were recorded for the following organs and tissues: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, stomach, testes with epididymides, and thyroid/parathyroids. Histopathology was performed on all surviving animals in the control group and at 100 mg/kg bw/day and any gross lesions from animals in all groups, and the stomachs in all animals at 50 mg/kg bw/day and females at 25 mg/kg bw/day. The following organs were examined microscopically: adrenals, aorta, brain, cervical spinal cord, colon, caecum and rectum, duodenum, jejunum, ileum and esophagus, exorbital lacrimal glands, eyes, femur, heart, kidneys, lesions, liver, lumbar spinal cord, lung, mammary gland, mesenteric lymph node, mid-thoracic spinal cord, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerve, seminal vesicles, skin, spleen, sternum with bone marrow, stomach, testes with epididymides, thigh musculature, thymus, thyroid with parathyroids, trachea, urinary bladder, and uterus with vagina and cervix.

The test material, D1470.01, described as a white powder, was received from the Sponsor, and stored at room temperature. For the purpose of dosing, the purity of the test material was assumed to be 100%. Information on the methods of synthesis and stability and data on composition or other characteristics which define the test material are on file with the Sponsor.

Results

Stability of the test material was determined at concentrations of 2 and 50 mg/ml and the results indicated that the formulations were stable for 10 days at room temperature and under refrigeration.

All animals at 400 mg/kg bw/day died by day 4. At 200 mg/kg bw/day animals died or were killed in extremis within 5 days after administration. At 100 mg/kg bw/day 3 males and 1 female died in week 1 and another male died in week 2. No mortality occurred in the 0, 25 and 50 mg/kg bw/day.

Clinical signs were observed at 100 mg/kg bw/day and above and included hypoactivity, mucoid/soft faeces, salivation, dyspnea, partially closed eyes, and crust formation at perinasal, perioral and front limbs.

Body weight loss was noted in week 1 in males at 100 mg/kg bw/day. Body weight gain was reduced at all dose levels (surviving animals): 80-86%, 68-76% and 49-77% of control at 25 (not statistically significant), 50 and 100 mg/kg bw/day.

Food consumption was significantly decreased at 100 mg/kg bw/day (78 and 83% of control for males and females, respectively) and decreased at 50 mg/kg bw/day in males (91% of control).

The haematological examination revealed that the erythrocyte count, haemoglobin and haematocrit were significantly increased in males at 100 mg/kg bw/day (108, 107 and 111% of control, respectively). Monocytes were present in males at 50 and 100 mg/kg bw/day in a dose-related manner compared to none in the control group.

Aspartate aminotransferase was increased at 50 and 100 mg/kg bw/day in males (not dose-dependent: 162 and 136% of control, respectively). Alanine aminotransferase was significantly increased at 50 mg/kg bw/day in males (215% of control) and at 100 mg/kg bw/day in both sexes (203-258% of control); the change was dose-related in males. Albumin was increased in males at 100 mg/kg bw/day (113% of control). Calcium was increased in both sexes at 100 mg/kg bw/day (109% of control).

Significant increases in relative organ weights were observed at 100 mg/kg bw/day for adrenals (males only), stomach (both sexes), brain (males only), heart (males only), testis and thyroid (females only). These changes were, according to the study report authors, attributed to the decreased body weights, except for the increases in relative stomach weights (which also were observed at 50 mg/kg bw/day although not significant) based on alterations in histopathology.

No apparent treatment-related effects were noted on gross pathology. The microscopic evaluation revealed acanthosis in the non-glandular stomach of 2/4 males and 4/7 females at 100 mg/kg bw/day and of 2/8 females at 50 mg/kg bw/day with concomitant necrosis/erosion in one female at each level. These histopathological changes were dose-related in incidence and severity, resulting in mortality for animals at 100 mg/kg bw/day and higher.

Conclusion

In conclusion, the target organ for CPC was the stomach and at dose levels from 50 mg/kg bw/day, it produced a localised toxic (i.e. irritant) action near or at the site of administration, specifically in the non-glandular region of the stomach. The NOAEL was considered to be 25 mg/kg bw/day in this study, based on histomorphological alterations in the non-glandular region of the stomach, i.e. forestomach.

Ref.: 4

SCCS comment

The SCCS notes that the purity of the test material was assumed to be 100% according to the study report; no information was provided by the Sponsor of the study.

The SCCS agrees on the NOAEL of 25 mg/kg bw/day for local effects in the forestomach probably due to the irritative properties of the test substance. The SCCS also considers 25 mg/kg bw/day as a NOAEL for systemic toxicity (decreased body weight gain) although it can be argued that the decreased body weight gain might be secondary to the local effects in the stomach.

Revision of the Opinion on Cetylpyridinium chloride (P97)

Guideline: /
Species/strain: Beagle dogs
Group size: 3/sex/group
Test substance: Cetylpyridinium chloride (CPC) (D1470.01)
Batch: /
Purity: /
Vehicle: /
Dose levels: 0, 5, 25, 125, 250, 500 mg/kg bw (in gelatin capsule)
Dose volume: /
Route: Oral
Administration: Gelatin capsule
GLP: In compliance
Study period: March 1993 to March 1994

CPC was administered once daily in a gelatin capsule to Beagle dogs (3 animals/sex/group) at dose levels of 0, 5, 25, 125, 250, 500 mg/kg bw for 28 days. Dogs were observed for mortality, moribundity or other overt signs of toxicity twice daily. Specific observations for pharmacotoxic signs were conducted each day just prior to dosing and about 2 hours after dosing. All animals were subjected to a detailed clinical examination once a week and included pharmacological and toxicological findings, as well as the occurrence, size, location and description of palpable masses. Food consumption and body weight were measured weekly. Ophthalmoscopic and electrocardiographic examinations were performed on each animal prior to dosing initiation and at week 4, just prior to study termination. Prior to scheduled sacrifice, blood and urine samples were collected for haematology, clinical chemistry, and urine analyses, except for high-dose animals where blood and urine samples were collected on day 11 from surviving animals prior to sacrifice. Complete necropsies were performed on all animals. Organ weights were recorded for all surviving animals for the following organs and tissues: adrenals, brain, heart, kidneys, liver, spleen, thymus, ovaries, pituitary, testes, and thyroid/parathyroids. Histopathology was performed on all target organs (thymus, stomach, oesophagus, duodenum, ileum, colon, rectum, and the mandibular, mesenteric and tracheobronchial lymph nodes) of all animals. Histopathology was also performed on the liver and kidney of all surviving animals at all dose levels. In addition, histopathology was performed on all surviving animals at dose levels of 25, 125, 250, 500 mg/kg bw for the following organs: adrenals, aorta, bone with bone marrow (femur), bone marrow smear, brain, cervical spinal cord, caecum, jejunum, eyes, heart, gross lesions, lumbar spinal cord, lung, mammary gland, thoracic spinal cord, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerve, skeletal muscle, skin, spleen, testes with epididymides, thyroid, parathyroids, trachea, urinary bladder, uterus, vagina and cervix.

Results

Clinical signs were observed at 25 mg/kg bw/day and above and included dehydration, abnormal head movements, decreased activity, decreased defaecation, emesis, mucoid diarrhoea, discoloured faeces and ptialism, and at 125 mg/kg bw/day also nasal discharge. At 500 mg/kg bw/day one male and one female were sacrificed in extremis and the surviving animals at this dose level were sacrificed on day 11. At 250 mg/kg bw/day 2 animals of each sex and at 125 mg/kg bw/day 2 males and 1 female were found dead or sacrificed in extremis.

The animals at 500 mg/kg bw/day showed marked body weight loss before death/sacrifice. Body weight loss was also noted in males at 250 mg/kg bw/day (81% of pretest weight) and in females at 25 mg/kg bw/day and above (92, 74 and 74% of pretest at 25, 125 and 250 mg/kg bw/day, respectively). Animals in the 5 and 25 mg/kg bw/day groups did not

vary significantly from their pretest weight, although the 25 mg/kg bw/day group did display some tendency toward a decrease in body weight.

Food consumption was markedly decreased at 25 mg/kg bw/day and higher, including the first 1.5 weeks at 500 mg/kg bw/day in both sexes (46-88% and 35-68% of control for males and females, respectively).

Erythrocyte count (128-129% of control), haemoglobin (124-126% of control), haematocrit (124-125% of control) and activated partial thromboplastin time (APTT, 152-160% of control) was increased in females at 125 and 250 mg/kg bw/day with most of the values being significant from controls and outside the historical reference range for the laboratory. In all animals that died during the study haemoglobin, haematocrit and erythrocyte count were increased; in most of these animals APTT was increased and several animals had increased leukocyte count, mainly segmented neutrophil count. Increased haemoglobin, haematocrit, erythrocyte count and leukocyte count (segmented neutrophils) were also seen on day 11 in surviving animals at 500 mg/kg bw/day.

Sodium, chloride and aspartate aminotransferase (ASAT) were decreased at 125 and 250 mg/kg bw/day in females. Urea nitrogen and globulin were increased at 125 and 250 mg/kg bw/d in females and concomitantly the A/G ratio was decreased. Most of the animals that died during the study had decreased sodium and chloride values, and increased in urea nitrogen, phosphorus, creatinine, total protein, glucose, alanine aminotransferase, aspartate aminotransferase and cholesterol values.

Increased relative adrenal and kidney weights were noted in females at 125 mg/kg bw/day and were considered by the study report authors to be secondary to the decreased body weight. The relative thymus weight was decreased (not statistically significant) in males at 125-500 mg/kg bw/day (39-68% of control) and in females at 5-500 mg/kg bw/day (28-83% of control) in a dose-related manner.

Macroscopic effects found in animals that died, were killed in extremis or killed after 11 days of exposure at 500 mg/kg bw/day or at termination of the study at 5, 25, 125 and 250 mg/kg bw/day were all local effects in the gastrointestinal tract and included red discolouration or foci in the stomach, duodenum, jejunum, ileum, caecum, colon and/or rectum at all dose levels in males and at 125 mg/kg bw/day and above in females; and erosions/ulcers in the stomach and colon of females. At the higher dose levels the effects were found further down the gastrointestinal tract.

These local effects were confirmed by histopathology. Erosions, ulcers and/or acute/subacute inflammation in the esophagus, stomach (cardia, fundus, pylorus), duodenum, ileum, colon and/or rectum were observed in both sexes at 125 and 250 mg/kg bw/day (animals dying during study and after 28-d exposure) and after 11 days exposure at 500 mg/kg bw/day. In the stomach, cardia erosion was noted in one male at 5 mg/kg bw/day and inflammation in one male at 25 mg/kg bw/day. Necrosis of the pylorus was noted in 1/2 females at 500 mg/kg bw/day and in the duodenum in 1/3 females at 125 mg/kg bw/day. Haemorrhage of the stomach fundus was noted in 1/2 females at 500 mg/kg bw/day and of the colon in 1/3 males at 125 mg/kg bw/day. Other histopathological findings included atrophy of the thymus at 125 and 250 mg/kg bw/day in both sexes, and lymphoid depletion and/or lymphadenitis in one or more lymph nodes (mandibular, mesenteric and tracheobronchial) in one or more animals at 125 and 250 mg/kg bw/day.

