Scientific Committee on Consumer Safety

SCCS

OPINION ON

Soytrimonium chloride

COLIPA n° P72

The SCCS adopted this opinion at its 14th plenary meeting

of 27 March 2012

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

Jürgen Angerer, Ulrike Bernauer, Claire Chambers, Qasim Chaudhry, Gisela Degen, Elsa Nielsen, Thomas Platzek, Suresh Chandra Rastogi, Vera Rogiers, Christophe Rousselle, Tore Sanner, Jan van Benthem, Jacqueline van Engelen, Maria Pilar Vinardell, Rosemary Waring, Ian R. White

Contact
European Commission
Health & Consumers
Directorate D: Health Systems and Products
Unit D3 - Risk Assessment
Office: B232 B-1049 Brussels
Sanco-SCCS-Secretariat@ec.europa.eu

ISSN 1831-4767 ISBN 978-92-79-30760-7
Doi:10.2772/82578 ND-AQ-12-010-EN-N

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

ACKNOWLEDGMENTS

Prof. J. Angerer
Dr. U. Bernauer
Dr. C. Chambers
Prof. G. Degen
Dr. W. Lilienblum (associated scientific advisor, rapporteur)
Dr. S.C. Rastogi
Prof. V. Rogiers
Prof. T. Sanner (chairman)
Dr. J. van Engelen
Prof. R. Waring
Dr. I.R. White

Keywords: SCCS, scientific opinion, preservative, P72, soytrimonium chloride, Directive 768/76/EEC, CAS 61790-41-8, EC 263-134-0

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on soytrimonium chloride, 27 March 2012

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

TABLE OF CONTENTS

1. BACKGROUND

Soyatrimethylammonium chloride (CAS No 61790-41-8; EC No 263-134-0) with the INCI-name soytrimonium chloride is a mixture of C16 and C18, saturated and unsaturated alkyl trimethylammoniun chlorides.

According to Colipa[1], soytrimonium chloride falls under the definition of Alkyl (C16, C18, and C22) trimethyl ammonium chloride.

Many alkyl trimethylammonium compounds are currently listed in Annex VI, entry 44 and covered by "Alkyl (C12-C22) trimethyl ammonium, bromide and chloride (*)" of the Cosmetics Directive (76/768/EEC) as preservatives. The asterix (*) indicates that these substances are allowed for other uses than preservation.

The first scientific opinion (SCCP/917/05) on Alkyl (C16, C18, C22) trimethylammonium chloride for other uses than as a preservative was adopted on 17 April 2006, a second on 21 March 2007.

The 3rd scientific opinion (SCCP/1246/09) on Alkyl (C16, C18, C22) trimethylammonium chloride for other uses than as a preservative was adopted on 8 December 2009 with the conclusion:

"*As far as systemic and local toxicity are concerned, the chemical analogy between the three compounds seems to permit a read-across approach for cetrimonium chloride, steartrimonium chloride and behentrimonium chloride.*

The calculation of the Margin of Safety leads to a value of 192.

Apart from the fact that quaternary ammonium derivative formulations have the potential to be irritative, especially when combinations of the concerned compounds are used, the SCCS is of the opinion that the use of cetrimonium chloride, steartrimonium chloride and behentrimonium chloride does not pose a risk to the health of the consumer under the following concentration limits:

Cetrimonium chloride (C16), steartrimonium chloride (C18):
Rinse-off hair care products up to 2.5%
Leave-on hair care products up to 1.0%
Leave-on facial cream products:
Sum of cetrimonium chloride and steartrimonium chloride up to 0.5%
Behentrimonium chloride (C22):
Rinse-off hair care products up to 5.0%
Leave on hair care and facial cream products up to 3.0%"

The current submission concerns the use of soytrimonium chloride in hair dyes products for purposes other than as a preservative.

[1] Colipa: The European Cosmetics Association

2. TERMS OF REFERENCE

1. *Does SCCS consider soytrimonium chloride safe when used in oxidative and non-oxidative hair colorants products in a concentration on-head up to maximum 3.0% for purposes other than as a preservative?*

2. *And/or does the SCCS have any further scientific concern with regard to the use of soytrimonium chloride in cosmetic products?*

3. OPINION

Preamble

The applicant submitting the dossier on soytrimonium chloride provided the following comparison of soytrimonium chloride with related materials and argumentation for a read-across approach:

Soytrimonium Chloride, a C_{16}/C_{18}-saturated/C_{18}-unsaturated n-alkyl trimethylammonium chloride mixture is structurally closely related to Cetrimonium chloride (C_{16}-saturated), Steartrimonium chloride (C_{18}-saturated) and trimethylammonium tallow alkyl chlorides (CAS# 8030-78-2). The toxicity of the family of alkyl trimethylammonium chlorides has been reviewed in the fatty nitrogen derived (FND) cationics High Production Volume (HPV) Chemicals Challenge program [5]. The C_{16} and C_{18} n-alkyl trimethylammonium derivates reviewed in the HPV program are identical to those reviewed in SCCS/1246/09 [1]. The trimethylammonium tallow alkyl chloride is a related material to the C_{16} and C_{18} derivatives and is structurally very comparable to Soytrimonium Chloride discussed in this submission. Tallow derived materials typically have an approximate hydrocarbon chain length distribution of 2.5% C_{14}, 29% C_{16}, 23% C_{18}, 2% palmitoleic acid, 41.5% oleic acid (1 carbon-carbon double bond) and 3% linoleic acid (2 carbon-carbon double bonds) derived hydrocarbon chains. Similar to Soytrimonium Chloride, the tallow derived material also consists of mainly C_{18} saturated and unsaturated n-alkyl chains.

Within the context of the HPV Chemicals Challenge program, the FND cationics category surfactants are considered as one category in a single HPV chemical dossier based on the following generalities:

- *Structural and functional similarities of cationic surfactants,*
- *similar measured and modelled physical properties such as melting point, boiling point,*
- *vapour pressure, partition coefficient (log Kow) and water solubility,*
- *comparable moderate to negligible mammalian toxicity, and*
- *comparable anticipated metabolism.*

In general, the measurement and prediction of physical/chemical properties for surfactants, including the cationic surfactants discussed here, are complicated by their behaviour in test systems and the environment. Although predictions within the category vary, the overall data and knowledge of the chemicals support the conclusion that the n-alkyl trimethylammonium chlorides have closely related structures and behave similarly from the perspective of physical/chemical

properties. This conclusion can be extended to Soytrimonium Chloride which contains compounds (about 30%) that are structurally identical to other members of this category. The main difference of Soytrimonium Chloride compared to the other n-alkyl trimethylammonium compounds discussed here is that its predominant component, oleyl trimethylammonium chloride (about 70% of the mixture), contains an unsaturated hydrocarbon chain, in contrast to the saturated hydrocarbon chains of the other substances.

Toxicity data are available for a number of chemicals across the HPV FND trimethylalkyl category and show similar effects. They have minimal observable toxicity below acutely toxic doses; they are unlikely to have mutagenic activity or cause reproductive effects, and are not developmental toxicants. All materials show skin and eye irritation or corrosive properties, depending on the concentrations tested. Dermal absorption and metabolism are considered comparable across the members of the category, including saturated and unsaturated alkyl chains.

In the present opinion the SCCS combines major parts of SCCP/1246/09 and the earlier SCCS opinions mentioned above with the newly introduced information on *soytrimonium chloride*, thus presenting an overview of the data introduced for n-*alkyl (C_{16}, C_{18}, C_{22}) trimethylammonium* chloride and bromide in terms of a chemical category, and the final SCCS conclusion, taking into account the full data package.

The development and use of chemical groups (categories, analogues) for bridging of data gaps by (Q)SAR, trend analysis or read-across within chemical groups is accepted in general. Some guidance for the conduct of such non-testing approaches has been provided for instance by the OECD and the EU (Ref. 29, 30). Although there are some established general rules available to perform read-across within a given chemical category (or smaller analogue group), read across is often a matter of case-by-case expert judgement considering the (non)-similarities among the chemical group, the size and stringency of the data base and last not least the quality of the available data.
By use of a data base for only a few n-alkyl trimethylammonium chlorides, the applicant suggests read-across essentially for oleyl trimethylammonium chloride which constitutes the main fraction of the Soytrimonium chloride mixture. The SCCS considers this as an analogue approach requiring reliable data of good quality because of the small number of substances and inevitably a small data base. The question is whether the data base and data quality are sufficient to conclude that the existing data gaps for Soytrimonium chloride can be bridged with confidence. In particular, the carbon-carbon double bond in oleyl trimethylammonium chloride represents an additional biologically active functional group that requires careful consideration.

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

Soyatrimethylammonium Chloride

3.1.1.1. Primary name and/or INCI name

Soytrimonium chloride (INCI name)

3.1.1.2. Chemical names

Soyatrimethylammonium chloride
N-(SoyAlkyl)-N, N, N-Trimethyl Ammonium Chloride

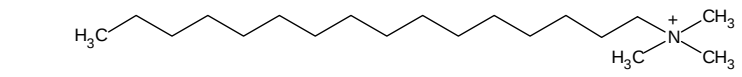
3.1.1.3. Trade names and abbreviations

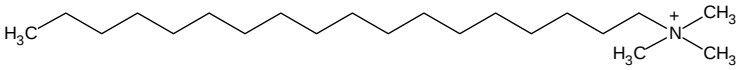
Arquad SV-60PG (58-62% active)
Arquad PC SV-60PG
Quaternium-9
COLIPA P72

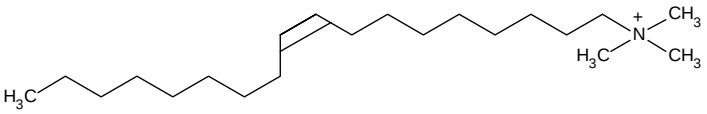
3.1.1.4. CAS / EC number

CAS: 61790-41-8
EC: 263-134-0

3.1.1.5. Structural formula







plus Cl^- counter-ion

Alkyl chain distribution (typical): C16: ± 15%
C18: ± 85% (± 15% saturated, ± 70% unsaturated)

Comment
The C18 mono-unsaturated chain derives from oleic acid (cis-9-octadecenoic acid).

The C18 unsaturated hydrocarbon chain(s) may also contain 2 double bonds, presumably derived from linoleic acid (cis,cis-9,12-octadecadienoic acid) (ref. 17).

3.1.1.6. Empirical formula

Formula: C16 saturated $C_{19}H_{42}NCl$
C18 saturated $C_{21}H_{46}NCl$
C18 unsaturated $C_{21}H_{44}NCl$

3.1.2. Physical form

Yellow liquid

3.1.3. Molecular weight

Molecular weight of the trimethylalkonium moiety (cationic part):
C16 saturated: 284 g/mol

C18 saturated: 312 g/mol
C18 unsaturated: 310 g/mol

Molecular weights including the counterion chloride:
C16 saturated 320 g/mol
C18 saturated 348 g/mol
C18 unsaturated 346 g/mol

3.1.4. Purity, composition and substance codes

Arquad SV-60PG – 58-62% soytrimonium chloride in propylene glycol
Other name: GTS72278
Batch No. 1009228 (purity 60.3% Soytrimonium Chloride; 0.6% free amine/amine HCL)

Arquad SV-100 (reference standard in the dermal absorption study)
Lot No. 2245-22-A (purity 98.4% w/w)
Chain distribution: C16 saturated: 14.80%
C18 saturated: 9.65%
C18 (mono)unsaturated: 72.43%

Ref.: 17

3.1.5. Impurities / accompanying contaminants

Free amine and amine hydrochloride: ≤ 2%

3.1.6. Solubility

Soluble in water

The test substance Arquad SV-60 and the reference substance Arquad SV-100 are both soluble in water/methanol 1:1.

Ref.:17

3.1.7. Partition coefficient (Log P_{ow})

According to the earlier submission on n-alkyl (C_{16}, C_{18}, C_{22}) trimethylammonium chloride, an inherent property of cationic surfactants is that they accumulate at the interface between polar (water) and hydrophobic phases, which makes the accurate measurement of the P_{ow} for any surfactant unfeasible.

Ref.: 1, 5

3.1.8. Additional physical and chemical specifications

Melting point: 5-6 °C
Boiling point:
Flash point:
Vapour pressure:
Density: 950 g/L at 20 °C
Viscosity: 240 mPa s Ref.: 18
Surface tension:
pH: 8.3 – limits 6.0 – 9.0 (10% solution in water)
Refractive index:

3.1.9. Homogeneity and Stability

3.1.9.1 Homogeneity

Comparison of Soytrimonium Chloride with Cetrimonium Chloride and Steartrimonium Chloride

	Soytrimonium Chloride (Arquad SV-60PG)	Cetrimonium Chloride	Steartrimonium Chloride
Alkyl chain: C14 and shorter	<1%	5% max	5-34%
C16	15%	94% min	
C18	~15% saturated ~70% unsaturated	4% max	80-92%
C20	Not present		2%
C22			
C24			
Appearance	liquid	liquid	Liquid or pellets
Colour	Colourless to slightly yellow	Colourless to slightly yellow	Colourless to slightly yellow
Ingredient	58-62%	24-30%	49-51% or 78-82%
Solvent	Propylene glycol 38-42%	Water 70-76%	49-51% water or 18-22% water/isopropanol
pH	6.0-9.0	5.0-7.35	5.0-8.0

Alkyl chain blends from plant oil are used for the production of alkyltrimethylammonium chlorides. Therefore, the resulting products are always comprised of a predominating alkyl chain length, after which the product is named. However it should be noted that certain percentages of shorter or longer alkyl chains are always present in the product.