Conclusion

The NOAEL for systemic toxicity was considered to be 25 mg/kg bw/day in this study, based on mortality and decreased thymus weight confirmed by thymus atrophy at 125 mg/kg bw/day and above. Since the organ weight effects at 5 and 25 mg/kg bw/day were not confirmed by histopathological changes, they were not considered to be toxicologically relevant.

The NOAEL for local effects was < 5 mg/kg bw/day in this study, based on effects in the gastrointestinal tract at all dose levels.

SCCS comment

The SCCS notes that no information on the purity and stability of the test material was provided.

Overall SCCS comment to the submitted subacute toxicity studies

Two oral 28-day studies – one in rats and the other in dogs – are included in Submission II and in the Company Submission and described above. The SCCS notes that these two 28-day subacute toxicity studies were also included in Submission I. The SCCP concluded that the quality of the dossier was such that an adequate assessment of Cetylpyridinium chloride (P97) was not possible.

In addition to these two studies, Submission II (and Submission I) also includes two other oral 28-day studies. None of these studies have been evaluated in this Opinion because they were either submitted as a poorly legible pdf-file or were only described in secondary literature and therefore, are not considered to add any valuable information for the safety assessment of cetylpyridinium chloride as a cosmetic ingredient.

The full reports of a 14-day palatability rat study of cetylpyridinium chloride and of two 28-day feeding studies of cetylpyridinium chloride (one in rats and the other in dogs) were submitted to EFSA for the evaluation of the safety and efficacy of Cecure[®] (Ref.: 24); the active ingredient in Cecure[®] is cetylpyridinium chloride. The two rat studies which are also considered of relevance for the safety assessment of cetylpyridinium chloride as a cosmetic ingredient are summarised below, based on the descriptions in the EFSA opinion. The CEF Panel considered that no conclusions could be drawn from the 28-day dog study since as a result of treatment with CPC, the feed intake was strongly diminished, not allowing establishment of a dose-response. Furthermore, the number of animals was insufficient to allow characterisation of the observed effects. Thus, this study is not considered of relevance for the safety assessment of cetylpyridinium chloride as a cosmetic ingredient and therefore, not included in this Opinion.

In a 14-day palatability study, Sprague Dawley rats (5/sex/group) were given 0, 100, 500, 1000, 1500 and 2000 ppm of CPC in the diet (equivalent to 0, 5, 25, 50, 75 and 100 mg/kg bw/day) (Redfield Laboratories, 2001a, as cited in Ref.: 24). The study was performed under GLP and according to international guidelines. Regular observations included clinical parameters, body weights and feed consumption.

Thinness was observed in one high-dose female; the effect correlated with lower feed consumption and was considered treatment related. Body weight gains were decreased at 50 mg/kg bw/day and above for males and at 75 mg/kg bw/day and above for females. Decreased feed consumption was reported starting at 25 mg/kg bw/day and above, but only statistically significant at 50 mg/kg bw/day and above.

In a 28-day study, Sprague Dawley rats (10/sex/group) were administered 0, 125, 250, 375, 500, 750 and 1000 ppm of CPC in the diet (equivalent to 0, 6.25, 12.5, 18.7, 25, 37.5 and 50 mg/kg bw/day) (Redfield Laboratories, 2001b, as cited in Ref.: 24). The study was performed under GLP and according to international guidelines. Observations included body weight and feed consumption measured weekly. Haematology, clinical chemistry and urinalysis were evaluated at termination. All animals underwent gross necropsy and specific tissues underwent histopathology examination.

Body weights and body weight gains were significantly lower at 37.5 and 50 mg/kg bw/day; these effects were considered treatment-related and were attributed to a direct effect on

Revision of the Opinion on Cetylpyridinium chloride (P97)

feed consumption. Similar findings were reported in animals from the remaining treated groups but they were inconsistent. A dose-related decrease in feed consumption was reported in both sexes at 37.5 and 50 mg/kg bw/day and in females at 18.7 and 25 mg/kg bw/day. There were some changes in the haematology and in clinical chemistry, including lower total bilirubin concentration at all doses in females and higher glucose and higher aspartate aminotransferase activity in high-dose males. Several differences in absolute and relative organ weights were reported without histopathology changes. In males, there was a consistent dose-related increase in relative weight for caecum and testes being statistically significant from the 37.5 and the 50 mg/kg bw/day groups, respectively. In females, there was a consistent dose-related increase in relative weight for caecum (18.7, 37.5 and 50 mg/kg bw/day groups), although not statistically significant at 25 mg/kg bw/day.

3.3.5.2. Sub-chronic (180 / 90 days) oral toxicity
--

Guideline:	OECD TG 407 (1981), but with a 6-month exposure period
Species/strain:	Sprague Dawley CrI:CD rats
Group size:	20/sex/group
Test substance:	Cetylpyridinium chloride (CPC) (D1470.01)
Batch:	/
Purity:	Assumed to be 100%
Vehicle:	Deionised water
Dose levels:	0, 5, 15, 40, 75 mg/kg bw
Dose volume:	10 ml/kg bw
Route:	Oral
Administration:	Gavage
GLP:	In compliance
Study period:	July 1993 to January 1995

CPC was administered once daily by gavage (vehicle: deionised water) to Sprague Dawley rats (20 animals/sex/group) at dose levels of 0, 5, 15, 40, 75 mg/kg bw for 6 months. The study was performed according to OECD TG 407 with the following deviations: Instead of 10 animals, 20 animals were used per dose; functional observations were not performed; total cholesterol was not measured. The deviations were not considered to have influenced the validity of the study. Rats were observed for mortality and moribundity twice daily at least 6 hours apart. A thorough physical examination was conducted at each weighing interval. A careful cageside observation for obvious indications of toxic effects was performed once daily. Food consumption and body weights were measured weekly. An ophthalmoscopic examination was performed on each animal prior to treatment and during the final week of treatment. Prior to scheduled sacrifice, blood and urine samples were collected for haematology, clinical chemistry, and urine analyses. Necropsies were performed on all animals. Organ weights were recorded on all animals for the following organs and tissues: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary, spleen, stomach, testes, thymus, and thyroid/parathyroids. Histopathology was performed on all surviving animals in the control group and at 75 mg/kg bw/day and all animals that died. The following organs were examined microscopically: adrenals, aorta, brain, bone marrow (sternum), cervical spinal cord, colon, caecum and rectum, duodenum, jejunum, ileum and esophagus, exorbital lacrimal glands, eyes, femur (including bone marrow), heart, kidneys, lesions, liver, lumbar spinal cord, lung, mammary gland, mesenteric lymph node, mid-thoracic spinal cord, ovaries, pancreas, pituitary, prostate, salivary glands (mandibular), sciatic nerve, seminal vesicles, skin, spleen, stomach, submandibular lymph nodes, testes with epididymides, thigh musculature, thymus, thyroid with parathyroids, trachea, urinary bladder, uterus, vagina and cervix. Gross lesions, lungs, livers, stomachs (glandular and non-glandular portions), and kidneys from all the animals were examined microscopically.

The test material, D1470.01, described as a white powder, was received from the Sponsor and stored at room temperature. For the purpose of dosing, the purity of the test material was assumed to be 100%. Information on the methods of synthesis and stability and data on composition or other characteristics which define the test material are on file with the Sponsor.

Results

Stability of the test material was determined at a concentration of 5 mg/ml and the results indicated that the formulation was stable for 10 days at room temperature and under refrigeration.

Adjusted survival rates at week 26 were 100, 95, 100, 94, and 95% for males at 0, 5, 15, 40, 75 mg/kg bw/day, respectively, and 100% for each of the female groups.

Clinical signs observed in males and females at 75 mg/kg bw/day included few/soft faeces, salivation, abnormal respiratory sounds and urine stains (females). Males and females at 40 and males at 15 mg/kg bw/day also showed an increased incidence of abnormal respiratory sounds.

Body weights were decreased from week 4 onwards in animals at 75 mg/kg bw/day and from week 16 and 6 onwards in males and females, respectively, at 40 mg/kg bw/day. Body weight loss was noted occasionally in animals at 75 mg/kg bw/day. Body weight gain was significantly reduced at 40 and 75 mg/kg bw/day in males (64-81% of control) and females (69-80% of control) and in females at 15 mg/kg bw/day (86% of control).

Food consumption was significantly decreased at 75 mg/kg bw/day in males (89% of control) and in females (92% of control).

Erythrocyte count and haematocrit were significantly increased at 75 mg/kg bw/day in males (both 104% of control) and females (107 and 106% of control), and erythrocyte count was statistically significantly increased at 40 mg/kg bw/day in females (104% of control). Haemoglobin was increased in females at 75 mg/kg bw/day (104% of control) and mean cell haemoglobin and mean cell haemoglobin concentration were significantly decreased in these females (97 and 98% of control, respectively). Platelet count was significantly increased at 75 mg/kg bw/day in females (114% of control). Prothrombin times were significantly longer at 15-75 mg/kg bw/day in females (104-107% of control).

Glucose was significantly decreased at 40 and 75 mg/kg bw/day in males and females (82-88% of control) in a dose-related manner. Urea was significantly increased at 40 and 75 mg/kg bw/day in males (117-125% of control) in a dose-related manner. Total protein was decreased in both sexes at 75 mg/kg bw/day (91-94% of control) and albumin was decreased in females at 75 mg/kg bw/day (90% of control). Aspartate aminotransferase was significantly increased at 75 mg/kg bw/day in both sexes (both 118% of control) and alanine aminotransferase was significantly increased at 75 mg/kg bw/day in males (205% of control) and females (160% of control).

The relative stomach weight was significantly increased in both sexes at 75 mg/kg bw/day (152-163% of control) and at 40 mg/kg bw/day (123-130% of control), and in males at 15 mg/kg bw/day (110% of control). The relative adrenal weight was significantly increased in males at 40 mg/kg bw/day (124% of control) and in both sexes at 75 mg/kg bw/day (135-148% of control).

Gross pathology revealed thickened mucosa of the nonglandular stomach at 75 mg/kg bw/day in both sexes and of the glandular stomach at 75 mg/kg bw/day in males. A higher incidence of a distended caecum was observed in males at 15-75 mg/kg bw/day and in females at 75 mg/kg bw/day.

The histopathology data showed acanthosis (hyperplasia) in the non-glandular stomach with an increased incidence at 40 and 75 mg/kg bw/day with concomitant necrosis/erosion in one male at each dose level, and of 4 and 2 females at 40 and 75 mg/kg bw/day, respectively. Acanthosis was also observed in 3 males at 15 mg/kg bw/day. Oedema was noted in one male at 15 and 40 mg/kg bw/day and in 2 males at 75 mg/kg bw/day, and in 3 and 2 females at 40 and 75 mg/kg bw/day, respectively.

Conclusion

The NOAEL for systemic toxicity was considered to be 15 mg/kg bw/day in this study, based on decreased glucose levels in 40 and 75 mg/kg/d animals of both sexes.

The NOAEL for local effects was 5 mg/kg bw/day in this study, based on acanthosis of the non-glandular region of the stomach, i.e. forestomach.

Ref.: 6

SCCS comment

The SCCS notes that the purity of the test material was assumed to be 100% according to the study report; no information was provided by the Sponsor of the study.

The SCCS agrees on the NOAEL of 5 mg/kg bw/day for local effects in the stomach probably due to the irritative properties of the test substance. The SCCS also considers 5 mg/kg bw/day as a NOAEL for systemic toxicity (decreased body weight gain in females, 86% of control) although it can be argued that the decreased body weight gain might be secondary to the local effects in the stomach.

Overall SCCS comment to the submitted subchronic toxicity studies

An oral 26-week study in rats is included in Submission II and in the Company Submission and described above. The SCCS notes that this study was also included in Submission I. The SCCP concluded that the quality of the dossier was such that an adequate assessment of Cetylpyridinium chloride (P97) was not possible.

In addition to this study, Submission II (and Submission I) also includes several other oral subchronic studies. None of these studies have been evaluated in this Opinion because they were either submitted as a poorly legible pdf-file, or were only described in secondary literature and therefore, are not considered to add any valuable information for the safety assessment of cetylpyridinium chloride as a cosmetic ingredient.

The full reports of two 90-day feeding studies of cetylpyridinium chloride (one in rats and the other in dogs) were submitted to EFSA for the evaluation of the safety and efficacy of Cecure[®] (Ref.: 24); the active ingredient in Cecure[®] is cetylpyridinium chloride. In addition, a third 90-day rat study was crucial for the setting of the NOAEL for cetylpyridinium chloride in the EFSA Opinion. The two rat studies which are also considered of relevance for the safety assessment of cetylpyridinium chloride as a cosmetic ingredient are summarised below, based on the descriptions in the EFSA Opinion. The CEF Panel considered that the 90-day dog study was not suitable for the derivation of a NOAEL; thus, this study is not considered of relevance for the safety assessment of cetylpyridinium chloride as a cosmetic ingredient and therefore, not included in this Opinion.

In a 13-week study, Sprague-Dawley albino rats (10/sex/group) were administered 0, 125, 250, 500, and 1000 ppm of CPC in the diet, corresponding to an average consumption of approximately 0, 9, 18, 35 and 70 mg/kg bw/day for males, and to 0, 11, 22, 42 and 84 mg/kg bw/day for females, respectively (Charles River Laboratories, 2006a, as cited in Ref.: 24). The study was performed under GLP conditions and according to international guidelines.

Regular observations included clinical parameters, body weight and feed consumption, ophthalmology and neurological examinations such as functional observation tests performed on all animals (passive home cage, interactive cage behaviour, response to handling, etc.; no open field assessments were performed). Further observations included haematology and coagulation parameters, serum chemistry, urinalysis, organ weights,

histopathology on all tissues from all animals in groups 0 and 1000 ppm, in all early death animals and on all gross lesions.

In males and females, from the 1000 ppm group, Mean body weights were significantly lower in high-dose animals than in controls and were related to decreased feed consumption. Haematological examination showed statistically significant increases in red blood cell counts and haematocrit values in high-dose females; mean corpuscular haemoglobin concentration in those animals was decreased consistently, but did not reach statistical significance at the end of the study. Serum chemistry showed significant lower levels of alkaline phosphatase (males) and creatinine levels (both sexes) in the 1000 ppm group. In males, relative caecum weights were statistically significantly increased at 500 and 1000 ppm and relative weights of brain, heart and testis were statistically increased in males at 1000 ppm. In females relative weights of adrenals glands, brain, caecum, heart, kidney, liver, lung, ovary and spleen were significantly increased at 1000 ppm. No histopathological changes were reported.

According to the CEF Panel, taking into account the increase in caecum weights in males in the 500 ppm group, a NOAEL of 250 ppm (18 mg/kg bw/day) can be identified.

Rats (6/sex/group, strain not specified) were administered 0, 125, 300, 800, 2000, 5000, 10000 ppm CPC in the diet (equivalent to approximately 0, 6.25, 15, 40, 100, 250 and 500 mg/kg bw/day) for 90 days (USAEH-HT, 1969, as cited in Ref.: 24).

All animals administered 250 and 500 mg/kg bw/day died within three weeks after initiation of the test. Unspecified differences in body weight gain, relative liver and kidney weights and food utilisation at 100 mg/kg bw/day were reported for both sexes. A positive correlation was noted between dietary levels of CPC and increases in relative caecum weight (statistically significant at 15 mg/kg bw/day and above in females and at 40 mg/kg bw/day and above in males). Gross and microscopic examination of liver, kidneys, lung, spleen, caecum and testis from any of the administered groups were reported to show no appreciable differences compared to controls. It was noted that, as the concentration of CPC increased, the total number of microorganisms in the caecal contents decreased in both sexes.

The CEF Panel identified a NOAEL of 18 mg/kg bw/day, based on increased relative caecum weights in male rats in the 90-day study. The CEF Panel considered the increase in caecum weight as relevant for risk characterisation of Cecure® as an increase in relative caecum weight has been consistently positively correlated with increased dietary levels of CPC in sub-chronic rat studies. Furthermore, in one of these studies it was noted that as the concentration of CPC increased, the total number of microorganisms in the caecal contents decreased in both sexes. An increase in caecum weight in animals has also been associated elsewhere with modification on the composition of the intestinal microbiota and therefore the CEF Panel considered that the possibility of a potential similar effect of CPC occurring in the gastrointestinal microflora of humans should not be disregarded.

Overall SCCS conclusion on repeated dose toxicity

Based on the submitted 6-month repeated dose toxicity study in rats described above, a NOAEL for local effects of 5 mg/kg bw/day is considered. The local effects observed in the stomach of rats are probably due to the irritative properties of cetylpyridinium chloride and related to the administration of the test material as aqueous solution (by gavage in water). The SCCS also considers 5 mg/kg bw/day as a NOAEL for systemic toxicity (decreased body weight gain in females, 86% of control) although it can be argued that the decreased body weight gain might be secondary to the local effects in the stomach.

However, in the dietary studies reported in the EFSA Opinion, decreased body weight gain was also reported for rats. No local effects (irritation) were observed in the gastrointestinal tract indicating that the irritative properties of cetylpyridinium chloride could be avoided by

administration in the feed instead of an aqueous solution (by gavage in water). A NOEL for decreased body weight gain of 25 mg/kg bw/day can be considered based on the sub-acute studies and of around 40 mg/kg bw/day based on the 90-day studies. From the 90-day studies, the CEF Panel identified a NOEL of 18 mg/kg bw/day, based on increased relative caecum weights in male rats in one of the 90-day study. The CEF Panel considered the increase in caecum weight as relevant for risk characterisation. The SCCS agrees with the evaluation of the EFSA CEF Panel.

For dermal application, the dietary studies reported in the EFSA opinion are considered as more relevant for the safety assessment of cetylpyridinium chloride than the gavage studies submitted by the applicant. Consequently, the NOEL of 18 mg/kg bw/day identified by the CEF Panel is used for the MOS calculation for use of cetylpyridinium chloride as a preservative in skin lotions and creams, as well as in anti-perspirant deodorants, cf. Terms of Reference.

For oral application, the gavage studies submitted by the applicant are considered as more relevant for the safety assessment of cetylpyridinium chloride than the dietary studies reported in the EFSA Opinion. Consequently, the NOEL of 5 mg/kg bw/day identified in the submitted 6-month study is used in the safety assessment for the MOS calculation for use of cetylpyridinium chloride as a preservative in mouthwashes cosmetic products, as well as in all other oral hygiene cosmetic products, cf. Terms of Reference.

3.3.5.3. Chronic (> 12 months) toxicity

No data have been submitted.

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline:	/
Species/strain:	<i>S. typhimurium</i> strains TA98, TA100, TA1535, TA1537, TA1538
Replicate:	triplicates in 2 independent experiments
Test substance:	D0751.01
Solvent:	water
Batch:	/
Purity:	/
Concentrations:	experiment 1: 0.33, 1, 3.3, 10 and 33 µg/plate without S9-mix 3.3, 10, 33, 100 and 333 µg/plate with S9-mix experiment 2: 1, 3.3, 10, 33 and 100 µg/plate with S9-mix
Treatment:	/
GLP:	in compliance
Date:	15 December 1987 – 1 March 1988

D0751.01 was investigated for the induction of gene mutations in *Salmonella typhimurium* strains (Ames test). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the level of toxicity in a concentration range-finding study with TA100 both without and with S9-mix. Toxicity, as a reduction in the number of spontaneous revertant colonies and/or clearing of the bacterial background lawn, was evaluated for 10 concentrations ranging from 33 up to

20000 µg/plate. Negative and positive controls were in accordance with the present guideline.

Results

The results of the concentration range-finding study indicated that the appropriate maximum concentrations to be plated in this gene mutation test in bacteria were 333 µg/plate in the presence of S9-mix and 33 µg/plate in the absence of S9-mix.

Both in the presence and in the absence of S9-mix, biologically relevant increases in the number of revertants due to D0751.01 treatment were not observed in any of the strains tested at any concentration level.

Conclusion

Under the experimental conditions used, D0751.01 was not genotoxic (mutagenic) in this gene mutation tests in bacteria.

Ref.: 7

SCCS comment

As D0751.01 is used as a disinfectant and biocide, the gene mutation test in bacteria, although no effect on cytotoxicity has been shown at the concentrations tested, is not a suitable test to investigate the genotoxic (mutagenic) potential.

Morover, the test was not performed according to the OECD TG 471. Batch number, purity and the incubation method were not mentioned in the report.

Therefore, this test has limited value.