The predominating alkyl chain in Soytrimonium Chloride is the C_{18}-mono-unsaturated chain (~70%), with C_{16} and C_{18} saturated chains accounting for approximately 15% each. The C_{16} and C_{18} saturated chains are common to the other n-alkyl trimethylammonium chlorides, in particular cetrimonium chloride and steartrimonium chloride, respectively, as reviewed in SCCS/1246/09.

3.1.9.2 Stability

No data submitted

General comments on section 3.1

Some instability of the test substance Arquad SV-60 and Arquad SV-100 (reference standard in the dermal absorption study) in water/methanol and in the hair dye formulations applied has been reported.

Ref.: 17

3.2. Function and uses

Many alkyl trimethylammonium compounds are currently listed in Annex VI, entry 44 and covered by "Alkyl (C12-C22) trimethyl ammonium, bromide and chloride (*)" of the Cosmetics Directive (76/768/EEC) as preservatives. The asterix (*) indicates that these substances are allowed for other uses than preservation.

With regards to other uses than preservation, the SCCS concluded in its Opinion in 2009 (SCCP/1246/09):
"Apart from the fact that quaternary ammonium derivative formulations have the potential to be irritative, especially when combinations of the concerned compounds are used, the SCCS is of the opinion that the use of cetrimonium chloride, steartrimonium chloride and behentrimonium chloride does not pose a risk to the health of the consumer under the following concentration limits:

Cetrimonium chloride (C16), steartrimonium chloride (C18):
Rinse-off hair care products up to 2.5%
Leave-on hair care products up to 1.0%
Leave-on facial cream products:
Sum of cetrimonium chloride and steartrimonium chloride up to 0.5%
Behentrimonium chloride (C22):
Rinse-off hair care products up to 5.0%
Leave on hair care and facial cream products up to 3.0%"

The dossier submitted addresses Soytrimonium Chloride, a C_{16}/C_{18}-saturated/C_{18}-unsaturated n-alkyl trimethylammonium chloride mixture, and its specific use in oxidative and non-oxidative hair colorant products at on-head levels up to 3%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1 Acute oral toxicity
3.3.1.2 Acute dermal toxicity

No new data

Data compiled from SCCS/1246/09 (Ref. 1)

Acute oral Toxicity

Substance	Species, year	Dosage (mg/kg bw)	Test	Results, effective dose (mg/kg bw)
Cetrimonium chloride	Mouse, 1978	300, 400, 500, 600	LD-50	400-600
Cetrimonium chloride	Wistar rat, 1984	630 - 4000	OECD TG 401	LD50, males: 2970 LD50, females: 1550
Cetrimonium chloride	SD rat, 1997	125, 500	OECD TG 420, Fixed dose	125: no deaths 500: 5 deaths from 10 animals

Steartrimonium chloride	Mouse, 1989	Range not stated	OECD TG 401	LD50, males: 633 LD50, females: 536
Steartrimonium chloride	SD rat, 1996	Ca. 550, Limit test	OECD TG 401	550: no deaths
Steartrimonium chloride *)	Wistar rat, 1996	Males: 800, 2000 Females: 650-2000	OECD TG 401	Females: LD50 ca. 700 Males. 800, no deaths, 2000, 5 deaths of 5 animals
Acute dermal Toxicity				
Cetrimonium chloride	New Zealand white albino rabbits, 1977	Only 1 dose, 4.3 ml/kg bw		3 of 6 animals died.

*) The test substance contained about 80% steartrimonium chloride and about 20% isopropanol.

3.3.1.3 Acute inhalation toxicity

No data submitted

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

A. Cetrimonium chloride - skin irritation rabbit - study 1

Taken from SCCS/1246/09

Guideline: OECD TG 404, Annex V to Dir. 67/548/EEC, Method B.4: Acute Dermal Irritation/Corrosion
Species/strain: New Zealand white albino rabbit
Group size: 3 animals
Test substance: Genamin CTAC (28-30% cetrimonium chloride in water)
Batch: E06112547 (04.10.1983)
Purity: Not stated
Dose: 0.5 ml of test substance on 6 cm²
Observation period: 21 days
GLP/QAU: not available (test performed before EU GLP-Guidelines)
Date of test: Jun-Jul 1984

A patch with 0.5 ml test material was placed on a ± 6cm² area of the shaved skin of three female rabbits and covered with semi-occlusive dressing for 4 hours. After the 4 hour application time, the patch was removed and the area was wiped with a cellulose tissue. Skin reactions were evaluated 30 minutes, 60 minutes, 24 hours, 48 hours, 72 hours, 7 days, 14 days and 21 days after patch removal.

Results
No mortality or other clinical effects were observed. Slight erythema and oedema were observed 30 minutes after patch removal. At the 24, 48 and 72 hours time points, grade 2-3 erythema and grade 1-2 oedema were observed. Dry and brownish patchy skin was observed at 48 hours, 72 hours and 7 days. Other aspects of the application site were: hardened skin at 7 days, ablation of large scales at 7 and 14 days and shiny skin at 14 days. The mean score values of the 24, 48 and 72 hour readings were 2.9 for erythema and

1.6 for oedema. At 7 and 14 days, no oedema, but grade 2 erythema was found. At day 21, adverse skin reactions were absent.

Conclusion
The study authors conclude that Genamin CTAC is irritating to skin when applied for 4 hours at an active concentration of 29%.

Ref.: 10 (subm 2004)

B. Cetrimonium chloride - skin irritation rabbit - study 2

Taken from SCCS/1246/09

Guideline:	OECD TG 404, Annex V to Dir. 67/548/EEC, Method B.4: Acute Dermal Irritation/Corrosion
Species/strain:	New Zealand white albino rabbit
Group size:	3 animals
Test substance:	Quartamin 60W25 (24-26% cetrimonium chloride in water)
Batch:	3-4
Purity:	Not stated
Dose:	0.5 ml of test substance on 6 cm²
Observation period:	14 days
GLP/QAU:	In compliance
Date of test:	Feb-Mar 1997

A patch with 0.5 ml test material was placed on a ± 6cm² area of the shaved skin of three female rabbits and covered with semi-occlusive dressing for 4 hours. After the 4 hour application time, the patch was removed and the area was wiped with a cellulose tissue. Skin reactions were evaluated 30 minutes, 60 minutes, 24 hours, 48 hours, 72 hours, 7 days and 14 days after patch removal.

Results
No mortality or other clinical effects were observed. Slight erythema and oedema were observed at 30 minutes after patch removal. Grade 2-3 erythema was observed at all time points up to 14 days. Grade 1-2 oedema was found between 60 minutes and 7 days; at 14 days two rabbits showed no oedema, while grade 2 oedema was found in the third rabbit. Dryness of skin was noted at 24, 48 and 72 hours and at 7 and 14 days in 1, 1, 2, 3 and 1 rabbit(s), respectively. The mean score values of the 24, 48 and 72-hour readings were 3.0 for erythema and 1.9 for oedema.

Conclusion
The study authors conclude that Quartamin 60W25 is irritating to skin when applied to the skin for 4 hours at an active concentration of 25%.

Ref.: 11 (subm 2004)

C. Steartrimonium chloride - skin irritation rabbit - study 1

Taken from SCCS/1246/09

Guideline:	OECD TG 404, Annex V to Dir. 67/548/EEC, Method B.4: Acute Dermal Irritation/Corrosion
Species/strain:	New Zealand white albino rabbit
Group size:	3 females/dose
Test substance:	2 or 20% of Quartamin 86W (unknown percentage of steartrimonium chloride) in water
Batch:	1841 (batch No. not documented)
Purity:	Not stated
Dose:	0.5 ml of 2% or 20% of test substance in distilled water on 6 cm²

Observation period: 14 days
GLP/QAU: In compliance
Date of test: Sep 1996

A patch with 0.5 ml test material was placed on a ± 6cm² area of the shaved skin of three female rabbits per dose group and covered with semi-occlusive dressing for 4 hours. After the 4 hour application time, the patch was removed and the area was wiped with cotton wool soaked in distilled water. Skin reactions were evaluated 1, 24, 48 and 72 hours after patch removal.

Results
20% Quartamin 86W: no mortality or other clinical effects were observed. Grade 2 erythema was observed at all time points between 1 and 72 hours, except for one rabbit showing grade 1 at 1 hour. Evaluation at 7 days was impaired by crust formation. Grade 1 oedema was found at all time points between 1 and 72 hours, except one rabbit showing a grade 2 at 1 hour. No oedema was noted at 7 and 14 days. The mean score values of the 24, 48 and 72 hour readings were 2.0 for erythema and 1.0 for oedema.

2% Quartamin 86W: no mortality or other clinical effects were observed. Grade 1 erythema was observed at 1, 24 and 48 hours in 1 of 3 rabbits. No erythema was found at later time points. No oedema was noted at any time points between 1 hour and 14 days. The mean score values of the 24, 48 and 72 hour readings were 0.2 for erythema and 0.0 for oedema.

Conclusion
The study authors conclude that a 20% solution of Quartamin 86W (unknown percentage of steartrimonium chloride) in water was irritating to the skin when applied for 4 hours, while a 2% solution showed to be non-irritating.

Ref.: 12 (subm 2004)

D. Steartrimonium chloride - skin irritation rabbit - study 2

Taken from SCCS/1246/09

Guideline:	OECD TG 404, Annex V to Dir. 67/548/EEC, Method B.4: Acute Dermal Irritation/Corrosion
Species/strain:	New Zealand white albino rabbit
Group size:	3 females exposed for 3 minutes, 1 female exposed for 4 hours
Test substance:	Genamin STAC (79.2% steartrimonium chloride, 19.1% isopropanol and 0.9% water)
Batch:	1061969521
Purity:	79.2% (DIN ISO 2871)
Dose:	500 mg of test substance
Observation period:	22 days
GLP/QAU:	GLP-statement available; no QAU statement
Date of test:	Jan-Feb 1996

A patch with 0.5 g test material wetted with 0.3 ml physiological saline was placed on the shaved skin of four female rabbits and covered with semi-occlusive dressing. After the respective application times of 3 minutes in three rabbits and 4 hours in one rabbit, patches were removed and the area was wiped with a cellulose tissue. Skin reactions were evaluated 30 minutes, 60 minutes, 24 hours, 48 hours, 72 hours, 7 days, 14 days and 22 days after patch removal.

Results
No mortality or other clinical effects were observed. In the rabbit exposed for four hours, grade 2 erythema was observed at all time periods between 1 and 22 days; grade 1 oedema was found at time points between 1 and 7 days. No erythema or oedema was noted

at any time point after the 3 minute exposure. The treated skin area of the rabbit exposed for 4 hours was found sporadically dry, rough, indurated, encrusted, chapped and discoloured beige. 22 days after application, pink coloured new skin and a scar were noted. The mean score values of the 24, 48 and 72 hour readings were 2.9 for erythema and 1.6 for oedema.

Conclusion
The study authors conclude that Genamin STAC (79.2% steartrimonium chloride, 19.1% isopropanol and 0.9% water) caused severe skin burns when applied for 4 hours, but did not lead to any visual signs of irritation when applied for 3 minutes.

Ref.: 13 (subm 2004)

E. Behentrimonium chloride – skin irritation

No data submitted

F. Soytrimonium chloride – skin irritation, Episkin ® reconstituted human epidermis model

Data from Submission Dec 2010

Testing Guideline:	Method followed the available draft OECD test guideline (now OECD Testing Guideline 439 (2010)[19])
Method:	In Vitro Reconstituted Human Epidermis Model
Model:	Episkin ®™
Test item:	Arquad SV60PG - GTS72278 (60.3% active Soytrimonium Chloride)
Concentration, vehicle and quantity:	GTS72278 - undiluted and at 5% v/v solution in sterile water (equivalent to 60.3% and 3.015% active Soytrimonium Chloride respectively) (identity, concentrations and purity of the test substance not confirmed)
Negative control:	Sterile water
Positive control:	Sodium dodecyl sulphate (conc. 5% w/v)
Duration of contact:	15 minute exposure period and a 42±1 hr post-treatment incubation period
Measurements:	Absorbance, optical density and subsequent histopathological evaluation.
GLP statement:	Yes
Study period:	Feb – Nov 2010

The skin irritation potential of Soytrimonium Chloride was evaluated in the *in vitro* Reconstituted Human Epidermis Model (Episkin®). The test material (Arquad SV-60PG - GTS72278) was applied both undiluted (60.3% active Soytrimonium Chloride) and at a 20-fold dilution (3.015% active Soytrimonium Chloride). The diluted concentration is equivalent to the maximum on-head hair colorant usage concentration.

Results
When applied neat and at a concentration of 5% (equivalent to 60.3% and 3.015% active Soytrimonium Chloride), no cytotoxicity was induced. Relative mean viability was 106.3 ± 4.8% for the undiluted material and 121.3 ± 1.1% for the diluted material. Histological evaluation showed no to minimal epidermal effects with the 5% (3.015% active) solution. The neat material also resulted in no to minimal epidermal effects in two tissues, with the third tissue showing slight epidermal effects. In general, the histopathological evaluation confirmed the absence of cytotoxicity in the MTT section of the study at both concentrations thus, according to the applicant, indicating that the test material Arquad SV-60PG (60.3% active Soytrimonium Chloride) is only slightly irritating to the skin.

Absorbance Results

Treatment	Tissue	Individual absorbance values	
Undiluted	1	0.847	0.797
	2	0.764	0.741
	3	0.789	0.771
5% in sterile water	1	0.924	0.886
	2	0.937	0.844
	3	0.905	0.878
Negative control	1	0.800	0.758
	2	0.751	0.739
	3	0.662	0.718
Positive control	1	0.129	0.131
	2	0.068	0.072
	3	0.155	0.136

Optical Density and Viability Results

Treatment	Individual OD_{540} values	Mean OD_{540}	Standard deviation	Relative individual tissue viability	Relative mean viability %	Standard deviation
Undiluted	0.822	0.785	0.035	111.4	106.3	4.8
	0.752			101.9		
	0.780			105.7		
5% in sterile water	0.905	0.895	0.008	122.6	121.3	1.1
	0.890			120.6		
	0.891			120.7		
Negative control	0.779	0.738	0.045	105.6	100	6.4
	0.745			100.9		
	0.690			93.5		
Positive control	0.130	0.115	0.040	17.6	15.6	5.3
	0.070			9.5		
	0.145			19.6		

Histology summary:

Treatment	Tissue	Histological observations[1]
Undiluted	1	Comparable to negative control - grade 0
	2	Loss of granulation among stratum granulosum cells – grade 1
	3	Minimal vacuolation of cells in all layers of the epithelium – grade 1
5% in sterile water	1	Slight vacuolation of cells in all layers of the epithelium – grade 2
	2	Comparable to negative control - grade 0
	3	Minimal vacuolation of cells in all layers of the epithelium – grade 1
Negative control	1	Grade 0 in 3/3 samples
	2	
	3	
Positive control	1	Complete necrosis in 3/3 samples
	2	
	3	

[1] *Grading scale for histological observations*
Grade 0 = Absence of any epithelial changes
Grade 1 = Minimal cellular alterations characterised by loss of granulation of cells in the stratum granulosum, isolated nectrotic cells of cytoplasmic vaculation of superficial cells
Grade 2 = Moderate numbers of necrotic or vacuolated cells more especially in surface layers.
Grade 3 = Marked number of necrotic or vacuolated cells at all levels including basal layer with loss of cell boundaries, orientation and nuclear detail
Grade 4 = Complete degeneration/necrosis of all cell layers

Ref: 3 (subm 2010)

Comments
The reference to historical data of the model is missing. This should include, but is not limited to:

i) Acceptability of the Quality Control data with reference to historical batch data
ii) Acceptability of the positive and negative control values with reference to positive and

negative control means and ranges (see OECD TG 439 [19]).

Both concentrations (equivalent to 60.3% and 3.015% active Soytrimonium Chloride) did not show marked skin irritation in this test. Based on these results, as well as the findings for the n-alkyl trimethylammonium chlorides in general, as detailed in SCCS/1424/09 [1], it is reasonable to conclude that soytrimonium chloride only has a slight/minimal potential for skin irritancy (CLP Category 2, not warranted).

The above data provide no information on the irritant potential of Soytrimonium chloride under oxidative conditions. Appropriate studies with oxidised soytrimonium chloride would be required to determine irritant potential.

3.3.2.2. Mucous membrane irritation

A. Cetrimonium chloride - eye irritation rabbit - study 1

Taken from SCCS/1246/09

Guideline:	OECD TG 405, Annex V to Dir. 67/548/EEC, Method B.5: Acute Eye Irritation/Corrosion
Date of test:	Jun-Jul 1984
Species/strain:	New Zealand white albino rabbit
Group size:	3 animals
Test substance:	Genamin CTAC (28-30% cetrimonium chloride in water)
Batch:	E06112547 (04.10.1983)
Purity:	not stated
Dose:	0.1 ml of test substance
Observation period:	21 days
GLP/QAU:	not available (test performed before EU GLP-Guidelines)

A volume of 0.1 ml of the test substance was placed into the conjunctival sac of the left eye of each animal. After 24 hours, treated eyes were rinsed thoroughly with physiological saline warmed to 37 °C. The untreated right eye served as control. Ocular reactions were evaluated 1, 24, 48, and 72 hours after instillation of the test article. When ocular reactions were noted at 72 hours, additional examinations were performed at 7, 14 and 21 days after the instillation. At 24 and 72 hours after instillation, additional examinations using fluorescein solution were performed.

Results:
Grade 1-2 corneal opacity was found at time points between 1 and 72 hours. At 7, 14 and 21 days, grade 3 corneal opacity was noted. Grade 1 iritis was found at all time points between 1 hour and 7 days. No iritis was reported at 14 days and grade 1 iritis in 1/3 animals at 21 days. Conjunctival irritation was evident as grade 1-3 redness and grade 3-4 swelling at time points 24, 48 and 72 hours and still persisted as grade 1-2 redness and grade 2 swelling at 21 days.
Fluorescein staining could not be evaluated at 24 hours and in one rabbit at 48 hours due to swelling of the conjunctivae; at 48 hours 1/2 to 3/4 of the corneal surface was affected in the other two rabbits. The mean score values at 24, 48 and 72 hours were 1.9 for opacity, 1.0 for iritis, 2.3 for conjunctival redness and 3.7 for conjunctival chemosis.

Conclusion:
The study authors conclude that Genamin CTAC (28-30% cetrimonium chloride in water), as tested, caused irreversible ocular damage, corneal opacity and conjunctival irritation which persisted throughout the test period until day 21.

Ref.: 16 (subm 2004)

B. Cetrimonium chloride - eye irritation rabbit - study 2

Taken from SCCS/1246/09

Guideline:	OECD TG 405, Annex V to Dir. 67/548/EEC, Method B.5: Acute Eye Irritation/Corrosion
Date of test:	Feb-Mar 1997
Species/strain:	New Zealand white albino rabbit
Group size:	3 animals
Test substance:	Quartamin 60W25 (24-26% cetrimonium chloride in water)
Batch:	3-4
Purity:	Not stated
Dose:	0.1 ml of test substance
Observation period:	21 days
GLP/QAU:	In compliance

A volume of 0.1 ml of the test article was placed into the conjunctival sac of the right eye of each male rabbit. The untreated left eye served as control. Ocular reactions were evaluated 1, 24, 48, and 72 hours after instillation of the test article according to the scoring system of the test guidelines. When ocular reactions were noted at 72 hours, additional examinations were performed at 7, 14 and 21 days after the instillation.

Results
The behaviour and physical condition of the rabbits were normal throughout the study. Grade 1 corneal opacity was observed at 1 hour and grade 3-4 opacity was found at all later time points including day 21. The iris could not be evaluated due to the corneal opacity. Conjunctival irritation was evident as grade 2-3 redness and grade 3-4 swelling at all time points including day 21. The mean score values at 24, 48 and 72 hours were 2.8 for opacity, 2.4 for conjunctival redness and 4.0 for conjunctival chemosis.

Conclusion
The study authors conclude that Quartamin 60W25 (24-26% cetrimonium chloride in water), as tested, causes irreversible ocular damage under the form of corneal opacity and conjunctival irritation which persisted throughout the test period until day 21.

Ref.: 17 (subm 2004)

C. Cetrimonium chloride - eye irritation rabbit - study 3

Taken from SCCS/1246/09

The submission contains a study report of 1985 which describes an ocular irritation study according to the so-called "Low Volume Procedure", in which a volume of 10 µl instead of 100 µl is instilled in the rabbit eye. The tested formulation contains 8% of 25% active Cetrimonium chloride and 60% of 30% active unidentified surfactant. Corneal opacity was observed in 3/6 animals, iritis was found in 4/6 rabbits and conjunctival irritation was evident in all animals. The study authors conclude that cetrimonium chloride showed to be reversely irritating to the eyes tested at an active concentration of 2.0% in an aqueous shampoo matrix.

Ref.: 18 (subm 2004)

D. Steartrimonium chloride - eye irritation rabbit

Taken from SCCS/1246/09

Guideline:	OECD TG 405, Annex V to Dir. 67/548/EEC, Method B.5: Acute Eye Irritation/Corrosion
Date of test:	Sep 1996

Species/strain:	New Zealand white albino rabbit
Group size:	3 females/dose
Test substance:	2% of Quartamin 86W (unknown percentage of steartrimonium chloride) in distilled water
Batch:	1841 (batch No. not documented)
Purity:	Not stated
Dose:	0.1 ml of test substance
Observation period:	14 days
GLP/QAU:	In compliance

A volume of 0.1 ml of the test substance was placed into the conjunctival sac of the left eye of each animal. The untreated right eye served as control. Ocular reactions were evaluated 1, 24, 48, and 72 hours after instillation of the test substance. When ocular reactions were noted at 72 hours, additional examinations were performed at 7 and 14 days after the instillation.

Results
Neither corneal opacity nor iritis was found at any time point. Conjunctival irritation was evident as grade 2 redness in all animals at time points between 1 and 72 hours; at 7 days grade 1 redness was present. Grade 1-2 swelling was found at time points 1, 24, and 48 hours. At 72 hours grades 0, 1 and 2 were found in the three rabbits. After 14 days, all ocular reactions had disappeared. The mean score values at 24, 48 and 72 hours were 0.0 for opacity, 0.0 for iritis, 1.8 for conjunctival redness and 1.4 for conjunctival chemosis.

Conclusion
The study authors concluded that Quartamin 60W25 (unknown percentage of steartrimonium chloride), as a 2% solution in distilled water, produced transient conjunctival irritation.

Ref.: 19 (subm 2004)

E. *Behentrimonium chloride - eye irritation*

No data submitted

F. Soytrimonium chloride – eye irritation, isolated chicken eye assay in vitro

New Test, Submission Dec 2010

Method:	In Vitro Isolated Chicken Eye Test - OECD Guideline No. 438
Eye species:	Chicken
Number of eyes:	3 per test group
Site of application:	Cornea
Test material:	Arquad SV60PG - GTS72278 (60.3% active Soytrimonium Chloride) (identity and purity not confirmed)
Test substance:	Soytrimonium chloride
Concentration, vehicle and quantity:	GTS72278 - undiluted and at 5% v/v solution in sterile water (equivalent to 60.3% and 3.015% active Soytrimonium Chloride respectively)
Negative control:	Physiological saline, 0.9%
Positive control:	Benzalkonium chloride, 5% w/v aqueous
Duration of contact:	10 seconds followed by rinsing with 20ml saline
Evaluation times:	0, 30, 75, 120, 180 and 240 minutes
GLP statement:	Yes
Study period:	Feb – Nov 2010

The test material (Arquad SV-60PG - GTS72278) was applied both undiluted (60.3% active Soytrimonium Chloride) and at a 20-fold dilution (3.015% active Soytrimonium Chloride). The diluted concentration is equivalent to the maximum on-head hair colorant usage concentration.

Results
The undiluted material produced severe irritation in this assay with an irritation index of 141/200. Histological examination of the corneas revealed moderate erosion of the epithelium with no effects on the stroma and endothelium being observed.

The 5% (v/v) aqueous dilution of the test material caused very slight swelling (5%), slight opacity (1.0) and slight to moderate fluorescein retention (1.5). The calculated Irritation Index was 55/200. Histological examination of the corneas treated with the diluted material revealed very slight or slight erosion of the epithelium, with no effects on the stroma and endothelium being observed.

Overall, these results indicate that the undiluted test material has the potential to induce severe eye irritation, in line with the other n-alkyl trimethylammonium materials detailed in SCCS/1246/09, whereas the diluted material can be considered to be irritating.

Treatment	Maximum mean score for Swelling %	Opacity	Fluorescein retention	Irritation Categories [1]	Irritation Index	Classification[2]
Undiluted	21	3	3	III;IV;IV	141	Category 1
5% in sterile water	5	1.0	1.5	I;II;II	55	Not classified
Negative control	0	0.0	0.0	Not applicable	Not applicable	Not classified
Positive control	23	3.0	3.0	III;IV;IV	143	Category 1

[1] *I = no effect, II = slight effect, III = moderate effect, IV = severe effect*
[2] *Classification according to EU CLP*

Histology summary

Treatment	Tissue	Histological observations
Undiluted	1	Moderate erosion of the epithelium
	2	Moderate erosion of the epithelium
	3	Moderate erosion of the epithelium
5% in sterile water	1	Very slight erosion of the epithelium
	2	Very slight erosion of the epithelium
	3	Slight erosion of the epithelium
Negative control	1	No effects observed
	2	
	3	
Positive control	1	Moderate erosion of the epithelium
	2	Severe erosion of the epithelium
	3	Severe erosion of the epithelium

Conclusions
Applying the UN-GHS and the EU-CLP classification criteria of the ICE, the undiluted test material (60.3% active ingredient) is classified as Category 1 (irreversible effects on the eye/serious damage to the eye). Some eye irritation potential of the 5% (v/v) aqueous dilution of the test material was observed in the ICE test.

Ref.: 2 (subm 2010)

Comment
Firm conclusions whether the dilution tested has the potential of only minimal or transient eye irritation cannot be drawn based on the ICE test.

3.3.3. Skin sensitisation

Taken from SCCS/1246/09 - Summary

Tests conducted:

A. *Cetrimonium chloride* - sensitisation, Maximisation test

B. *Cetrimonium chloride* - sensitisation, Buehler test

C. *Steartrimonium chloride* - sensitisation, Maximisation test

D. *Steartrimonium chloride* - sensitisation, Buehler test

E. *Behentrimonium chloride* - sensitisation, Buehler test - study 1

F. *Behentrimonium chloride* - sensitisation, Buehler test - study 2

Submission 2010

G. Soy*trimonium chloride* - sensitisation

No data submitted

General comments with regard to skin sensitisation (taken from SCCS/1246/09)

The majority of the presented sensitisation studies suffer a number of significant shortcomings, such as the lack of preliminary test data, the lack of individual positive control data, etc. Since the tested quaternary ammonium compounds are known to exhibit profound irritative properties, the lacking data would be helpful for the interpretation of the sensitisation results, which for the time being remain difficult to interpret.
Nevertheless, it must be recognised that quaternary ammonium compounds are not known to be sensitising, but merely corrosive. There are some rare clinical reports (Ref.: A (subm 2004)), but considering the many years of use of these compounds, they are considered to be of minor importance. Therefore, there is no reason to consider *cetrimonium chloride*, *steartrimonium chloride* or *behentrimonium chloride* as important skin sensitisers.