***In vitro* mammalian cell gene mutation test**

Guideline:	OECD 476
Cells:	L5178Y <i>tk</i> ^{+/-} mouse lymphoma cells
Replicates:	duplicates in 4 independent assays
Test substance:	Cetylpyridinium chloride, monohydrate (CPC)
Batch:	00217109
Purity:	99.9%
Vehicle:	RPMI 1640 culture medium
Concentrations:	assay 1: 0.063, 0.125, 0.25, 0.5, 0.75, 1. 1.5 and 2 µg/ml without S9-mix
	assay 2: 0.5, 0.75, 1. 1.5, 2, 2.5, 5 and 7.5 µg/ml with S9-mix
	assay 3: 0.0125, 0.025, 0.05, 0.075, 0.1, 0.2, 0.3 and 0.4 µg/ml without S9-mix
	assay 4: 0.25, 0.5, 0.75, 1. 1.5, 2, 3 and 5 µg/ml with S9-mix
Treatment:	assay 1, 2 and 4: 4 h treatment without and with S9-mix; expression period 48 h and selection period of 9 days
	assay 3: 24 h treatment without S9-mix; expression period 48 h and selection period of 9 days
GLP:	in compliance
Study period:	2 June 2003 – 8 August 2003

CPC was assayed for gene mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of S9 metabolic activation. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of an initial toxicity test with concentrations up to the prescribed maximum concentration of 5000 µg/ml measuring relative suspension growth and exposure times were similar to those in the main test. In the main test, cells were treated for 4 h or 24 h (without S9-mix assay 3) followed by an expression period of 48 h to fix the DNA

Revision of the Opinion on Cetylpyridinium chloride (P97)

damage into a stable *tk* mutation. Toxicity was measured in the main experiments as percentage relative total growth of the treated cultures relative to the total growth of the solvent control cultures. To discriminate between large (indicative for mutagenic effects) and small colonies (indicative for a clastogenic effect) colony sizing was performed. Negative and positive controls were in accordance with the OECD guideline.

Results

Precipitation of CPC occurred at concentrations of 150 µg/ml and above. This was of no consequence, as the toxicity of CpC demanded the use of much lower test concentrations in the mutation assays.

In the initial toxicity test the highest test concentration causing a relative total growth above 10% were 0.5 µg/ml without S9-mix and 1.5 µg/ml with S9-mix. The appropriate level of toxicity (10-20% relative total growth) was reached in assay 1 at 0.750 µg/ml, in assay 2 at 1.5 µg/ml, in assay 3 at 0.1 µg/ml and in assay 4 at 1.0 µg/ml. These concentrations are considered as the highest test concentrations. Results of higher concentrations were not taken into account.

In all 4 assays, a biologically relevant increase in the number of mutant colonies was not observed independent of the presence or absence of S9-mix.

Conclusion

Under the experimental conditions used, CPC was not mutagenic in this mouse lymphoma assay using the *tk* locus as reporter gene.

Ref.: 8

***In Vitro* Mammalian Chromosome Aberration Test**

Guideline:	OECD 473 (1983)	
Cells:	Chinese hamster ovary (CHO) cells	
Replicate:	duplicate cultures in 2 independent experiments	
Test substance:	Cetylpyridinium chloride, monohydrate (CPC)	
Batch:	00217109	
Vehicle:	Ham's F10 culture medium	
Purity:	99.9%	
Concentrations:	experiment 1:	0.3, 1 and 3.3 µg/ml without and with S9-mix
	experiment 2:	1, 4 and 6 µg/ml with S9-mix
		0.25, 0.5 and 2 µg/ml without S9-mix 24 h harvest
		0.25, 0.5 and 1 µg/ml without S9-mix 48 h harvest
Treatment	experiment 1:	6 h treatment without and with S9-mix; harvest time 24 h after the start of treatment
	experiment 2:	6 h treatment with S9-mix; harvest time 24 h after the start of treatment
		22 h treatment without S9-mix; harvest time 24 and 48 h after the start of treatment
GLP:	in compliance.	
Date:	28 May 2003 – 3 September 2003	

CPC has been investigated for the induction of chromosome aberrations in CHO cells both in the absence and presence of metabolic activation. Liver S9-fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Test concentrations for experiment 1 were based on the results of a preliminary test without and with metabolic activation with 9 concentrations up to 100 µg/ml. In the main test, cells were treated for 6 h (without and with S9-mix) or 22 h (without S9-mix) and harvested 24 h or 48 h after the start of treatment. Approximately 2 h before harvest, each culture was treated with

colcemid (0.1 µg/ml culture medium) to block cells at metaphase of mitosis. Chromosome (metaphase) preparations were stained with Giemsa and examined microscopically for chromosomal aberrations and polyploidy. Toxicity was measured by cell counts; a concentration was toxic if the cell count was reduced to less than 50% of the mean vehicle control culture values. Negative and positive controls were in accordance with the OECD guideline.

Results

In both experiments, a biologically relevant increase in cells with chromosome aberrations was not found independent of the presence or absence of S9-mix and of treatment times. CPC did not induce an increase in cells with polyploidy in both the presence and absence of S9-mix in cultures harvested 24 h or 48 h post treatment.

Conclusion

Under the experimental conditions used, CPC was not genotoxic (clastogenic) in the chromosome aberration test in CHO cells.

Ref.: 9

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

***In vivo* Mammalian Bone Marrow Micronucleus Test**

Guideline:	OECD 474 (1997)
Species/strain:	CD-1 mice
Group sizes:	5 mice/sex/group 10 mice/sex/group in the high dose group
Test substance:	Cetylpyridinium chloride, monohydrate (CPC)
Batch:	00217109
Purity:	99.9%
Vehicle:	water
Dose level:	0, 25, 50, 100 mg/kg bw/day
Treatment:	twice orally, 24 h apart
Sacrifice times:	48 h after the start of the first treatment.
GLP:	in compliance
Study period:	11 January 2005 – 8 April 2005

CPC was investigated for induction of micronuclei in the bone marrow cells of CD-1 mice. Dose selection was based on the result of a dose range finding test for toxicity in which 1 mice/sex were treated twice orally 24 h apart with 50, 125, 350, 800 and 2000 mg/kg bw/day CPC. The animals were examined for clinical signs and mortality around 1 min, 0.5, 1, 2 and 4 h post dosing, then twice daily until the end of the observation period. In the main toxicity test mice were exposed twice orally 24 h apart to 75, 100 and 125 mg/kg bw/day. The animals were examined for clinical signs and mortality as during the dose range finding test.

In the micronucleus test, mice were exposed twice orally 24 h apart to 0, 25, 50 and 100 mg/kg bw/day. Health status checks on the animals were done at frequent intervals after dosing and prior to the scheduled kill.

Bone marrow cells were collected 48 h start of treatment. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and total erythrocytes (PCE/NCE ratio). Bone marrow preparations were stained and examined microscopically for the PCE/NCE ratio and micronuclei. Negative and positive controls were in accordance with the OECD guideline.

Results

In the dose range finding test, the accompanied clinical signs were subdued behaviour, hunched appearance, laboured breathing, rolling gait, piloerection, pale, cold, unwilling to move, unable to stand, prostrate and extremities dark. In the main toxicity test, with lower doses, the accompanied clinical signs were subdued behaviour, hunched appearance, laboured breathing, rolling gait, piloerection, cold, tremors, unable to stand, prostrate and eyes closed. Based on these toxicity investigations the maximum tolerated dose of CPC was judged to be in the region of 100 mg/kg bw/day.

In the micronucleus test, 3 animal deaths occurred in the high dose group. A clinical sign of subdued behaviour was observed.

The decrease in the PCE/NCE ratio, indicating toxic effects of CPC to bone marrow cells, confirmed bone marrow cell exposure.

A biologically relevant increase in the number of bone marrow cells with micronuclei was not observed at any dose level after administration of CPC.

Conclusion

Under the experimental conditions used, CPC did not induce micronuclei in bone marrow cells of treated mice and, consequently, CPC was not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 23

SCCS comment

At the highest dose group, the PCE/NCE ratio was not decreased. Strangely, the clinical signs that were found in the main toxicity test at the highest dose group were not observed in the micronucleus test.

3.3.7. Carcinogenicity

No data have been submitted.

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No studies have been included in the Company Submission. Submission II includes an older multigeneration study (from 1970). This study (also included in Submission I) has not been evaluated in this Opinion because it was only described in the form of a 14-page English translation of an Italian study report.

3.3.8.2. Teratogenicity

Guideline:	/
Species/strain:	Sprague Dawley Crl:CD rats
Group size:	30 females/group
Test substance:	Cetylpyridinium chloride (CPC) (D1470.01)
Batch:	/
Purity:	/
Vehicle:	Distilled water

Revision of the Opinion on Cetylpyridinium chloride (P97)

Dose levels: 0, 5, 15, 60 mg/kg bw
Dose volume: 10 ml/kg bw
Route: Oral
Administration: Gavage
GLP: In compliance
Study period: July 1993 to February 1994

CPC was administered once daily by gavage (vehicle: distilled water) to Sprague Dawley rats (30 females/group) at dose levels of 0, 5, 15, 60 mg/kg bw during day 6 to 16 of gestation. The dose levels were based on a range-finding study (5 females/group) with dose levels of 0, 5, 50, 100, 200, 300 mg/kg bw/day in which mortality was noted at 100 mg/kg bw/day and above and body weight gain reduction/loss was noted at 50 mg/kg bw/day and above.

Rats were observed for mortality and signs of overt toxicity twice daily throughout the study. The presence and duration of clinical signs of toxicity were recorded daily on gestation days 6 to 20. Females that did not survive until scheduled euthanasia were necropsied. All maternal gross lesions were saved for possible future examination. Food consumption and body weight were recorded on gestation day 0, 6, 9, 12, 16 and 20. On gestation day 20, all surviving animals were euthanised followed immediately by caesarean section examination. The gravid uterine weight was recorded and the foetuses were removed. The location of viable and non-viable foetuses, early and late resorptions, and the number of total implantations and corpora lutea were recorded. Individual foetuses were weighed, sexed, tagged and examined for external malformations and variations. Approximately one-half of the foetuses were examined for soft-tissue malformations and variations and the other one-half for skeletal malformations and variations.

Results

Test article preparations stored for 10 days under ambient conditions contained 99-108% of the initial day 0 levels confirming stability under the conditions of storage.

No mortality was seen among treated females. An increased incidence of decreased defaecation, hair loss, scabbed area and laboured breathing was noted at 60 mg/kg bw/day only. Body weight gain was reduced during day 6-16 at 60 mg/kg bw/day (49% of control) due to body weight loss during day 6-9 and reduced body weight gain during the rest of the treatment period. Body weight gain recovered during day 16-20. Food consumption was decreased during the whole treatment period at 60 mg/kg bw/day and was comparable to the control from day 16-20. The number of pregnant animals was 24, 25, 23 and 24 at 0, 5, 15, 60 mg/kg bw, respectively. Gravid uterus weight was comparable between treated and control groups. No treatment-related macroscopic findings were noted.

There were no significant differences in the number of corpora lutea, early/late resorptions, live foetuses, post-implantation loss, mean foetal body weight or in the foetal sex ratio. External foetal examination revealed no treatment-related findings. No treatment-related changes in the incidence of soft tissue anomalies or skeletal malformations were noted. An increased incidence of skeletal variations was observed at 60 mg/kg bw/day and included reduced ossification of the jugal, incomplete ossification of the cervical vertebral arches and ischium, presence of 14th thoracic vertebra and/or 5th lumbar vertebra, unossified 5th sternebra, bent rib, 12 full pairs of ribs, a rudimentary rib and/or full unilateral rib; the slightly higher incidences of these developmental variations were not considered indicative of a teratogenic effect by the applicant.