Specific comment on Soy*trimonium Chloride:*
Unlike the above mentioned quaternary ammonium compounds containing saturated alkyl chains, Soytrimonium Chloride is a mixture of quaternary n-alkyl trimethylammonium compounds, one of them predominant (about 70%) and containing an unsaturated alkyl chain. The double bond and the adjacent allylic positions of hydrocarbons are a structural alert for autoxidation in presence of air oxygen. The formation of hydrocarbon hydroperoxides at the allylic positions may readily occur. According to general experience, oxidation of unsaturated hydrocarbon chains occurs slowly in presence of air oxygen but is expected to happen much more rapidly under the oxidative conditions of hair dyeing. Hydrocarbon hydroperoxides formed may act as prehaptens (see Opinion on Fragrance Allergens in Cosmetic Products, SCCS/1459/11; Ref. 28).
Hence, appropriate studies, according to the Notes of Guidance, with oxidised soytrimonium chloride are required to determine a sensitisation potential.

3.3.4. Dermal / percutaneous absorption

3.3.4.1 *In vitro* dermal / percutaneous absorption

A. Cetrimonium chloride - dermal absorption in vitro - pig skin

Taken from SCCS/1246/09, modified

Guideline:	Draft OECD TG 428: Percutaneous Absorption: *in vitro* Method (1994)
Test system:	Excised, dermatomed (1000µm) pig skin (back and flank of castrated male pig) on a static diffusion cell
N° of samples:	6 (not stated whether they originated from one or more animals)
Test substance:	Hair care formulation containing 3.5% *cetrimonium chloride* (see also remarks below)
Batch:	Not stated
Purity:	Not stated
Applied amount:	25 mg/cm² (100 mg on 4 cm²) of the formulation (corresponding to 0.87 mg/cm^2 of the active ingredient), rinsed off with shampoo & water after 30 min
Duration of study:	72 hours
GLP/QAU:	In compliance
Study period:	July - Aug 1997

Preparations of dermatomed pig skin measuring 1000 µm in thickness with stratum corneum, epidermis and parts of the dermis were used. Six skin samples were mounted in parallel in Teflon diffusion chambers which were continuously rinsed with receptor fluid (0.9% sodium chloride in distilled water). Prior to the experiment, a skin integrity test was conducted using the marker substance caffeine. The integrity of the skin disks could be demonstrated with cumulative amounts over 5 hours from 0.05 to 0.21% of an applied caffeine dose. The test formulation reported to contain 3.5% *cetrimonium chloride* was applied to the skin disks at an area dose of 25 mg/cm² (100 mg on 4 cm²) for an exposure period of 30 minutes and subsequently rinsed off with a neutral shampoo and water.

Concentrations of *cetrimonium chloride* in receptor fluid were determined at the start of the experiment (0 hours) and after 16, 24, 40, 48, 64 and 72 hours by HPLC/ESI/MS detection. In addition, the test compound was analysed in different skin layers and in the rinsing fluid in order to enable calculation of total recovery.

Results
At any of the different sampling times, small quantities of the test compound could be detected in the horny layer (1.25-14.25 µg/cm²) and in residual skin (0.75-7.25 µg/cm², corresponding to 0.086-0.83%, with a mean of 0.27±0.28%). The total recovery was about 108%. The following table shows the exact values expressed as µg/cm²:

Amount of *cetrimonium chloride* in:	**Expressed as µg/cm² [mean ±SD (range)]**
Receptor fluid	below detection limit (100 ppb)
Stratum corneum	6.1 ± 5.3 (1.25 - 14.25)
Dermis	2.3 ± 2.5 (0.75 - 7.25)
Rinsing solution	742 ± 38 (701.8 - 805.8)
Spatula/swabs/pipette	140 ± 51 (64.5 - 215.5)
Total recovery	891 ± 43 (842.0 - 960.5)

Conclusion
The study authors conclude that, viewing the fact that the horny layer of the skin has not been completely separated; the worst case situation should be considered. They propose to use the amount found in the dermis (7.25 µg/cm² as upper level) for quantitative exposure assessment for rinse-off products.

Ref.: 31 (subm 2004)

Comments

-	The exact composition of the test substance is unknown. The following information can be found in different sections of the study report.
p.9:	One formulation of *cetrimonium chloride* (an emulsion with 3.5% content of active ingredient) was tested …..
p.11:	As a hair-care formulation was used, application was performed over a time span of 30 minutes, ...
p.11:	*cetrimonium chloride* was used in a formulation with 3.5% content of active ingredient; more information can be found in Appendix I
Appendix I:	Chemical data of Henkel KgaA 25% *cetrimonium chloride* aqueous solution (no trade name stated, though in Appendix III, Dehyquart A-CA is mentioned)
p.13:	The test compound *cetrimonium chloride* was applied ...

Taking all the above information together, it can be assumed that the test was performed with a hair-care formulation containing 14% of a commercial 25% *cetrimonium chloride* aqueous solution. The composition of the hair care formulation is not stated.

The results expressed as a percentage are not displayed in this report. They are not considered relevant since the applied dose was clearly in excess. Therefore the percentages would provide an underestimation of the real dermal absorption.

For the purposes of the risk assessment, a conservative value (limit of quantification, LOQ) for the receptor fluid may be taken as a worse case value with the assumption that the amounts in the receptor fluid were at the respective LOQ value. For the duration of 24 h, a mean value of about 3.3 µg/cm² (range 2.4-3.7 µg/cm²) can be calculated from the data in the table in Appendix III of the study. Adding the amount of the test substance in dermis would result in **10.6 µg/cm²** as a worst case value.

B. Steartrimonium chloride - dermal absorption in vitro

No data submitted

C. Behentrimonium chloride - dermal absorption in vitro

No data submitted

D. Soytrimonium chloride - dermal absorption in vitro - human skin

New study, submission 2010

Guideline:	OECD guideline 428 (2004)
Tissue:	human skin (2 thigh, 1 abdomen, 2 with unknown location from 5 females) for each of both test items thickness nominally 400 µm
Membrane integrity:	Only skin samples with TER values >10 kΩ were used
Diffusion cell:	glass diffusion cells, static system (exposed membrane area 2.54 cm^2)
No. of chambers:	12 from 5 donors for each of both test items 3 untreated control chambers for each of both test items
Test substance:	Soytrimonium chloride (Arquad SV-60PG) (non-radio-labelled)
Reference substance:	Arquad SV100, lot No. 2245-22A (non-radio-labelled)
Purity:	/
Test item 1:	1a). Oxidative hair dye formulation containing nominally 10% GTS72278 and 6.03% soytrimonium chloride (STC) (not certified) Batch No. SWF2750-2 1b) Oxidative Hair Dye Developer,

	Batch No. CTT123-19 *1a) and 1b) were mixed in a ratio 1:1(v/v) before application*
Area dose:	808 µg STC/cm^2
Test item 2:	2) Semi-permanent hair dye formulation containing nominally 5% GTS72278 and 3.015% soytrimonium chloride (STC) (not certified) Batch No. DTF0819053BF01
Area dose:	912 µg STC/cm^2
Time period:	30 min (samples of receptor fluid after 0.5, 1, 2, 4, 6, 8, 10, 12, 16 and 24 hours)
Receptor fluid:	phosphate buffered saline (pH 7.4)
Solubility in receptor:	/
Stability:	/
Method of Analysis:	liquid chromatography-mass spectroscopy (LC–MS) of the two unsaturated C_{18} species of soytrimonium chloride (combined response of m/z 310 and 308 ions)
GLP statement:	Yes
Study period:	May - Dec 2010

The penetration and distribution of soytrimonium chloride (STC) in GTS72278 from an oxidative hair dye formulation (test item 1) and a semi-permanent hair dye formulation (test item 2), both containing a nominal 3.015% STC has been determined in vitro through human skin. The test items were each investigated in 12 samples of human skin obtained from five female donors.

Results
Most analytical values were found to be below the limit of quantification (LOQ) or close above. Study data were partly re-calculated and compiled in the table below because of shortcomings in considering LOQs or values close above by the study authors.

Test fraction	**Amount of dose recovered (expressed as µg/cm^2) *)**			
	Oxidative conditions		**Non-oxidative conditions**	
	Mean	S.D.	Mean	S.D.
Donor chamber	5.70	5.66	5.67	7.97
Skin wash after 0.5 h	731	84	830	36
Skin wash after 24 h	(≤65.0)	n.a.	(≤69.4)	n.a.
Stratum corneum	≤3.12 **a)**	n.a.	≤6.49 **c)**	n.a.
Dermis/epidermis	≤7.19 **b)**	n.a	(≤4.05)	n.a
Receptor fluid	(≤0.234)	n.a	(≤0.246)	n.a
Total recovery	≤812	56	≤916	32
Dose applied	808		912	
Absorbed dose	≤7.424	n.a	≤4.296	n.a

n.a., not applicable
*) Values in bracket are limits of quantification (LOQ)
a) Two of 8 sample values were close above the LOQ of 2.70 µg/cm^2.
b) Two of 8 sample values were close above the LOQ of 6.50 µg/cm^2.

c) One of 9 sample values was close above the LOQ of 6.36 µg/cm^2.

Oxidative Hair Dye/Developer mixture (test item 1):
The analysed concentration of the test substance was 4.04% (w/w) (808 µg/cm^2). The amount assumed to be systemically available (i.e., the amount in the remaining epidermis/dermis added to the amount in the receptor fluid) was less than **7.42 µg/cm^2** or **0.92%** of the STC applied.
The mean total recovery was about 100%. Four cells from the oxidative hair dye/developer mixture group were excluded from the means, as the total recoveries were outside the sponsor's 85 – 115% exclusion criteria (no investigation on possible reasons or explanation of the deviations was reported).

Semi-permanent hair dye formulation (test item 2):
The analysed concentration of the test substance was 4.56% (w/w) (912 µg/cm^2).. The amount assumed to be systemically available was less than **4.30 µg/cm^2** or **0.47%** of the STC applied.
The mean total recovery was about 100% of the STC applied. Three cells from the semi-permanent hair dye group were excluded from the means, as the total recoveries were outside the sponsor's 85 – 115% exclusion criteria (no investigation of possible reasons or explanation of the deviations was reported).

Ref.: 4 (subm 2010)

Comments
The study has several shortcomings regarding characterization of the test and reference substance and analytical determinations:

- Purity of the reference item (Arquad SV-100):
The purity (98.4%) stated in the certificate of analysis (CoA) is questionable. As the chain distribution of the material was not certified, the purity could not be determined.
- Analytical problems: Due to differences between test substance and reference substance regarding the chain distributions, both C_{18} unsaturated chains (one and two double-bonds) were finally determined.
-Stability of the test substance:
The stability and the fate of the test substance both under non-oxidative and oxidative study conditions remain unclear. The validation study (ref. 17) revealed stability problems with the test and reference substance which could apparently not be completely solved until the end of the study. Stability testing was reported only for a few days, but not for the envisaged time period of one month. The latter time period would have been required to assess the stability of the study samples, as these were stored for several weeks before analysis. According to the last amendment (no. 7) to the validation study, an amendment to the final report of the validation study regarding stability testing and accuracy determination was requested by the sponsor. This amendment to the final report is missing in the documentation of the submission.

- No documentation on the technical ability of the performing laboratory and the validity of the method was included in the study report (see Basic Criteria, SCCS/1358/10, Ref. 20)

- The LOQs considerably vary between different matrices and in part appear to be unusually high.

Addendum to the above validation study (submission May 2012)

After the publication of the Opinion in April 2012, an Addendum to the above validation study (ref. 17) was provided by the applicant in May 2012. The Addendum had been requested by the study sponsor from the test facility in a study plan amendment (no. 7, signed 13 Dec 2010) to the validation study. GLP was stated. Both the Addendum initiation

date and the date of the initiation of the experimental phase were not reported. The completion date of the experimental phase of the Addendum was 20 May 2011 and the Addendum completion date was 22 March 2012. In the Addendum, the test facility aimed at providing data and evidence on the accuracy/precision on the analytical method and on the stability of the test substance soytrimonium chloride under experimental dermal absorption, storage and work up conditions.

Results
The accuracy and precision of the methodology for the quantification of the test substance, when evaluated in both oxidative and semi-permanent formulation, was generally established on skin, tape strip and cotton swab. However, several exceptions were noted, in particular in the low concentration range where 2/3 of the mean accuracy values did not meet the accuracy criterion of ±20%. Deviations of the accuracy values were up to 54%.
The stability of the test substance in both formulation types when stored at room temperature for 4 weeks was established. The stability of extracts of both formulation types at room temperature for 48 hours was established. Some deviations were explained by errors in the sample preparation/work up procedure. The stability of the test substance in the receptor fluid stored at room temperature for 11 weeks was not established. The concentrations of the test substance were found significantly reduced (21-28% of the nominal concentrations) over the storage period. The losses were explained to be caused by the test substance material adhering the walls of the glass containing vessels during storage. Experimental data confirming this explanation were not reported.

Ref. 17a, submitted May 2012

Conclusion
Due to the above described shortcomings of the validation study including the Addendum, the study data on dermal absorption is not acceptable. It cannot be excluded that losses of the test substance in study samples occurred both under non-oxidative and oxidative conditions and that the low amounts/concentrations of the test substance in skin or receptor fluid, reported below the LOQs or close above, are artefacts under experimental or storage conditions.

3.3.4.2 *In vivo* dermal / percutaneous absorption

A. Cetrimonium chloride - dermal absorption in vivo - rat

Taken from SCCS/1246/09

A publication of 1979 describes a dermal absorption study in the rat with *laurtrimonium bromide (dodecyl trimethyl ammonium bromide)*, which was included in the submission because of the chemical analogy between the tested compound and cetrimonium chloride (hexadecyl trimethyl ammonium chloride). The specific activity of the (1-^{14}C) compound was 24.7 µCi/mg. Three different settings and their results are described:

1) Dermal application with rinsing: 200 µl volume of a 1% laurtrimonium bromide aqueous solution was applied to a 10 cm² clipped skin area (0.2 mg/cm²) of 5 rats for 18 minutes (no occlusion) and subsequently rinsed off.
Of the radioactivity applied, 13.2% remained on the skin. The total absorption was 0.59±0.13% of the applied radioactivity, corresponding to 1.18±0.26 µg/cm². Most of the absorbed amount was excreted in the urine (0.35% of the applied dose during the first 24 hours). The total recovery was 95.3±2.4%.

2) Dermal application with rinsing: 261-293 mg of a hair rinse-off solution containing 0.42% laurtrimonium bromide were applied to the clipped skin area (0.123 mg/cm²) of 5 rats for 8 minutes (no occlusion) and subsequently rinsed off.
Of the applied radioactivity, 4.11% remained on the treated skin site. Only 0.016% of the amount applied was excreted during the first 24 hours. No significant radioactivity was

detected in the blood during the experiment. The total absorption was 0.093±0.061% of the applied radioactivity, corresponding to 0.11±0.075µg/cm². The total recovery was 92.6±3.1%.

3) Dermal application without rinsing: 240 µl of a 3% laurtrimonium bromide aqueous solution was non-occlusively applied to a clipped skin area of 8 cm² (0.90 mg/cm²) of 3 rats. While in the experiment with rinsing, the excretion of radioactivity was always lower on day 2 compared to day 1, in this experiment there was a marked increase in absorption on day 2. The study authors suggested that this was caused by slight but invisible skin damage. The percutaneous absorption of the applied radioactivity was 3.15±1.65%, corresponding to 28.4±14.9 µg/cm². The total recovery was 96.4±7.1%.

Ref.: 32 (subm 2004)

B. Steartrimonium chloride - dermal absorption in vivo

No data submitted

C. Behentrimonium chloride - dermal absorption in vivo

No data submitted

D. Soytrimonium chloride - dermal absorption in vivo

No data submitted

General comments on Section 3.3.4

Low dermal absorption values were reported in the in vitro dermal absorption studies with Cetrimonium Chloride, Soytrimonium Chloride and an in vivo study with Laurtrimonium Bromide (data summarized in the table below). Both in vitro studies have shortcomings. In particular the varying LOQs higher than the concentrations of the test substances in the receptor fluid and epidermis/dermis make an assessment of the doses dermally absorbed difficult.

Comparison of study data on dermal absorption of n-alkyl trimethylammonium compounds

Substance	Test system	Conditions of dermal application	Area dose (mg/cm^2)	Exposure, (duration)	Absorbed dose ($\mu g/cm^2$)	Absorbed dose (%)
Soytrimonium chloride	Human skin, 400 µm	1) 4.04% in an oxidative hair dye formulation	0.808	30 min, (24 h)	7.42 *), (worst case)	0.92 *)
		2) 4.56% in a semi-permanent hair dye formulation	0.912	30 min, (24 h)	4.30 *) (worst case)	0.47 *)
Cetrimonium chloride (C_{16} alkyl)	Pig skin, 1000 µm	3.5% in an emulsion (hair care formulation)	0.87	30 min, (24 h; up to 72 h)	10.6 (24 h) (worst case)	1.22
Laurtrimonium bromide	Rat, in vivo	1) 1% in an aqueous solution	0.2	18 min, (24 h)	1.18±0.26	0.59±0.13

(C_{12} alkyl)		2) 0.42% in a hair rinse-off solution	0.123	8 min, (24 h)	0.11±0.075	0.093±0.061
		3) 3% in an aqueous solution	0.90	leave-on	28.4±14.9	3.15±1.65

*) Values are not considered reliable due to severe shortcomings of the analytical part of the study.

The *in vivo* study with Laurtrimonium Bromide is considered relevant for comparison for the following reasons:

- The test was performed in two rinse-off settings and one leave-on setting with an analogous compound to Soytrimonium Chloride.
- The analogous compound (Laurtrimonium Bromide) has a similar structure and chain length which means that its dermal absorption is likely to be similar compared to the substance mixture Soytrimonium Chloride.
- The *in vivo* study was performed in the rat, which is known to display a dermal absorption normally much higher than human skin. On the other hand, for the rinse-of settings, lower concentrations of Laurtrimonium Bromide and shorter exposure times on the skin compared to the in vitro study with Soytrimonium Chloride were used, thus reducing the dermal absorption in rat skin.

Taken together, the dermal absorption data of all studies conducted were comparable and of the same magnitude. However, all these studies suffer from shortcomings.

The values assessed for dermal absorption of Soytrimonium Chloride in human skin *in vitro* are considered not reliable due to severe shortcomings of the analytical part of the study. Instead, by use of an analogue approach, the dermal absorption values of Cetrimonium Chloride in pig skin could be used for the calculation of the Margin of Safety, despite the shortcomings of the study.

3.3.5. Repeated dose toxicity

3.3.5.1 Repeated dose (28 days) oral / dermal / inhalation toxicity

No new data.

Taken from SCCS/1246/09 (Ref. 1) (re-assessed)

A. *Cetrimonium chloride* - 28-day oral administration in the rat

Guideline:	Annex V to Dir. 67/548/EEC, Method B.7: Repeated dose (28 days) toxicity (oral)
Date of test:	Aug - Sep 1988
Species/strain:	Sprague Dawley rat
Group size:	10 animals/sex/dosage group and 5 animals/sex/recovery group
Test substance:	Dehyquart A-CA (24-26% *cetrimonium chloride* in water)
Batch:	548050
Purity:	Not stated
Dosages:	0 - 30 - 100 - 300 mg/kg bw/day (corresponding to about 7.5, 25, 75 mg/kg bw/day of the active substance in the dose groups)
Observation period:	56 days
GLP/QAU:	In compliance

Groups of 10 male and 10 female rats received 30, 100 and 300 mg/kg bw/day of Dehyquart A-CA by oral gavage for 28 days (5 days per week). Control animals received 10 ml of distilled water per kg bw/day. Control and high dose groups were supplemented with

5 rats/sex in order to study the reversibility of treatment-related effects after a subsequent 28-day treatment-free period. Twice daily clinical observations and mortality checks were scheduled, while body weights and food consumption were recorded weekly. At the end of the exposure period, ophthalmologic examinations, blood biochemical and haematological investigations were performed. At terminal sacrifice, all animals were subjected to gross necropsy and the organs were weighed. A large number of organs and tissues from animals of all study groups were preserved and the majority of these specimens were subjected to histopathological examination.

Results
The following effects were noted:

30 mg/kg bw/day: no adverse effects noted

100 mg/kg bw/day: no adverse effects noted

300 mg/kg bw/day:
- statistically significant increases in serum alanine aminotransferase (ALT) activity in males (2-fold) and females (1.6-fold) (both outside the range of the historical controls);
- males: slight increase in absolute and relative adrenal weights and slight decrease in absolute and relative spleen weight
- macroscopic examination: thickening of the forestomach mucosa, associated with oedema and sporadic ulceration in male and female rats;
- microscopic examination: inflammatory oedema of the forestomach mucosa, sporadic ulceration and acanthosis up to papillomatous hyperplasia in both sexes;
- no histopathological alterations were found in adrenals and spleen or any other organs

All animals survived the study and all treatment-related changes were shown to be reversible following the recovery period.

There were no effects on food consumption and body weight development. The mean water intake of the males of the high dose group was higher than that of controls. Ophthalmologic and haematological results revealed no treatment-related changes in any group.

Conclusion (taken from Ref. 1):
The study authors conclude that the forestomach and stomach changes observed at 300 mg/kg bw/day can be considered to be a result of local irritation and therefore are not indicative of systemic toxicity. The slight weight changes of spleen and adrenals and the increase in serum ALT activity were regarded as possible signs of some systemic toxicity.
The dosage of 100 mg/kg bw/day was considered to be the no-observed-adverse-effect-level (NOAEL) of Dehyquart A-CA (24-26% *cetrimonium chloride* in water, corresponding to about 25 mg/kg bw/day of the active substance) in this study.

Ref.: 33

B. *Cetrimonium chloride* - 28-day dermal administration in the rabbit

In a study from 1978, 5 New Zealand albino rabbits/sex/group were treated cutaneously with the test substance for 5 days/week for 4 weeks at a dose of 0 or 10 mg/kg bw/day (0 or 0.5% aqueous solutions, respectively). The dosage volume was 2.0 ml/kg bw with an approximate exposure period of 6.5 to 7 hours. Body hair was clipped as needed on approximately 25% of the body surface area. The skin of all rabbits was abraded with a clipper head prior to each application. The animals were restrained with collars during the exposure period. Following the exposure period, the treated skin surface was cleaned with

water. All rabbits were examined daily for clinical signs and mortality. Dermal irritation readings were recorded daily. The animals were weighed weekly during the exposure period. Blood was collected for haematology measurements before initiation of dosing and prior to termination. Liver and kidneys were weighed at necropsy. A complete list of tissues was collected for histopathological evaluation.

Two control group animals died during the study. Slight to moderate erythema was observed in all treated rabbits between days 4 and 8, but disappeared in 4 rabbits by day 17. Very slight to slight oedema was observed between days 6 and 12 in 4 rabbits and subsided by day 17. Two rabbits had intermittent slight oedema during week 4, and one rabbit developed oedema on day 20. No evidence of desquamation or leather-like skin was present in these animals. In the other rabbits, slight atonia occurred up to week 4 in 3 animals. Slight skin fissuring was observed in most of the rabbits but typically disappeared by the end of the study. There were no treatment-related effects on body weight, haematology, organ weight, gross necropsy findings or histopathology, except for treated areas of the skin that showed mild to marked acanthosis with active mitosis, hyperkeratosis, and partial to extensive necrosis of the epidermis and hair follicles, partly with encrustation and exudate.

The US National Toxicology Programme report concluded that the toxic response only consists of skin irritation, and proposed a NOAEL value of 10 mg/kg bw/day for systemic effects.

Ref. 34 (= pp.170-171 of Ref.1, submission 2004)

Conclusion

There were no treatment-related effects on body weight, haematology, organ weight, gross necropsy findings or histopathology, except for treated areas of the skin that showed mild to marked acanthosis with active mitosis, hyperkeratosis, and partial to extensive necrosis of the epidermis and hair follicles, partly with encrustation and exudate.

The US National Toxicology Programme report concluded that the toxic response only consists of skin irritation, and proposed a NOAEL value of 10 mg/kg bw/day for systemic effects.

C. *Steartrimonium chloride* - 28-day oral/dermal administration

No data submitted

D. *Behentrimonium chloride* - 28-day oral/dermal administration

No data submitted

E. *Soytrimonium chloride* - 28-day oral/dermal administration

No data submitted

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

No data submitted

3.3.5.3 Chronic (> 12 months) toxicity

No new data.

Taken from SCCS/1246/09

A. *Cetrimonium bromide* - chronic toxicity

A publication of 1976 reports the adverse effects observed in groups of 10 male and 10 female Sprague Dawley rats when treated orally for one year with 10, 20 and 45 mg *cetrimonium bromide*/kg bw/day in drinking water. The following effects were noted:

10 mg/kg bw/day:
- slightly increased body weight (not statistically significant)

20 mg/kg bw/day:
- slightly increased body weight (not statistically significant)
- increased relative caecum weight in males

45 mg/kg bw/day:
- significantly reduced mean body weights in both sexes after 3 weeks, persisting till end of study in males and for 9 weeks in females
- males: significantly decreased efficiency of food conversion
- significantly reduced skeletal growth (judged by the growth of the tail) in both sexes
- wetting and discoloration of ventral fur, often associated with a brown discoloration of the fur
- males: reduced relative liver weight
- increased relative caecum weight in both sexes

No compound related changes were observed in haematological and clinical laboratory analyses of blood and urine. No gross necropsy changes were seen, and no microscopic alterations were found in the wall of stomach and small intestine of treated rats. No other tissues were subjected to histopathological examination.

The authors conclude that *cetrimonium bromide*, when continuously administered in large doses, may potentially prevent proper nutrition by increasing the rate of gastric emptying and intestinal transit and/or by interfering with the absorption of nutritional substances.

Ref.: 35 (subm 2004)

Comment
Slight systemic effects were observed. Suppression of growth was also observed with another quaternary ammonium compound, alkyl dimethyl benzyl ammonium chloride, at a dosage of about 63 mg/kg b.w./day (LOAEL, no NOAEL derived; study cited in Ref. 31) For the calculation of the MoS, the NOAEL of 10 mg/kg bw/day for the analogous substance *Cetrimonium bromide* appears appropriate. Although this NOAEL may be the consequence of a local effect in the gastrointestinal tract, this NOAEL could be used as a conservative approach in the MoS calculation.

B. *Steartrimonium chloride* - chronic toxicity

No data submitted

C. *Behentrimonium chloride* - chronic toxicity

No data submitted

D. *Soytrimonium chloride* - chronic toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

No new data.

Taken from SCCS/1246/09

A. *Cetrimonium chloride* - Bacterial reverse mutation assay

B. *Steartrimonium chloride* - Bacterial reverse mutation assay 1

C. *Steartrimonium chloride* - Bacterial reverse mutation assay 2

D. *Steartrimonium chloride* - Bacterial reverse mutation assay 3

E. *Behentrimonium chloride* - Bacterial reverse mutation assay

F. *Cetrimonium chloride* - *in vitro* chromosome aberration test

The presented mutagenicity studies were all negative. Testing was limited to low concentrations due to the high cytotoxicity.