Conclusion

No evidence of developmental toxicity was observed in the 60 mg/kg bw/day group nor in any of the dose groups. The NOAEL of cetylpyridinium chloride with regard to maternal toxicity was 15 mg/kg/day, and 60 mg/kg/day with respect to developmental toxicity.

Ref.: 10

SCCS comment

The SCCS notes that the purity of the test material was not provided.

Guideline:	/
Species/strain:	New Zealand white rabbits
Group size:	15 females/group
Test substance:	Cetylpyridinium chloride (CPC)
Batch:	/
Purity:	/
Vehicle:	Distilled water
Dose levels:	0, 2.5, 12, 100/25 mg/kg bw
Dose volume:	2 ml/kg bw
Route:	Oral
Administration:	Gavage
GLP:	In compliance
Study period:	January 1979 to October 1979

CPC was administered once daily by gavage (vehicle: distilled water) to New Zealand white rabbits (15 females/group) at dose levels of 0, 2.5, 12, 100 mg/kg bw during day 7 to 18 of gestation. The high dose (100 mg/kg bw/day) was selected on the basis of the rabbit oral LD₅₀ value (450 mg/kg bw) and a 28-day study, where 100 mg/kg bw/day was given to younger rabbits with only minimal effects. The dose level of 100 mg/kg bw/day was reduced to 25 mg/kg bw/day (exact time not given) because six dams died after 3-6 daily treatments. The two lower dose levels (2.5 and 12 mg/kg bw/day) were selected as 100 and 500-fold exaggerations of the probable human exposure.

Food consumption and body weight were recorded on gestation day 0 and every three days. On gestation day 29, the dams were weighed and sacrificed. The doe's abdomen and thorax was opened and examined grossly noting any pathology. The numbers of corpora lutea were recorded. The uterine horns were excised and the numbers and positions of live or dead foetuses and any resorption sites were noted and recorded. The foetuses were weighed, sexed, tagged and examined for external abnormalities. One-third of the foetuses were examined for skeletal abnormalities and the remaining two-third for soft-tissue abnormalities.

Results

Six dams at 100 mg/kg bw/day died after 3-6 daily treatments. Another three dams in each of the groups received 1-3 treatments; one dam died and two were sacrificed at term. The remaining six untreated dams were dosed daily (days 7-18) with 25 mg/kg bw/day. One dam at 12 mg/kg bw/day and one at 25 mg/kg bw/day died after receiving all of the daily treatments. The post-mortem examinations of the animals that had died revealed gastric ulcers and severe diarrhoea.

Body weight loss was seen at 25 mg/kg bw/day and body weight gain was reduced at 12 mg/kg bw/day (60% of control). Food consumption was significantly reduced at 12 and 25 mg/kg bw/day in a dose dependent manner (76 and 49% of control, respectively).

Five does aborted their foetuses: one at 2.5 mg/kg bw/day, two at 12 mg/kg bw/day and two at 25 mg/kg bw/day; the abortions were considered by the study report authors probably related to the maternal toxicity which included weight loss.

The number of implantations was increased at 25 mg/kg bw/day. The number of resorptions and dead foetuses was increased at 25 mg/kg bw/day. Foetal weight at 25 mg/kg bw/day was significantly decreased in females (73% of control). These effects were all considered by the study report authors to be related to the maternal toxicity. No treatment-related effect on the number of corpora lutea and sex ratio were noted. No treatment-related

Revision of the Opinion on Cetylpyridinium chloride (P97)

changes in the incidence of soft tissue anomalies or skeletal malformations were noted. An increased incidence of skeletal variations was observed at 25 mg/kg bw/day and included missing sternebrae and 13th rib variations.

Conclusion

The data demonstrated that these materials were not teratogenic in rabbits at doses ranging up to those fatal to the dam; therefore, the NOAEL for teratogenicity was greater than or equal to 100 mg/kg bw/day.

Ref.: 11

SCCS comment

The SCCS notes that the purity and stability of the test material was not provided. Furthermore, the study was limitedly reported with only summarised tables available for the findings observed in the study. Thus, this study is considered inadequate for the safety assessment of cetylpyridinium chloride.

Overall SCCS comment to the submitted teratogenicity studies

Two teratogenicity studies – one in rats and the other in rabbits – are included in Submission II and in the Company Submission and described above. The SCCS notes that these two studies were also included in Submission I. The SCCP concluded that the quality of the dossier was such that an adequate assessment of Cetylpyridinium chloride (P97) was not possible.

3.3.9. Toxicokinetics

Guideline:	/
Species/strain:	Charles River CD rats
Group size:	5/sex
Test substance:	Cetylpyridinium chloride (CPC) (D1470.01)
Batch (unlabelled):	/
Purity (unlabelled):	99.2%
Test substance (labelled):	D1470.01R (6.06 mCi; ¹⁴ C; position of radiolabel not indicated)
Batch (radiolabelled):	/
Purity (radiolabelled):	>98%
Vehicle:	Saline solution (0.9%)
Dose level:	25 mg/kg bw
Dose volume:	5 ml/kg bw
Route:	Oral
Administration:	Gavage
GLP:	In compliance
Study period:	June 1993 to March 1994

The distribution and excretion in rats was studied following single oral gavage administration of CPC. The radiolabelled test substance was administered to CD rats (5/sex) at a dose level of 25 mg/kg bw (vehicle: 0.9% sodium chloride solution); no control group was included in the study. The target level of total radioactivity to be administered was 60 µCi. Following dosing, each animal was placed in a glass metabolism chamber for the duration of the study (72 hours). Rats were observed for mortality, moribundity and overt toxicity at least twice daily throughout the study. Individual body weights were recorded just prior to test substance administration and at scheduled necropsy. Exhaled air, urine and faeces were collected for the 0-12, 12-24, 24-48 and 48-72 hours intervals. Cage wash

Revision of the Opinion on Cetylpyridinium chloride (P97)

was collected at 24, 48 and 72 hours. Prior to euthanasia, blood samples were obtained for the determination of radiocarbon in whole blood. Tissues (adipose, bone, bone marrow, brain, gastrointestinal tract, gastrointestinal tract contents, heart, kidneys, liver, lungs muscle, pancreas, spleen, testes/ovaries and carcass) were collected at necropsy.

The test material, D1470.01, described as a white powder, was received from the Sponsor. Documentation of the derivation, characterisation and stability testing of the test material are on file with the Sponsor.

Results

No mortality and no overt toxicity were observed. All animals gained weight, except for one male that lost weight.

The major part of radioactivity was excreted via the faeces with approximately 87 and 84% for males and females, respectively. In urine, 4.7 and 3.3% was excreted by males and females respectively within 72 hours. Excretion via exhaled air was negligible (less than 0.01% of the administered dose as radiolabelled carbon dioxide). Recovery of administered radioactivity was approximately 99 and 94% for males and females, respectively.

Most of the applied radioactivity in tissues was recovered from the gastrointestinal tract and its contents (0.21-0.44% for gastrointestinal tract and 0.49-1.11% for gastrointestinal tract contents). The carcass contained 3.1 and 3.3% for males and females, respectively, and cage rinse 1.2 and 1.6%, respectively.

Conclusion

Oral absorption following administration of 25 mg/kg bw CPC as a single dose by gavage was estimated to be approximately 14% in rats. No appreciable amount of the dosed radioactivity was eliminated as expired radiolabelled carbon dioxide in either sex. Less than 5% of the dosed radioactivity remained in the body of either sex at 72 hours following dosing.

Ref.: 12

SCCS comment

The figures in the tables of the original study report are poorly legible and thus, this study could not be evaluated by the SCCS.

Guideline:	/
Species/strain:	Charles River CD rats
Group size:	5/sex
Test substance:	Cetylpyridinium chloride (CPC) (D1470.01)
Batch (unlabelled):	/
Purity (unlabelled):	99.2%
Test substance (labelled):	D1470.01R (6.06 mCi; ¹⁴ C; position of radiolabel not indicated)
Batch (radiolabelled):	/
Purity (radiolabelled):	>98%
Vehicle:	Saline solution (0.9%)
Dose level:	2.5 mg/kg bw
Dose volume:	1 ml/kg bw
Route:	Intravenous
GLP:	In compliance
Study period:	June 1993 to March 1994

The distribution and excretion in rats was also studied following single intravenous bolus injection of CPC. The radiolabelled test substance was administered at a dose level of 2.5

Revision of the Opinion on Cetylpyridinium chloride (P97)

mg/kg bw (vehicle: 0.9% sodium chloride solution) to CD rats (5/sex); no control group was included in the study. The target level of total radioactivity to be administered was 6 μ Ci. Following dosing, each animal was placed in a glass metabolism chamber for the duration of the study (72 hours). Rats were observed for mortality, moribundity and overt toxicity at least twice daily throughout the study. Individual body weights were recorded just prior to test substance administration and at scheduled necropsy. Exhaled air, urine and faeces were collected for the 0-12, 12-24, 24-48 and 48-72 hours intervals. Cage wash was collected at 24, 48 and 72 hours.

The test material, D1470.01, described as a white powder, was received from the Sponsor. Documentation of the derivation, characterisation and stability testing of the test material are on file with the Sponsor.

Results

No mortality, no overt toxicity and no effect on the body were noted.

The major part of radioactivity was excreted via the urine and faeces with 36 and 26% for males and females, respectively, in the urine and 32 and 37% for males and females, respectively, in the faeces. Excretion via exhaled air was negligible. The carcass contained 13 and 15% for males and females, respectively. Recovery of administered radioactivity was approximately 84 and 80% for males and females, respectively.

Conclusion

Following administration of 2.5 mg/kg bw CPC as a single dose by intravenous injection to rats, the major part of applied radioactivity was excreted in urine and faeces in similar amounts. No appreciable amount of the dosed radioactivity was eliminated as expired radiolabelled carbon dioxide in either sex.

Ref.: 13

SCCS comment

The figures in the tables of the original study report are poorly legible and thus, this study could not be evaluated by the SCCS.