G. *Soytrimonium chloride* - mutagenicity / genotoxicity

No data submitted

Comment
Apart from some shortcomings in the above tests conducted, the results do not indicate a mutagenic/genotoxic potential of *Cetrimonium, Steartrimonium* or *Behentrimonium Chloride*. However, due to the unsaturated hydrocarbon chain of oleyl trimethylammonium in the mixture, Soytrimonium Chloride is considered chemically less stable than the tested substances above, in particular under oxidative conditions, so that a read-across in terms of mutagenicity/genotoxicity is not possible. Consequently, appropriate studies according to the Notes of Guidance with Soytrimonium Chloride are required to determine a mutagenicity/genotoxicity potential.

3.3.7. Carcinogenicity

No *in vivo* studies are available.

Taken from SCCS/1246/09

Cetrimonium chloride - *in vitro* cell transformation assay

A publication of 1979 describes how cryo-preserved primary Syrian hamster embryo cells were cultivated and incubated with 0.1, 1.0 and 5.0 µg *cetrimonium chloride*/ml. The substance showed to be toxic at the highest concentration tested, though did not produce transformation at any of the doses tested.

Ref.: 1, 40 (subm 2004)

3.3.8. Reproductive toxicity

No new data.

Taken from SCCS/1246/09

A. *Cetrimonium chloride,* dermal administration, rabbit

Under the test conditions used, *cetrimonium chloride* was not found to be foetotoxic and teratogenic. The NOEL for maternal systemic toxicity and embryo-foetal toxicity appeared to be 40 mg *cetrimonium chloride*/kg bw/day.

Ref.: 41 (subm 2004)

B. *Steartrimonium chloride* - dermal administration, rat

Under the test conditions used, *steartrimonium chloride* was not found to be foetotoxic and teratogenic. The NOEL for maternal systemic toxicity and embryo-foetal toxicity was about 12.5 mg *steartrimonium chloride*/kg bw/day (highest concentration tested).

Ref.: 1 (subm 2004)

Taken from SCCS/1246/09

A publication of 1983 summarizes a study in which the potential embryotoxic effects of *dimethyldistearylammonium chloride*, *benzyldimethylstearylammonium chloride* and *trimethylstearylammonium chloride* were investigated upon topical application of concentrations up to 9.9%, 6.6% and 2.5%, respectively. The authors conclude that, within the limitations of the study, none of the tested quaternary ammonium compounds exerted any selective embryopathic activity when applied topically to pregnant rats during the organogenic period and using dosage regimes eliciting adverse maternal reactions (local skin irritation).

Ref.: 42 (subm 2004)

C. *Behentrimonium chloride*

No data submitted

D. *Soytrimonium chloride*

No data submitted

3.3.9. Toxicokinetics

No new data

Taken from SCCS/1246/09

A. *Cetrimonium chloride/bromide*

A publication of 1975 reports a toxicokinetic study in rats using ^{14}C-labeled *cetrimonium bromide*. After administration of 0.8 mg/kg by oral gavage, about 80% of the dose of radioactivity was found in the gastrointestinal tract 8 hours after the administration, only small amounts were found in the blood plasma and about 2% of the administrated radioactivity was excreted in the bile during the first 12 hours after treatment. Only small amounts of radioactivity were found in the liver (about 0.8% of administered radioactivity), kidneys, spleen, heart, lung and skeletal muscles. Within three days of ingestion 92% of the radioactivity was excreted via the faeces and 1% via urine. The authors conclude that *cetrimonium bromide* is poorly absorbed in the gastro-intestinal tract and not readily metabolised in the rat body.

Intraperitoneal injections of ^{14}C-labeled CTAB to bile-duct cannulated rats showed that after 24 hours 36% of the radioactivity was excreted in the bile and 1% in the urine. The study indicated that CTAB was subjected to metabolic transformation, but the metabolites were not identified.

Ref.: 43 (subm 2004)

A publication of 1979 reports on the elimination and tissue distribution of ^{14}C-labelled *laurtrimonium bromide* (unsaturated C_{12}-alkyl chain) in rats. After a single intravenous injection of 0.5 ml of a 0.023% solution in saline, most radioactivity was eliminated from the body via the kidneys (58.9% after 24 hours and 68.1% after 48 hours) and faeces (11.6% after 24 hours and 14.1% after 48 hours). Radioactivity levels in the blood decreased rapidly and detected concentrations were 0.50 µg/ml at 3 minutes, 0.10 µg/ml at

9 minutes, 0.03 µg/ml at 30 minutes and 0.02 µg/ml at 120 minutes. The percentage of administered radioactivity in the liver and kidneys was 24.8% and 5.54%, respectively, after 15 minutes and 2.08% and 0.36%, respectively, after 24 hours. Only small amounts of radioactivity were found in other organs and there was no sign of accumulation of radioactivity in any organ.

Ref.: 32 (subm 2004)

B. *Steartrimonium chloride*

No data submitted

C. *Behentrimonium chloride*

No data submitted

C. *Soytrimonium chloride*

No data submitted

General comments on Section 3.3.9

In earlier submissions and in the Submission 2010, some aspects of metabolism have not or not adequately been addressed. In the Submission 2010, the applicant only considers the metabolism of the alkyl chains but does not take in account the fate of the trimethylammonium moiety of the substances. This reads as follows:
"The postulated mechanism of metabolism for even-carbon chain alkyl trimethylammonium chloride is degradation by a common pathway involving ω-oxidation, followed by β-oxidation, to yield metabolites with chain lengths of C_2 and C_4. Metabolism of odd numbered chains is postulated to follow a similar ω-, β-degradation pathway. Research on related surfactants (*e.g*. alkyl sulphates) indicates that the biotransformation of the hydrocarbon chain proceeds via the normal metabolic pathway of cytochrome P450 dependent ω-oxidation of aliphatic fatty acids [6]. Furthermore, a long hydrocarbon chain in the aliphatic fatty acids could undergo dehydrogenation metabolic pathway to form unsaturated hydrocarbon chain in vitro [7-10]. Literature report also indicated that unsaturated hydrocarbon chain in the oleic acid could be converted to corresponding saturated hydrocarbon chain in vivo via hydrogenation pathway [11]. In addition, a common Phase II mammalian detoxification pathway for aliphatic fatty alcohols involves enzymatically-mediated sulphate conjugation, followed by urinary excretion of the resulting polar, sulphate esters. Since the biotransformation of the surfactants involves shortening of the hydrophobic chain to yield more polar, excretable end-products, metabolism is expected to curtail the effects of surfactancy on systemic tissues distant from the site of application, thereby reducing toxicity."

However, similar to secondary and tertiary amines, the trimethylammonium structure is often considered as a structural alert for nitrosamine formation under appropriate reaction conditions, in particular depending on pH and nitrate/nitrite taken up, e.g., with food [21, 22, 23, 27]. Alkyl trimethylammonium compounds require cleavage of the alkyl carbon-nitrogen bond or methyl carbon-nitrogen bond to yield the respective tertiary amines, which may be further metabolized to the corresponding secondary amines. *Cetrimonium* Bromide and other alkyl/aryl trimethylammonium compounds have been shown to be metabolized to trimethylamine and dimethylamine (and possibly tertiary dimethylamines containing the alkyl chain) by rat liver microsomes [24, 25]. The velocity of metabolism and the formation of tertiary and secondary amines were dependent on the length and structure of the alkyl or aryl moiety of the molecule. However, the amines formed were only qualitatively determined (paper chromatography). Moreover, amine nitrosation by nitrite has been reported to be catalyzed in presence of the cationic surfactant decyltrimethylammonium

bromide and other micelle forming agents [26], suggesting amine impurities in such surfactants being prone to enhanced nitrosation risk (Ref. 27). Among the amines potentially formed from alkyl trimethylammonium compounds, at present only dimethylamine has been shown to be transformed to a carcinogenic nitrosamine whereas it is unknown whether carcinogenic nitrosamines may be formed from oleylmethylamine or other N-alkylmethylamines, which may be generated by the metabolism of Soytrimonium chloride. On the other hand, the bioavailability of alkyl trimethylammonium compounds is low. Apart from amine release by metabolism, there is some concern whether tertiary and secondary amines may be released from alkyl trimethylammonium compounds under oxidative conditions of hair dyeing. For these reasons, there exists some uncertainty about the extent of amine release by metabolism and on amine formation under oxidative conditions. Thus, the extent of amine release from Soytrimonium chloride by metabolism and by chemical degradation under oxidative conditions need clarification by the applicant. In case tertiary and secondary amines may be formed in the finished product or under the conditions of use, their concentrations should be restricted to the legal levels.

3.3.10. Photo-induced toxicity

No data submitted

3.3.11. Human data

No new data. See data in SCCS/1246/09

3.3.11.1 Single patch tests on human volunteers - skin irritation
(taken from SCCP/1087/07, revised 2009)

No new data. See data in SCCS/1246/09

A. – I. *Cetrimonium chloride* - single patch test 1 - 9

J. *Behentrimonium chloride* - single patch test 1

K. *Behentrimonium chloride* - additional studies

L. Re-evaluation of human skin irritation patch studies in the submission of July 2007

3.3.12. Special investigations (submission of July 2007)

No new data. See data in SCCS/1246/09

Report on post-marketing surveillance data

Due to the issues raised by the SCCP in 2007, the focus of the study was placed on skin irritation effects.

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Soytrimonium Chloride

Not applicable

3.3.14. Discussion

Parts of the discussion were taken from the Opinion SCCS/1246/09 (Ref. 1) as far as related to the n-alkyl trimethylammonium compounds Laurtrimonium chloride (C_{12} chain), Cetrimonium chloride (C_{16} chain), Steartrimonium chloride (C_{18} chain), and Behentrimonium chloride (C_{22} chain) which are used for an analogue approach to predict hazardous properties of Soytrimonium chloride and possible risks to humans exposed.

An analogue approach and read-across by comparison of Soytrimonium chloride with other n-alkyl trimethylammonium (Colipa P72) compounds is in principle considered possible, but needs careful consideration of the data base and data quality for each toxicological endpoint.

Chemical and physical specifications
Soytrimonium Chloride is a technical mixture of n-alkyl trimethylammonium compounds essentially containing unsaturated and saturated C_{16} and C_{18} hydrocarbon chains. Some shortcomings with regard to the data on the characterization, variability of the composition

and stability of Soytrimonium Chloride are described in Section 3.1. The stability of Soytrimonium Chloride, under both oxidative and non-oxidative conditions requires clarification. The stability of the quaternary ammonium function is unknown and there is concern about possible amine release from alkyl trimethylammonium compounds under oxidative conditions. There is also concern about hydroperoxide formation under oxidative conditions from oleyl trimethylammonium chloride which constitutes the major fraction (about 70%) of Soytrimonium Chloride. The dermal absorption study also provides an indication for the potential instability of Soytrimonium Chloride under oxidative and non-oxidative conditions of hair dyeing. Shortcomings with regard to the data on the identification, stability and physico-chemical properties of *Cetrimonium chloride*, *Steartrimonium chloride* and *Behentrimonium chloride* are mentioned in Opinion SCCS/1246/09 (Ref. 1).

Acute systemic toxicity

Cetrimonium chloride, *steartrimonium chloride* and *behentrimonium chloride* display relatively low acute systemic toxicity. The oral LD_{50} value of *cetrimonium chloride* was determined to be 400-600 mg/kg bw, while its dermal LD_{50} appeared to be much higher (4300 mg/kg bw). For *behentrimonium chloride* no data were available, while *steartrimonium chloride* was shown to have an oral LD_{50} in the rat of 536-633 mg/kg bw. It can be expected that the acute oral and dermal toxicity of Soytrimonium Chloride is in the same order of magnitude compared to the substances tested within the chemical category.

Local toxicity

As is the case for the majority of quaternary ammonium compounds, concentrated *Cetrimonium chloride*, *Steartrimonium chloride* and *Behentrimonium chloride* are corrosive/irritant to skin and eyes. This is confirmed by the presented skin and eye irritation studies on rabbits with different formulations containing the quaternary ammonium compounds (Submission of 2008). Similar properties apply to *Soytrimonium chloride*: Based on the results provided, it is reasonable to conclude that soytrimonium chloride only has a slight/minimal potential for skin irritancy (CLP Category 2, not warranted). As presumed from the read-across within the chemical analogue group, the potential of skin irritation, tested with the Episkin ® reconstituted human epidermis model, was confirmed as well as the strong eye irritation potential tested with the Isolated Chicken Eye test.

The submission of 2008 contains 8 human single patch tests with *Cetrimonium chloride*-containing shampoos and hair care formulations. Concentrations ranging from 0.4 to 3.5% were put in contact with the skin under occlusive or semi-occlusive patches, mostly for 24 or 48 hours. The effects on the skin of the volunteers ranged from no irritation up to moderate irritation. Despite some shortcomings of the studies, it is acknowledged that the skin area doses used in the human tests are higher than the skin area doses expected from the intended use levels in cosmetic products. Combined with post-marketing experiences presented for hair products from 5 companies, this compensates for the absence of more detailed results showing linearity. As such, one can assume that the compounds under study are unlikely to cause irritant effects under their intended use. However, a prediction of the local toxicity when using Soytrimonium Chloride under oxidative hair dye conditions is not possible from these human studies due to probable instability of the mixture under oxidative conditions and potential formation of irritating oxidation products.

The submission of 2008 contains sensitisation studies with 24-29% *Cetrimonium chloride*, 80% *Steartrimonium chloride* and 77-83% *Behentrimonium chloride*. For every individual study, the shortcomings are mentioned in section 3.3.3. of the Opinion SCCS/1246/09 (Ref. 1). The available animal in vivo tests and human data do not indicate a sensitizing potential of these quaternary ammonium n-alkyl chain substances. However, the situation may be different with regard to *Soytrimonium chloride*, since its predominant component contains an unsaturated hydrocarbon chain.

Oxidizable allylic positions adjacent to the double bond of oleyl trimethylammonium (about 70% of the *soytrimonium chloride* mixture) are structural alerts for skin sensitization. Possible hydroperoxide formation can lead to prehapten formation, in particular under oxidative conditions as in hair dyeing. The SCCS is of the opinion that an analogue approach and read-across for skin sensitization of Soytrimonium Chloride is not possible. Hence, appropriate studies, according to the Notes of Guidance, with oxidised soytrimonium chloride are required to determine a sensitisation potential.

Dermal absorption of *soytrimonium chloride* and similar trimethylammonium compounds

Studies with three test substances are available, *laurtrimonium bromide (C_{12}-n-alkyl trimethyl ammonium bromide, rat in vivo)*, *cetrimonium chloride* (C_{16}-n-alkyl *trimethylammonium chloride*, pig skin in vitro) and s*oytrimonium chloride* (human skin in vitro). In all three studies, dermal absorption values between 1.2 and 10.6 µg/cm^2 (0.6-1.2% of the dose applied) were reported under short-term exposure and rinse-off conditions. Apart from the P72 substances tested, data available on the dermal absorption of other cationic trimethylammonium alkyl compounds with different structures also suggest low dermal absorption of this substance group. One example is the dermal absorption of labelled *camphor benzalkonium methosulphate (Colipa No S57*) determined in living human epidermis/dermis and representing 0.65 ± 1.04 µg-eq/cm² (0.19 ± 0.31%) of the dose applied (SCCP/1202/08). Another example is HC Blue 16 (COLIPA B119) which was reported to be absorbed by about 0.703 +/- 0.069 micrg/cm² (SCCS/1478/12). Hence, it is conceivable that the low dermal absorption of various alkyl or aryl trimethylammonium compounds is strongly dependent on the cationic character of the trimethylalkonium moiety and less on the lipophilic alkyl or aryl moiety of the molecule and that an analogue approach in principle can be applied.

In general, in case of low absorption of a substance in an in vitro dermal absorption study, the analytical determination of the substance in dermis and receptor fluid may pose a challenge. It cannot be excluded that in cases such as *soytrimonium chloride* and *cetrimonium chloride* the comparably high dermal absorption values determined in vitro are artificially high and at least in part caused by the high analytical LOQs in these studies. The SCCS notes that these dermal absorption values determined in human and pig skin, respectively, are higher than with *laurtrimonium bromide* in rat skin *in vivo.* However, the values determined for dermal absorption of Soytrimonium Chloride in human skin *in vitro* are considered not reliable due to severe shortcomings of the analytical part of the study. In an analogue approach, use of the worst case value for dermal absorption of *cetrimonium chloride* in pig skin (also a study with considerable shortcomings and high analytical LOQs) would lead to a MoS lower than 100, in particular when the total exposure to the P72 group of substances is taken into account. In conclusion, a new dermal absorption study with *soytrimonium chloride* is required.

Toxicokinetics and metabolism

A toxicokinetic study indicated that ^{14}C-labelled cetrimonium bromide was only poorly absorbed in the gastro-intestinal tract after oral administration. Within three days of ingestion, 92% of the radioactivity was excreted via the faeces and 1% via urine. The study was not considered of sufficient quality as a basis for a re-calculation of a MoS.

Similar to secondary and tertiary amines, the trimethylammonium structure is often considered as a structural alert for nitrosamine formation under appropriate reaction conditions. The metabolic fate of the trimethylammonium moiety of *the Soytrimonium substances* needs clarification by the applicant. Specifically, the potential chemical degradation and formation of secondary and tertiary amines under oxidative conditions needs to be considered.

Repeated dose toxicity
A chronic (12 months) oral study with *Cetrimonium Bromide* in the rat indicated that the test compound may potentially prevent proper nutrition by increasing the rate of gastric emptying and intestinal transit and/or by interfering with the absorption of nutritional substances and reduced skeletal growth. The males displayed a reduced relative liver weight. None of these effects was observed at the lowest tested dosage level of 10 mg/kg bw/day. This dosage can be considered as a conservative NOAEL of *Cetrimonium Bromide.* This NOAEL is also comparable to or lower than other NOAELs of similar quaternary alkyl or aryl trimethylammonium compounds (reported range 12.5 – 125 mg/kg bw/d (Ref. 4, 31).

Developmental toxicity
Dermal developmental toxicity studies with *Cetrimonium chloride* in the rabbit and the rat revealed dose-dependent irritant effects, but no increased incidence of foetal malformations nor developmental variations in the treated groups compared to controls were observed. *Cetrimonium chloride* was found to be non-foetotoxic and non-teratogenic in both species. The NOEL for maternal systemic toxicity and embryo-foetal toxicity appeared to be 40 mg *Cetrimonium chloride*/kg bw/day for the rabbit and 12.5 mg *Cetrimonium chloride*/kg bw/day for the rat.

Genotoxicity/Mutagenicity
Apart from some shortcomings in the tests conducted, the results do not indicate a mutagenic/genotoxic potential of *Cetrimonium, Steartrimonium* or *Behentrimonium Chloride*. However, due to the unsaturated hydrocarbon chain of oleyl trimethylammonium, the predominant substance in the mixture, Soytrimonium Chloride is considered chemically less stable than the tested substances above, in particular under oxidative conditions, so that an analogue approach and read-across in terms of mutagenicity/genotoxicity is not possible. Consequently, appropriate studies according to the Notes of Guidance with Soytrimonium Chloride are required to determine a mutagenicity/genotoxicity potential.

Carcinogenicity
No *in vivo* carcinogenicity studies were available for Soytrimonium Chloride. An in vitro transformation study was negative.

4. CONCLUSION

The final evaluation of the safety of Soytrimonium Chloride for the intended uses of non-oxidative and oxidative conditions of hair dyeing is not possible due to some missing data and studies:

- No information on the irritant potential of Soytrimonium chloride under oxidative conditions in skin is available. Appropriate studies with oxidised soytrimonium chloride would be required to determine irritant potential.

- No information on the sensitizing potential of Soytrimonium chloride under oxidative conditions is available. Appropriate studies with oxidised soytrimonium chloride would be required to determine sensitizing potential.

- Values determined for dermal absorption of Soytrimonium Chloride in human skin *in vitro* are considered not reliable. A new dermal absorption study with Soytrimonium Chloride is required.

- Appropriate studies with Soytrimonium Chloride are required according to the Notes of Guidance to determine a mutagenicity/genotoxicity potential.

The SCCS considers that the missing data should be provided by the applicant by the end of 2012.

5. MINORITY OPINION

Not applicable

6. REFERENCES

Submission 2004 (Alkyl (C15, C18, C22) trimethylammonium chloride)

1. Toxicology/Regulatory Services, Inc. (2001). Fatty nitrogen derived cationics category-High production volume (HPV) chemicals challenge. Prepared for American Chemistry Council, Fatty Nitrogen Derivatives Panel, Cationics Task Group (2001), report dated 13.12.2001; available on the internet at the US-EPA HPV homepage (http://www.epa.gov/ chemrtk/volchall.htm) under http://www.epa.gov/chemrtk/amines/c14171rs.pdf
2. Clariant GmbH. Safety data sheet for Genamin 16. Clariant GmbH, Sulzbach, Germany.
3. Clariant GmbH. Safety data sheet for Genamin 18. Clariant GmbH, Sulzbach, Germany.
4. Clariant GmbH. Safety data sheet for Genamin 20/22. Clariant GmbH, Sulzbach, Germany.
5. Kao Corporation, S.A. Technical Data Sheet Quartamin 60W25, Ref. 1AQ014-V6 and Delivery Specifications Quartamin 60W25, delivery codeEAQ014. Kao Corporation S.A., Barbera del Valles, Spain.
6. Rupprich, N., and Weigand, W. (1984) Genamin CTAC - Prüfung der akuten oralen Toxizität and der männlichen und weiblichen Wistar-Ratte. Hoechst AG, Pharma Forschung Toxikologie, Germany. Unpublished Report No. 84.0440 dated 02.08.1984.
7. Mayordomo, L. (1997) Acute oral toxicity test in rats. Fixed dose method. Test substance: Quartamin 60W25. Centro de Investigacion y Desarrollo Aplicado, S.A.L., Spain. Unpublished Report No. CD-97/5482T dated 10.07.1997.
8. Hempstock, C. (1996) Quartamin 86W: Acute oral toxicity (Limit test) in the rat. Safepharm Laboratories Ltd., United Kingdom. Unpublished Report No. 140/438 dated 04.07.1996.
9. Jensch, U. (1996) Genamin STAC - Testing for acute oral toxicity in the male and female Wistar rat. Hoechst AG, Hoechst Marion Roussel, Non-Clinical Development, Drug Safety, Germany. Unpublished Report No. 96.0159 dated 25.03.1996.
10. Rupprich, N., and Weigand, W. (1984) Genamin CTAC - Prüfung auf akute dermale Reizwirkung / Ätzwirkung am Kaninchen. Hoechst AG, Pharma Forschung Toxikologie, Germany. Unpublished Report No. 84.0447 dated 25.07.1984.
11. Mayordomo, L. (1997) Primary skin irritation in rabbits. Test substance: Quartamin 60W25. Centro de Investigacion y Desarrollo Aplicado, S.A.L., Spain. Unpublished Report No. CD-97/5484T dated 29.04.1997.
12. Sanders, A. (1996) Quartamin 86W (20% & 2%): Acute dermal irritation test in the rabbit. Safepharm Laboratories Ltd., United Kingdom. Unpublished Report No. 140/530 dated 06.11.1996.
13. Kreiling, R. (1996) Genamin STAC - Test for primary dermal irritation in the rabbit. Hoechst AG, Pharma Development, Germany. Unpublished Report No. 96.0105 dated 19.02.1996.
14. Griffon, B. (2001) Acute dermal irritation in rabbits. Test substance: Behentrimonium chloride at a concentration of 10%. CIT, IFM Recherche, France. Unpublished Report No. 21879 dated 26.09.2001.
15. Griffon, B. (2001) Acute dermal irritation in rabbits. Test substance: Behentrimonium chloride at a concentration of 6.25%. CIT, IFM Recherche, France. Unpublished Report No. 23038 dated 28.02.2002.
16. Rupprich, N., and Weigand, W. (1984) Genamin CTAC - Prüfung auf akute Reizwirkung / Ätzwirkung am Auge bei Kaninchen. Hoechst AG, Pharma Forschung Toxikologie, Germany. Unpublished Report No. 84.0448 dated 25.07.1984.
17. Mayordomo, L. (1997) Primary eye irritation in rabbits. Test substance: Quartamin 60W25. Centro de Investigacion y Desarrollo Aplicado, S.A.L., Spain. Unpublished Report No. CD-97/5483T dated 10.07.1997.

18. Myer, J.A. (1985). Rabbit Eye Irritation (low volume procedure). Unpublished report No. 191-1077 dated 23.01.1985. International Research and Development Corporation, Mattawan, MI, USA.
19. Sanders, A. (1996) Quartamin 86W (2%): Acute eye irritation test in the rabbit. Safepharm Laboratories Ltd., United Kingdom. Unpublished Report No. 140/531 dated 06.11.1996.
20. Griffon, B. (2001) Acute eye irritation in rabbits. Test substance: Behentrimonium chloride at a concentration of 10%. CIT, IFM Recherche, France. Unpublished Report No. 21880 dated 26.09.2001.
21. Griffon, B. (2001) Acute eye irritation in rabbits. Test substance: Behentrimonium chloride at a concentration of 6.25%. CIT, IFM Recherche, France. Unpublished Report No. 23040 dated 28.02.2002.
22. Griffon, B. (2001) Acute eye irritation in rabbits. Test substance: Behentrimonium chloride at a concentration of 3.75%. CIT, IFM Recherche, France. Unpublished Report No. 23039 dated 28.02.2002.
23. Casadesus, A. (1997) Skin sensitisation in Guinea pigs (Maximisation test). Test substance: Quartamin 60W25. Centro de Investigacion y Desarrollo Aplicado, S.A.L., Spain. Unpublished Report No. CD-97/5485T dated 28.11.1997.
24. Bury, D. (1994) Genamin STAC - Prüfung auf sensibilisierende Eigenschaften an Pirbright-White-Meerschweinchen nach Buehler. Hoechst AG, Pharma Development, Germany. Unpublished Report No. 94.0846 dated 17.11.1994.
25. Driscoll, R. (1996) Quartamin 86W: Magnusson & Kligman maximisation study in the Guinea pig. Safepharm Laboratories Ltd., United Kingdom. Unpublished Report No. 140/440 dated 24.07.1996.
26. Bury, D. (1995) Genamin STAC - Prüfung auf sensibilisierende Eigenschaften an Pirbright-White-Meerschweinchen nach Buehler. Hoechst AG, Pharma Development, Germany. Unpublished Report No. 95.0263 dated 18.04.1995.
27. Bury, D. (1995) Genamin KDMP - Prüfung auf sensibilisierende Eigenschaften an Pirbright-White-Meerschweinchen nach Buehler. Hoechst AG, Pharma Development, Germany. Unpublished Report No. 95.0292 dated 05.05.1995.
28. Manciaux, X. (2001) Skin sensitisation test in Guinea pigs. Test substance: Behentrimonium chloride. CIT, IFM Recherche, France. Unpublished Report No. 20868 dated 17.07.2001.
29. Griffith, J.F., Nixon, G.A., Bruce, R.D., Reer, P.J., Bannan, E.A. (1980). Dose-response studies with chemical irritants in the albino rabbit eye as a basis for selecting optimum testing conditions for predicting hazard to the human eye. Toxicol. Appl. Pharmacol. 55, 501-513.
30. Diembeck, W., Beck, H., Benech-Kieffer, F., Courtellemont, P., Dupuis, J., Lovell, W., Paye, M., Spengler, J., and Steiling, W. (1999). Test guidelines for in vitro assessment of dermal absorption and percutaneous penetration of cosmetic ingredients. European Cosmetic, Toiletry and Perfumery Association. Food Chem. Toxicol. 37, 191-205.
31. Grötsch, W. (1997). Cutaneous permeation of cetrimoniumchloride through pig skin in vitro. L+S Gesellschaft für Mikrobiologie und biologische Qualitätsprüfung, Germany. Unpublished report No. 07260547 dated 24.11.1997.
32. Bartnik, F.G., and Wingen, F. (1979). Percutaneous absorption of dodecyltrimethylammonium bromide, a cationic surfactant, in the rat. Food Cosmet. Toxicol. 17, 633-637.
33. Potokar, M., Sterzel, W., and Pittermann, W. (1991). Dehyquart A-CA. 28-Tage-Test mit wiederholter oraler Verabreichung an Ratten. Henkel KGaA, Unpublished Report No. TBD 900322 dated July 1991.
34. Spicer, E.J.F. (1979) Subchronic percutaneous toxicity (twenty-eight days) in rabbits. International Research and Development Corporation, Mattawan, MI, USA, Unpublished Report No. 191-217. Cited in 1 and in 54.
35. Isomaa, B., Reuter, J., and Djupsund, B.M. (1976). The subacute and chronic toxicity of cetyltrimethylammonium bromide (CTAB), a cationic surfactant, in the rat. Arch. Toxicol. 35, 91-96.