Guideline:	/
Species/strain:	Beagle dogs
Group size:	5/sex (Phase I: 1/sex; phase II: 4/sex)
Test substance:	Cetylpyridinium chloride (CPC) (D1470.01)
Batch (unlabelled):	/
Purity (unlabelled):	99.2%
Test substance (labelled):	D1470.01R (6.06 mCi; 14 C; position of radiolabel not indicated)
Batch (radiolabelled):	/
Purity (radiolabelled):	>98%
Vehicle:	Saline solution (0.9%)
Dose level:	25 mg/kg bw
Dose volume:	5 ml/kg bw
Route:	Oral
Administration:	Gavage
GLP:	In compliance
Study period:	July 1993 to August 1994

The distribution and excretion in dogs was studied following single oral gavage administration of CPC. The radiolabelled test substance was administered to Beagle dogs (5/sex, Phase I: 1/sex; phase II: 4/sex) at a dose level of 25 mg/kg bw (vehicle: 0.9%

Revision of the Opinion on Cetylpyridinium chloride (P97)

sodium chloride solution); no control group was included in the study. The target level of total radioactivity to be administered was 300 µCi. Following dosing, each animal was placed in individual cages fitted with an excreta collection pan for the duration of the study (72 hours in phase I and 7 days in phase II). Dogs were observed for mortality, moribundity and overt toxicity at least twice daily throughout the study. Individual body weights were recorded pretest, just prior to test substance administration and at scheduled necropsy. Urine was collected for the 0-12, 12-24, 24-48 and 48-72 hours intervals (phase I), and additionally from hours 72-96, 96-120, 120-144 and 144-168 in phase II. Faeces and cage wash was collected for the 0-24, 24-48 and 48-72 hours interval (phase I), and additionally from hours 72-96, 96-120, 120-144 and 144-168 in phase II. Cage wash was collected at 24, 48 and 72 hours (phase I), and additionally at 96, 120, 144 and 168 hours in phase II. Prior to euthanasia, blood samples were obtained for determination of radiocarbon in whole blood and plasma. Tissues (adipose, bone (femur), bone marrow (femur), brain, gastrointestinal tract, heart, kidneys, liver, lungs muscle, spleen, testes/ovaries, uterus and carcass) were collected at necropsy.

The test material, D1470.01, described as a white powder, was received from the Sponsor. Documentation of the derivation, characterisation and stability testing of the test material are on file with the Sponsor.

Results

No mortality was observed. All animals gained weight, except for one male that lost weight. Clinical signs observed were soft stool, diarrhoea, vomiting and/or decreased defecation. The animals observed for 72 hours had lost weight, and those observed for 7 days had gained no or some weight.

In the animals observed for 72 hours, 61 and 76% was excreted in faeces for the male and the female dog, respectively, and 8 and 11% in urine, respectively. In the animals observed for 7 days, 41 and 39% was excreted in faeces for males and females, respectively, and 9 and 14% in urine, respectively. No tissue retained more than 0.2% of the administered radioactivity, except for the gastrointestinal tract and the carcass. Total recovery was 76% for the male dog and 95% for the female dog after 72 hours, and 56 and 57%, respectively, after 7 days. According to the study report authors, a lack of recovered radiolabel in the faeces seemed to account for the low recovery in phase II; no obvious cause for this difference was apparent.

Conclusion

In conclusion, these results seemed to indicate that the test article may be poorly absorbed in dogs following oral administration as evidenced by the predominance of faecal elimination of the test article.

Ref.: 14

SCCS comment

The figures in the tables of the original study report are poorly legible and thus, this study could not be evaluated by the SCCS.

Guideline:	/
Species/strain:	Beagle dogs
Group size:	5/sex (Phase I: 1/sex; phase II: 4/sex)
Test substance:	Cetylpyridinium chloride (CPC) (D1470.01)
Batch (unlabelled):	/
Purity (unlabelled):	99.2%
Test substance (labelled):	D1470.01R (6.06 mCi; ¹⁴ C; position of radiolabel not indicated)
Batch (radiolabelled):	/

Revision of the Opinion on Cetylpyridinium chloride (P97)

Purity (radiolabelled):	>98%
Vehicle:	Saline solution (0.9%)
Dose level:	2.5 mg/kg bw
Dose volume:	1 ml/kg bw
Route:	Intravenous
GLP:	In compliance
Study period:	July 1993 to August 1994

The distribution and excretion in dogs was also studied following single intravenous bolus injection of CPC. The radiolabelled test substance was administered to Beagle dogs (5/sex) at a dose level of 2.5 mg/kg bw (vehicle: 0.9% sodium chloride solution); no control group was included in the study. The target level of total radioactivity to be administered was 30 μ Ci. Following dosing, each animal was placed in individual cages fitted with an excreta collection pan for the duration of the study (72 hours in phase I and 7 days in phase II). Dogs were observed for mortality, moribundity and overt toxicity at least twice daily throughout the study. Individual body weights were recorded pretest, just prior to test substance administration and at scheduled necropsy. Urine was collected for the 0-12, 12-24, 24-48 and 48-72 hours intervals (phase I), and additionally from hours 72-96, 96-120, 120-144 and 144-168 in phase II. Faeces and cage wash was collected for the 0-24, 24-48 and 48-72 hours interval (phase I), and additionally from hours 72-96, 96-120, 120-144 and 144-168 in phase II. Cage wash was collected at 24, 48 and 72 hours (phase I), and additionally at 96, 120, 144 and 168 hours in phase II.

The test material, D1470.01, described as a white powder, was received from the Sponsor. Documentation of the derivation, characterisation and stability testing of the test material are on file with the Sponsor.

Results

All animals survived to study termination. Soft stool was noted in most animals. The animals observed for 72 hours gained no weight, and those observed for 7 days had gained weight.

In the animals observed for 72 hours, 7 and 9% was excreted in faeces for the male and the female dog, respectively, 45 and 40%, respectively, in urine, and 62 and 61%, respectively, remained in the carcass. The total recovery was >100%.

In the animals observed for 7 days, 10 and 13% was excreted in faeces for males and females, respectively, 32 and 42%, respectively, in urine, and 42 and 52%, respectively, remained in the body. The total recovery was 86% for males and 109% for females after 7 days.

Conclusion

In conclusion, urine was the major route for elimination of the radioactivity.

Ref.: 15

SCCS comment

The figures in the tables of the original study report are poorly legible and thus, this study could not be evaluated by the SCCS.

Guideline:	/
Species/strain:	Beagle dogs
Group size:	5/males
Test substance:	Cetylpyridinium chloride (CPC) (D1470.01)
Batch (unlabelled):	/

Revision of the Opinion on Cetylpyridinium chloride (P97)

Purity (unlabelled):	99.2%
Test substance (labelled):	D1470.01R (3.23 mCi; ¹⁴ C; position of radiolabel not indicated)
Batch (radiolabelled):	/
Purity (radiolabelled):	>98%
Vehicle:	Saline solution (0.9%)
Dose level:	25 mg/kg bw
Dose volume:	5 ml/kg bw
Route:	Oral
Administration:	Gavage
GLP:	In compliance
Study period:	July 1994 to March 1995

The distribution and excretion in dogs was studied following single oral gavage administration of CPC. The radiolabelled test substance was administered to male Beagle dogs (5 animals) at a dose level of 25 mg/kg bw (vehicle: 0.9% sodium chloride solution); no control group was included in the study. The target level of total radioactivity to be administered was 300 µCi. Following dosing, each animal was placed in individual cages fitted with an excreta collection pan for the duration of the study (5 days). Dogs were observed for mortality, moribundity and overt toxicity at least twice daily throughout the study. Individual body weights were recorded pretest, just prior to test substance administration and at scheduled necropsy. Individual food and water consumption values were recorded daily throughout the five days of sample collection.

Urine and faeces were collected for the 0-3, 3-6, 6-9, 9-12, 12-18, 18-24, 24-48, 48-72, 72-96 and 96-120 hours intervals. A 24-hour control urine sample was additionally collected before dose administration. Cage wash was collected at each collection interval and after the 120-hour collection interval.

Results

All animals survived to study termination. No overt toxicity was noted. One animal had lost weight after 5 days. No effect on food and water consumption was noted. The major part of the applied radioactivity was excreted in faeces (40%) followed by urine (22%). Cage wash accounted for 8% of the applied radioactivity. The total recovery was 69%.

Conclusion

In conclusion, the major part of the applied radioactivity was excreted in faeces (40%) followed by urine (22%).

Ref.: 16

SCCS comment

The figures in the tables of the original study report are poorly legible and thus, this study could not be evaluated by the SCCS.

Overall SCCS comment to the submitted toxicokinetic studies

Five toxicokinetic studies – two in rats and three in dogs – are included in Submission II and in the Company Submission and described above. The SCCS notes that these studies were also included in Submission I. The SCCP concluded that the quality of the dossier was such that an adequate assessment of Cetylpyridinium chloride (P97) was not possible. The SCCS agrees with the conclusion of the SCCP and therefore, the submitted toxicokinetic studies have not been evaluated by the SCCS in this opinion.

Overall SCCS conclusion on oral absorption based on the toxicokinetic studies

As the toxicokinetic studies could not be evaluated by the SCCS (see the SCCS comments above), the oral absorption of cetylpyridinium chloride from mouthwashes, cosmetic products containing up to 0.1% cetylpyridinium chloride or from all other oral hygiene cosmetic products containing up to 0.5% (w/w) cetylpyridinium chloride cannot be estimated.

According to the Notes of Guidance, 50% oral absorption is used in case inadequate data on oral absorption are available. However, if there is evidence to suggest poor oral bioavailability, it may be more appropriate to assume that only 10% of the administered dose is systemically available.

From the submitted oral toxicokinetic study in rats, the oral absorption was estimated to be approximately 14% following administration of 25 mg/kg bw cetylpyridinium chloride as a single dose by gavage. However, in another study with intravenous injection of 2.5 mg/kg bw cetylpyridinium chloride as a single dose to rats, the major part of applied radioactivity was excreted in urine and faeces in similar amounts. The latter study indicates that biliary excretion of cetylpyridinium chloride takes place in rats and therefore, the estimate of 14% absorption following oral administration based on faecal excretion in the oral rat study is not supported by the intravenous rat study.

In one of the submitted oral toxicokinetic studies in dogs, 10-15% of the radioactivity was excreted in the urine following administration of 25 mg/kg bw cetylpyridinium chloride as a single dose by gavage, whereas in the other submitted oral toxicokinetic studies in dogs, 22% of the radioactivity was reported to be excreted in the urine, also following administration of 25 mg/kg bw cetylpyridinium chloride as a single dose by gavage. However, the low recovery of the radioactivity in these studies is a major drawback for the interpretation of the results in both studies.

Overall, the available oral toxicokinetic data indicate an oral absorption of cetylpyridinium chloride of higher than 10% in both rats and dogs. Therefore, an oral absorption of 50% is used in the safety assessment for the calculation of the Margin of Safety (MoS).

3.3.10. Photo-induced toxicity

No data have been submitted.

3.3.11. Human data

Oral Mucosal Irritation:

As a follow-up to the commenting period, Industry response to the SCCS Opinion on the use of cetylpyridinium chloride as a preservative in cosmetic products with accompanying cosmetovigilance data for in market products in EU have been received (Information from Cosmetics Europe, 2015). The cosmetovigilance data are reproduced below:

"Three major Oral Care Industries collated all reported undesirable events associated with their products containing CPC, i.e. mouthwashes, for a period of 18 months, from 1 July 2013 to 31 December 2014, in their main European markets (between 3 and 28 Member States per company). The focus has been made on reports classified as an oral mucosal irritation considering that the participating companies do not manufacture personal care products with CPC.

The causality of the reported reactions has been evaluated according to the Colipa/Cosmetics Europe SUE Reporting Guidelines (2012). The relation of causality of the reported event to the product was assessed using a 5-level scale: very likely, likely, not clearly attributable, unlikely and excluded, using a decision tree provided in the guidelines.

Revision of the Opinion on Cetylpyridinium chloride (P97)

The causality assessment was carried out against the product as a whole and not against a specific ingredient. The possible effects of CPC can therefore not be evaluated independently from the other ingredients entering the composition of the evaluated product.

An overall industry rate could be calculated, using the sum of all company reports assessed as 'very likely' and 'likely' and dividing them by the sum of all units sold,

- *The overall European industry rate of reported reactions with a causality assessment "likely" or "very likely" is estimated as 0.76 per million units sold.*
- *The overall rate for undesirable effects assessed "likely" or "very likely" and classified as oral mucosal irritation is estimated at 0.35 per million units sold. None of those reports was classified as a Serious Undesirable Effect.*
- *No reports on dermal irritation was available from those companies, as could be expected considering the type of product.*

Considering the indication of mouthwashes, i.e. maintenance of oral hygiene and gum/mouth protection against bacteria, it is possible that some consumers may have a pre-existing or underlying oral condition (e.g., gingivitis). Consequently, some of the undesirable effects reported under oral mucosal irritation may be confounded by these conditions.

It is acknowledged that there is a possibility of oral mucosal irritation at site of contact. However, cosmetovigilance data collected across Europe by three major Oral Care Industries on CPC-based mouthwashes, for the period 1st July 2013 – 31 December 2014, indicate that oral mucosal irritation associated with oral rinse is typically mild, self-limiting and may be confounded by a pre-existing condition. In addition, it may not necessarily be attributable to CPC given the composition of the oral rinse formulations and the concurrent use of other oral hygiene products.

The frequency of those reports remains very low, especially for products used on the oral mucosa, and can be considered acceptable for consumers.

Those data support a safe use for consumers of CPC in Oral Care products marketed in EU."

SCCS comment

The SCCS acknowledges the receipt of the cosmetovigilance information and agrees that this information indicates that the oral mucosal irritation associated with oral hygiene cosmetic products is mild. However, cosmetovigilance data may be useful in indicating a problem with certain ingredients or specific products, but is in general of limited value in establishing safety or disproving a problem.

Skin sensitisation:

A number of human studies have been carried out to determine the sensitisation potential of cetylpyridinium chloride and cetylpyridinium chloride containing formulations. Generally, cetylpyridinium chloride has little or no sensitisation potential in humans. Cases of allergic contact dermatitis from gloves containing cetylpyridinium chloride have been recorded (Ref.: 25).

3.3.12. Special investigations

No relevant data have been submitted.

3.3.13. Safety evaluation (including calculation of the MoS)**CALCULATION OF THE MARGIN OF SAFETY****Oral application**

For oral application, the NOAEL of 5 mg/kg bw/day identified in the submitted 6-month study is used for the MOS calculation. Based on the toxicokinetic data, an oral absorption of 50% is considered. The adjusted NOAEL for the MOS calculation is 2.5 mg/kg bw/day.

Product	Max concentration (% in the finished product)	Total oral exposure (mg/kg bw/day)	Calculated SED based on an oral absorption of 50% (mg/kg bw/day)	MoS (adjusted NOAEL/SED)
Mouthrinse	0.1	0.033	0.016	156
Dentrifice ³ (toothpaste)	0.5	0.011	0.005	500
Aggregate exposure (cosmetics)			0.021	120
Denture adhesive ³	0.5	0.025	0.013	192
Denture cleanser ³	0.5	0.00013	0.00006	41700
Aggregate exposure (total)			0.034	74

In conclusion, the SCCS considers that the use of cetylpyridinium chloride in the single cosmetic products for oral application mentioned above is safe for the consumer. Aggregate exposure to cetylpyridinium chloride via cosmetic products and other consumer products (denture adhesive and cleanser) for these oral applications may be of concern for some consumers; however, it is considered unlikely that the oral applications mentioned above will be used simultaneously.

³ Data are from applicant's submission

Revision of the Opinion on Cetylpyridinium chloride (P97)

Oral exposure to cetylpyridinium chloride can also occur due to use of cetylpyridinium chloride containing solutions for the removal of microbial surface contamination of raw poultry products, as described in the EFSA opinion (Ref.: 24). The potential exposure to cetylpyridinium chloride was estimated by the CEF Panel in the EFSA opinion to be up to 5.7 µg/kg bw/day at the mean and to 17.8 µg/kg bw/day at the 95th percentile of treated poultry consumption. Aggregate oral exposure to cetylpyridinium chloride via cosmetic products and via treated poultry may therefore, be of concern for some consumers; however, it is considered likely that oral exposure from the applications mentioned above and exposure from treated poultry will not occur simultaneously very often.

Dermal application

For dermal application, the NOAEL of 18 mg/kg bw/day identified by the CEF Panel in the EFSA opinion is used for the MOS calculation. Based on the toxicokinetic data, an oral absorption of 50% is considered. The adjusted NOAEL for the MOS calculation is 9 mg/kg bw/day.

Product	Max concentration (% in the finished product)	Total dermal exposure (mg/kg bw/day)	Calculated SED based on a dermal absorption of 10% (mg/kg bw/day)	MoS (adjusted NOAEL/SED)
Body lotion	0.2	0.246	0.025	360
Face cream	0.2	0.048	0.0048	1875
Hand cream	0.2	0.065	0.0065	1385
Deodorant, non-spray	2.0	0.442	0.044	205
Aggregate exposure			0.080	113

In conclusion, the SCCS considers that the use of cetylpyridinium chloride in the single cosmetic products for dermal application mentioned above, as well as aggregate exposure to cetylpyridinium chloride via cosmetic products for these dermal applications is safe for the consumer.

Aggregate exposure

Aggregate exposure to cetylpyridinium chloride via cosmetic products for the oral and dermal applications may be of concern for some consumers; however, it is considered unlikely that all the oral and dermal applications mentioned above will be used simultaneously.

Oral exposure to cetylpyridinium chloride can also occur due to use of cetylpyridinium chloride containing solutions for the removal of microbial surface contamination of raw poultry products, as described in the EFSA opinion (Ref.: 24). Aggregate exposure to cetylpyridinium chloride via cosmetic products and via treated poultry may be of concern for some consumers; however, it is considered likely that exposure from cosmetic products and exposure from treated poultry will not occur simultaneously very often.

3.3.14. Discussion

Physico-chemical properties

The submission applies for inclusion of cetylpyridinium chloride in Annex VI of the Cosmetics Directive 76/768/ECC, soon in Annex V of the Cosmetic Regulation (EC) n.1223/2009. The proposed use of cetylpyridinium chloride in cosmetic formulations is as follows:

- *mouthwashes cosmetic products up to a concentration of 0.1 %*
- *all other oral hygiene cosmetic products up to a concentration of 0.5 %*
- *skin lotions and creams up to a concentration of 0.2 %*
- *anti-perspirant deodorants up to a concentration of 2.0 %*

The impurities on commercial cetylpyridinium chloride have not been described. The applicant has declared that "*No impurities of toxicological or ecotoxicological concern or impurities which exceed 1 g/kg are present in technical grade CPC.*" However, qualitative and quantitative impurities, which may be present at <1 g/kg level, have not been reported.

General toxicity

Cetylpyridinium chloride was of moderate acute oral toxicity in rats (oral LD₅₀ value of 560 mg/kg bw) in a study performed according to OECD TG 425, of low acute dermal in rats (dermal LD₅₀ value greater than 5000 mg/kg bw) in a study performed according to OECD TG 402, and of high acute inhalation toxicity in rats (inhalation LC₅₀ value between 0.054 and 0.51 mg/l) in a study performed according to OECD TG 403.

In the submitted 28-day studies performed with rats and dogs, a NOAEL of 25 and <5 mg/kg bw/day, respectively, can be identified based on local effects observed in the stomach. In the submitted 6-month study performed with rats, a NOAEL of 5 mg/kg bw/day can be identified based on local effects observed in the stomach. The local effects observed in the stomach are probably due to the irritative properties of cetylpyridinium chloride and related to the administration of the test material as aqueous solution (by gavage in water). Based on the submitted 6-month study, 5 mg/kg bw/day is also considered as a NOAEL for systemic toxicity (decreased body weight gain in females, 86% of control) although it can be argued that the decreased body weight gain might be secondary to the local effects in the stomach.

The EFSA CEF Panel has recently published a scientific Opinion on the evaluation of the safety and efficacy of Cecure[®] for the removal of microbial surface contamination of raw poultry products. The active ingredient in Cecure[®] is cetylpyridinium chloride. Two 28-day feeding studies and two 90-day feeding studies have been performed with cetylpyridinium chloride in rats and dogs, respectively, and were evaluated in the EFSA Opinion based on the full study reports; the 28-day and the 90-day studies in rats are considered relevant for the safety assessment of cetylpyridinium chloride as a cosmetic ingredient.

The CEF Panel identified a NOAEL of 18 mg/kg bw/day, based on increased relative caecum weights in male rats in one of the 90-day study; the CEF Panel considered the increase in caecum weight as relevant for risk characterisation. In these studies, decreased body weight gain was also reported for rats. No local effects (irritation) were observed in the gastrointestinal tract indicating that the irritative properties of cetylpyridinium chloride could be avoided by administration in the feed instead of an aqueous solution (by gavage in water). A NOAEL for decreased body weight gain of 25 mg/kg bw/day can be considered based on the sub-acute studies and of around 40 mg/kg bw/day based on the 90-day studies.

For dermal application, the dietary studies reported in the EFSA opinion are considered as more relevant for the safety assessment of cetylpyridinium chloride than the gavage studies submitted by the applicant. Consequently, the NOAEL of 18 mg/kg bw/day identified by the CEF Panel is used for the MOS calculation for use of cetylpyridinium chloride as a preservative in skin lotions and creams, as well as in anti-perspirant deodorants, cf. Terms of Reference.

For oral application, the gavage studies submitted by the applicant are considered as more relevant for the safety assessment of cetylpyridinium chloride than the dietary studies reported in the EFSA opinion. Consequently, the NOAEL of 5 mg/kg bw/day identified in the submitted 6-month study is used in the safety assessment for the MOS calculation for use of cetylpyridinium chloride as a preservative in mouthwashes cosmetic products, as well as in all other oral hygiene cosmetic products, cf. Terms of Reference.

In the teratogenicity study performed with rats, the NOAEL for maternal toxicity was 15 mg/kg/day, based on clinical signs of toxicity and decreased body weight gain; the NOAEL for developmental toxicity was 60 mg/kg/day (the highest dose level in the study). The teratogenicity study performed with rabbits was limitedly reported with only summarised tables available for the findings observed in the study and therefore, the study is considered inadequate for the safety assessment of cetylpyridinium chloride.

Irritation / sensitisation

Cetylpyridinium chloride at a concentration of 65% had a moderate to severe irritant potential to the skin of the rabbit in a study performed according to OECD TG 404. The neat substance has an extreme irritant potential to the eyes of the rabbit in a study performed according to OECD TG 405. No firm conclusions can be drawn on the irritant properties of solutions or formulations containing up to 2%.

Based on the submitted studies on oral mucosal irritation it is considered that cetylpyridinium chloride may be slightly irritating to the oral mucosa when used as a preservative in mouthwashes, cosmetic products up to a concentration of 0.1%, or in all other oral hygiene cosmetic products up to a concentration of 0.5%, cf. Terms of Reference. The cosmetovigilance information also indicates that the oral mucosal irritation associated with oral hygiene cosmetic products is mild. However, cosmetovigilance data may be useful in indicating a problem with certain ingredients or specific products, but is in general of limited value in establishing safety or disproving a problem.

Cetylpyridinium chloride did not show contact sensitising potential in two tests, one of these performed according to OECD TG 406. The skin sensitisation studies were based on the Buehler test method, which is regarded as less sensitive and may underestimate the sensitizing potential. In humans contact sensitisation to cetylpyridinium chloride has been reported.

Dermal absorption

Two *in vitro* dermal absorption studies have been submitted. The mean absorbed proportion was regarded as 0.054 µg/cm² (1.3% of dose) for a 0.2% (w/w) cetylpyridinium chloride oil-in-water formulation (simulating body lotion formulations) and as 0.597 µg/cm² (0.597% of dose) for a 2% (w/w) cetylpyridinium chloride glycol/water formulation (simulating deodorant formulations). Because of all the major deviations as noted by the SCCS for the two studies, the dermal absorption of cetylpyridinium chloride from body lotion formulations containing 0.2% (w/w) cetylpyridinium chloride or from deodorant formulations containing 2% (w/w) cetylpyridinium chloride cannot be estimated. According to the Notes of Guidance, 100% dermal absorption is used in case inadequate data on dermal absorption are available. However, as the submitted *in vitro* studies on dermal absorption indicate a low dermal absorption, a value of 10% will be used in the safety assessment for the calculation of Margin of Safety (MoS).

Mutagenicity / genotoxicity

Overall, the genotoxicity of cetylpyridinium chloride, monohydrate is sufficiently investigated in genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. Under *in vitro* conditions cetylpyridinium chloride, monohydrate did not induce gene mutations in mammalian cells. Cetylpyridinium chloride, monohydrate was not clastogenic. *In vitro* it did not induce an increase in the number of cells with chromosome aberrations in CHO cells. *In vivo* cetylpyridinium chloride, monohydrate exposure of mice did not result in an increase in cells with micronuclei. Consequently, based on the present reports cetylpyridinium chloride, monohydrate can be considered to have no genotoxic potential and additional tests are unnecessary.

Carcinogenicity

No data have been submitted.

Toxicokinetics

In rats administered ¹⁴C-cetylpyridinium chloride (position of radiolabel not indicated) as a single oral dose (gavage) of 25 mg/kg bw, the major part of radioactivity was excreted via the faeces (approximately 87 and 84% for males and females, respectively), and a minor part in the urine (4.7 and 3.3% for males and females, respectively) within 72 hours. Based on this information, oral absorption was estimated by the study report authors to be approximately 14%. However, in another study with intravenous injection of 2.5 mg/kg bw cetylpyridinium chloride as a single dose to rats, the major part of applied radioactivity was excreted in urine and faeces in similar amounts. The latter study indicates that biliary excretion of CPC takes place in rats and therefore, the estimate of 14% absorption following oral administration based on faecal excretion in the oral rat study is not supported by the intravenous rat study.

In one of the submitted oral toxicokinetic studies in dogs, 10-15% of the radioactivity was excreted in the urine following administration of 25 mg/kg bw cetylpyridinium chloride as a single dose by gavage, whereas in the other submitted oral toxicokinetic studies in dogs, 22% of the radioactivity was reported to be excreted in the urine, also following administration of 25 mg/kg bw cetylpyridinium chloride as a single dose by gavage. However, the low recovery of the radioactivity in these studies is a major drawback for the interpretation of the results in both studies.

According to the Notes of Guidance, 50% oral absorption is used in case inadequate data on oral absorption are available. However, if there is evidence to suggest poor oral bioavailability, it may be more appropriate to assume that only 10% of the administered

dose is systemically available. Overall, the available oral toxicokinetic data indicate an oral absorption of cetylpyridinium chloride of higher than 10% in both rats and dogs. Therefore, an oral absorption of 50% is used in the safety assessment for the calculation of the Margin of Safety (MoS).

4. CONCLUSION

1. *On the basis of the data provided, does the SCCS consider that cetylpyridinium chloride is safe for consumers, when used as a preservative in cosmetic products in the following specified concentrations:*

- *mouthwashes cosmetic products up to a concentration of 0.1 %*
- *all other oral hygiene cosmetic products up to a concentration of 0.5 %*
- *skin lotions and creams up to a concentration of 0.2 %*
- *anti-perspirant deodorants up to a concentration of 2.0 %*

Except for potential skin, eye and oral mucosal irritation, the SCCS considers that the use of cetylpyridinium chloride in a single cosmetic product for oral or dermal application is safe for the consumer. Aggregate exposure (based on worst case default assumptions for dermal and oral absorption) to cetylpyridinium chloride via cosmetic products and other consumer products (denture adhesive and cleanser) for the oral and dermal applications may be of concern for some consumers; however, it is considered unlikely that all the oral and dermal applications mentioned above will be used simultaneously.

2. *Does the SCCS have any further scientific concerns with regard to the use of cetylpyridinium chloride in cosmetic products?*

Oral exposure to cetylpyridinium chloride can also occur due to use of cetylpyridinium chloride containing solutions for the removal of microbial surface contamination of raw poultry products, as described in the EFSA Opinion (Ref.: 24). Aggregate exposure (based on worst case default assumptions for dermal and oral absorption) to cetylpyridinium chloride via cosmetic products and via treated poultry is of concern for consumers.

This Opinion covers only the use of cetylpyridinium chloride in non-spray cosmetic products.

5. MINORITY OPINION

/

6. REFERENCES

Submission II:

1. Vinegar MB, 1977. Delayed hypersensitivity study in guinea pigs of B7002, P&G No. BSBTS-407.
2. Ward R, 2003. Cetylpyridinium Chloride: *In Vitro* Absorption From a Oil-In-Water Emulsion through Human Epidermis. Central Toxicology Laboratory, Alderley Park Macclesfield, Cheshire, UK (2003a): Report Number: JV1763.
3. Ward R, 2003. Cetylpyridinium Chloride: *In Vitro* Absorption From a Glycol/Water Solution through Human Epidermis. Central Toxicology Laboratory, Alderley Park Macclesfield, Cheshire, UK (2003b): Report Number: JV1764.
4. Minnema DJ, 1994. 28-Day Repeated Dose Oral Toxicity Study in Rats with D1470.01, Report No. HWA 297672, P&G No. HPCR 0562.
5. Goldenthal EI, 1994. 28 Day Oral Toxicity of Cetylpyridinium Chloride in Dogs, Report No. 191-1576, P&G No.
6. Minnema DJ, 1995. Six-Month Toxicity Study in Rats with D1470.01, Report No. HWA 297-683, P&G No. HPCR 0593.
7. Lawlor TE, 1988. Salmonella/Mammalian Microsome Mutagenesis Assay, Laboratory Study No. T5845.501.
8. Riach CG, 2004. Mouse Lymphoma Cell Mutation Assay, Inveresk Report No. 23280.
9. Innés DC and Rans J, 2004. Chromosomal Aberration Assay with Chinese Hamster Ovary Cell Cultures *in vitro*, Inveresk Report No. 23284.
10. Stewart TA, 1994. Developmental Toxicity Study in Rats, Report No. 191-1595.
11. Nolen GA and Dierckman, TA 1979. The Effects of Cetylpyridinium Chloride (CPC) and a 9:1 Mixture of Cetyl Pyridinium Chloride and beta-Phenoxyethyl dimethyldodecyl-ammonium Bromide (DB) Administered Orally During Pregnancy on Fetal Development in the New Zealand Rabbit, Report No VE1165, P &G No. TGSE 1575.
12. Slauter RA, 1994. Distribution and excretion of a test article in rats following oral gavage administration, Report No 191-1591, P&G No. HPCR-0587.
13. Slauter RA, 1994. Excretion of a test article (D1470.01) in rats following intravenous administration, Report No 1911592, P&G No. HPCR-0587.
14. Slauter, 1994. Absorption, distribution and excretion of a test article (D1470.01) in Beagle dogs following oral gavage administration. Report No 191-1589, P&G No. HPCR-0588.
15. Slauter, 1994. Excretion of a test article (D1470.01) in Beagle dogs following intravenous administration, Report No 191-1590, P&G No. HPCR-0588.

16. Slauter, 1995. Absorption and excretion of a test article (D1470.01) in Beagle dogs following oral gavage administration. Report No 191-1617, P&G No. HPCR-0629.

Company Submission:

17. Durando BS, 2005. Acute oral toxicity up and down procedure in rats. Product Safety Laboratories, Report No. 16950, GLP, unpublished. Owner: Company.
18. Durando BS, 2005. Acute dermal toxicity in rats – Limit test. Product Safety Laboratories, Report No. 16853, GLP, unpublished. Owner: Company.
19. Durando BS, 2005. Acute inhalation toxicity study in rats. Product Safety Laboratories, Report No. 16854, GLP, unpublished. Owner: Company.
20. Durando BS, 2005. Primary skin irritation study in rabbits. Product Safety Laboratories, Report No. 16856, GLP, unpublished. Owner: Company.
21. Durando BS, 2005. Primary eye irritation study in rabbits. Product Safety Laboratories, Report No. 16855, GLP, unpublished. Owner: Company.
22. Durando BS, 2005. Dermal sensitization study in guinea pigs (Buehler method). Product Safety Laboratories, Report No. 16857, GLP, unpublished. Owner: Company.
23. Innés DC and Rans J, 2005. Cetyl Pyridinium Chloride – Micronucleus test in bone marrow cells of CD-1 mice – 0 h + 24 h oral dosing and 48 h sampling, Inveresk, Report No. 25436, GLP, unpublished. Owner: Company.

Others:

24. EFSA 2012. Scientific Opinion on the evaluation of the safety and efficacy of Cecure® for the removal of microbial surface contamination of raw poultry products. EFSA Panel on Biological Hazards (BIOHAZ) and EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), European Food Safety Authority (EFSA), Parma, Italy. EFSA Journal 2012;10(3):2612.
25. Pontén A, Hamnerius N, Bruze M et al. 2013. Occupational allergic contact dermatitis caused by sterile non-latex protective gloves: clinical investigation and chemical analyses. Contact Dermatitis 68:103-110.