36. Thompson, P.W. (1996) Quartamin 86W: Reverse mutation test "AMES test" using Salmonella typhimurium. Safepharm Laboratories Ltd., United Kingdom. Unpublished Report No. 140/442 dated 24.05.1996.
37. Müller, W. (1995) Genamin STAC - Study of the mutagenic potential in strains of Salmonella typhimurium (AMES test). Hoechst AG, Pharma Development, Germany. Unpublished Report No. 94.1032 dated 03.01.1995.
38. Müller, W. (1995) Genamin KDMP - Study of the mutagenic potential in strains of Salmonella typhimurium (AMES test). Hoechst AG, Pharma Development, Germany. Unpublished Report No. 95.0032 dated 08.02.1995.
39. Heidemann, A. (1989) Chromosom aberration assay in Chinese hamster V79 cells in vitro with cetyltrimethylammoniumchlorid (24-26% active ingredient in aqueous solution). Cytotest Cell Research GmbH & Co. KG, Germany. Unpublished Report No. 149106 dated 15.09.1989.
40. Inoue, K., Sunakawa, T., and Takayama, S. (1980). Studies of in vitro cell transformation and mutagenicity by surfactants and other compounds. Fd. Cosmet. Toxicol. 18, 289-296. 41 Aldridge, D. (1985) Teratology study in rabbits. Cetrimonium chloirde (100% active). International Research and Development Corporation, Mattawan, MI, USA. Unpublished Report No. 191-856. Cited in 1 and 54.
42. Palmer, A.K., Bottomley, A.M., Edwards, J.A., and Clark, R. (1983) Absence of embryotoxic effects in rats with three quaternary ammonium compounds (cationic surfactants). Toxicol. 26, 313 - 315. Also cited in 1 and in 54.
43. Isomaa, B. (1975) Absorption, distribution and excretion of [14C]CTAB, a quaternary ammonium surfactant, in the rat. Fd. Cosmet. Toxicol. 13, 231-237.
44. Röthlisberger, R. (1998). Epicutaneous patch test - Wellazid 7/0609/05/14/02. Cosmital SA, Marly, Switzerland. Unpublished report No. 169/98 dated 01.09.1998.
45. Laquieze, S. (1999). Etude de la tolerance cutanee de la Lotion stain remover Ref. 453061 en application simple sous patch-tests semi-occlusifs pendant une heure chez vingt-cinq volontaires sains et vingt-cinq volontaires polysensibles. Peritesco SARL, Paris, France. Unpublished report No. PO 642/D dated 24.06.1999.
46. Voß, W. (1996). Human patch test - Proficare Kräuterazid 8/5197/05/01/010. Dermatest - Gesellschaft für allergologische Forschung m.b.H, Münster, Germany. Unpublished report dated 19.07.1996.
47. Tronnier, H. (1998). Epicutaneous test for the analysis of the irritating effects caused by cosmetic products to the skin of humans - Kräuterazid 7/0609/05/05/02. Institut für experimentelle Dermatologie, Universität Witten/Herdecke, Witten, Germany. Unpublished report dated 18.12.1998.
48. Krächter, Matthies (1994). Dehyquart A-CA ex Henkel - Dehyquart A-CA ex Italien. Modifizierter Duhring-Kammer-Test. Henkel KGaA, Unpublished Report No. R 9300498 dated 10.11.1994.
49. Biltz, H. (1997). Examination of the Product "Wellazid" rinse-off (50%) 8/6161/05/07/465 by human patch test. Derma Consult GmbH, Alfter, Germany. Unpublished report dated 06.09.1997.
50. Häntschel, D. (1999). Koleston 99 0-Masse (83005095 B, 25%) epicutaneous patch test. Single application 24 h patch test. Skin Investigation and Technology Hamburg GmbH, Hamburg, Germany. Unpublished report No. 601-01-0008 dated 24.09.1999.
51. Häntschel, D. (1999). Epicutaneous patch test. SOP version 1.1 "Wella AG" from February 8th 1999. Skin Investigation and Technology Hamburg GmbH, Hamburg, Germany. Unpublished study protocol dated 08.02.1999.
52. Lockhart, L. (2001). Human irritation patch test (3-PAT). Unpublished report No. 01-108372-72 dated 05.07.2001. Hill Top Research Inc., St. Petersburg, Florida, USA.
53. Aust, L.B. (1999). Human irritation patch test (3-PAT). Unpublished report No. 98-101158-73 dated 02.02.1999. Hill Top Research Inc., Scottsdale, Arizona, USA.
54. CIR (1997). Final report of the safety assessment of cetrimonium chloride, cetrimonium bromide, and steartrimonium chloride. Int. J. Toxicol. 16, 195-220.
55. Havet, M. and Loquillard, A. (1998). Verification clinique de la tolerance cutanee du produit EVICHC2982130 apres shampooing Fle 433833 apres application unique sous pansement occlusif - patch test. EVIC-CEBA, Laboratoire de Recherche et

d'Experimentation, Vincennes, France. Unpublished report No. ECIC 98034 dated 02.10.1998.
56. Guillot, J.P. and Gavaud-Kennedy, C. (1998). Etude clinique pour la verification de la bonne tolerance locale epicutane, apres application unique sur la peau du dos et sous patch occlusif (large) pendant 48 heures, chez 50 volontaires adultes sans, de deux sexes: patch test simple unique. Institut d'Expertise Clinique, Bessenay, France. Unpublished report No. ECIC 98019 dated 02.06.1998.
57. Tronnier, H. (1986). Epikutantest - Genamin KDM-F (Behenyltrimethylammonium-chlorid). Hautklinik der Städtischen Kliniken, Dortmund, Germany. Unpublished report dated 12.12.1986.
58. Dosik, J.S. (2004). Human repeated insult patch study. Unpublished report No. DS101304- 1 dated 14.04.2004. TKL Research Inc., Paramus, New Jersey, USA.
59. Freeberg, F.E., Griffith, J.F., Bruce, R.D., BAY, P.H.S. (1984). Correlation of animal test methods with human experience for household products. J. Toxicol. Cutaneous Ocular Toxicol. 1, 53-64.
60. Freeberg, F.E., Nixon, G.A., Reer, P.J., Weaver, J.E., Bruce, R.D., Griffith, J.F., Sanders III., L.W. (1986). Human and rabbit eye responses to chemical insult. Fundam. Appl. Toxicol. 7, 626-634.
61. SCCNFP (2003b). Opinion concerning basic criteria for the in vitro assessment of dermal absorption of cosmetic ingredients, updated October 2003. SCCNFP/0750/03, adopted by the SCCNFP during the plenary meeting of 20 October 2003.
62. Hoechst AG, Analysenzertifikate [Certificates of Analysis] for Genamin CTAC batches No. E06112547 and E06178641. Hoechst AG, Frankfurt, Germany.
63. Cognis GmbH & Co. KG (1997). Data Profile Dehyquart A-CA. Revision 5-03.1997. Cognis GmbH & Co. KG, Düsseldorf, Germany.
64. Hoechst AG, Analysenzertifikate [Certificates of Analysis] for Genamin STAC batches No. 1061969521 and E061859561. Hoechst AG, Frankfurt, Germany.
65. Kao Corporation, S.A. Delivery Specifications Quartamin 86W, delivery code 162634. Kao Corporation S.A., Barbera del Valles, Spain.
66. Hoechst AG, Analysenzertifikate [Certificates of Analysis] for Genamin KDMP batch no E06186598. Hoechst AG, Frankfurt, Germany. L'Oreal S.A., Bulletin d'analyses matieres premieres [Certificates of Analysis] for Genamin KDMP batches no 0040242 and 20010400390104.

A. De Groot A.C., Weyland J.W. and Nater J.P. Contact allergy to preservatives: antimicrobials and antioxidants.
In: Unwanted Effects of Cosmetics and Drugs used in Dermatology, 3th edition, Elsevier, Amsterdam, 1994.
B. Colipa. Guidelines on the management of undesirable event reports. The European Cosmetic Toiletry and Perfumery Association, 23 August 2005.

Submission 2007

References in italics were submitted by industry as full reports in the submission dossier (2004). Corresponding reference numbers are indicated in brackets where appropriate.

1. Scientific Committee on Consumer Products (2006). Opinion on Alkyl (C16, C18, C22) trimethylammonium chloride, for other uses than as a preservative. SCCP/0917/05, adopted by the SCCP by written procedure on date 17 March 2006
2. EFfCI/COLIPA (2006). Comments on the SCCP Opinion on alkyl (C16, C18, C22) trimethylammonium chloride (SCCP/0917/05) and request to inform the SCCP and forward comments to the expert committee. Letter sent on 28 June 2006
3. Scientific Committee on Consumer Products (2007). SCCP Opinion on Alkyl (C16, C18, C22) trimethylammonium chloride, for other uses than as a preservative (COLIPA N°P72). SCCP/0000/00, adopted by the SCCP at its 11th plenary on 21 March 2007

4. Lockhart, L. (2001). Human irritation patch test (3-PAT). Hill Top Research Inc., St. Petersburg, Florida, USA. Unpublished report No. 01- 108372-72 dated 05 July 2001 (Reference #52 in Submission 2004)

5. Aust, L.B. (1999). Human irritation patch test (3-PAT). Hill Top Research Inc., Scottsdale, Arizona, USA. Unpublished report No. 98- 101158-73 dated 02 February 1999 (Reference #53 in Submission 2004)

6. Häntschel, D. (1999). Koleston 99 0-Masse (83005095 B, 25%) epicutaneous patch test. Single application 24 h patch test. Skin Investigation and Technology Hamburg GmbH, Hamburg, Germany. Unpublished report No. 601-01-0008 dated 24 September 1999 (Reference #50 in Submission 2004)

7. Biltz, H. (1997). Examination of the Product "Wellazid" rinse-off (50%) 8/6161/05/07/465 by human patch test. Derma Consult GmbH, Alfter, Germany. Unpublished report dated 06 September 1997 (Reference #49 in Submission 2004)

8a. Krächter, Matthies (1994). Dehyquart A-CA ex Henkel - Dehyquart A-CA ex Italien. Modifizierter Duhring-Kammer-Test. Henkel KGaA, Unpublished Report No. R 9300498 dated 10 November 1994 (Reference #48 in Submission 2004)

8b. Krächter, H.U. and Kleber M. (2007). Dehyquart® A: confirmation statement of test concentration used in study R 9300498

9. Tronnier, H. (1998). Epicutaneous test for the analysis of the irritating effects caused by cosmetic products to the skin of humans - Kräuterazid 7/0609/05/05/02. Institut für experimentelle Dermatologie, Universität Witten/Herdecke, Witten, Germany. Unpublished report dated 18 December 1998 (Reference #47 in Submission 2004)

10. Voß, W. (1996). Human patch test - Proficare Kräuterazid 8/5197/05/01/010. Dermatest - Gesellschaft für allergologische Forschung m.b.H, Münster, Germany. Unpublished report dated 19 July 1996 (Reference #46 in Submission 2004)

11. Laquieze, S. (1999). Etude de la tolerance cutanee de la Lotion stain remover Ref. 453061 en application simple sous patch-tests semi-occlusifs pendant une heure chez vingtcinq volontaires sains et vingt-cinq volontaires polysensibles. Peritesco SARL, Paris, France. Unpublished report No. PO 642/D dated 24 June 1999 (Reference #45 in Submission 2004)

12. Röthlisberger, R. (1998). Epicutaneous patch test - Wellazid 7/0609/05/14/02. Cosmital SA, Marly, Switzerland. Unpublished report No. 169/98 dated 01 September 1998 (Reference #44 in Submission 2004)

13. Upadhye, M.R. and Maibach, H.I. (1992). Influence of area of application of allergens on sensitization in contact dermatitis. Contact Dermatitis, 27, 281-286

14. Basketter D., Gilpin G., Kuhn M., Lawrence D., Reynolds F. and Whittle E. (1998). Patch tests versus use tests in skin irritation risk assessment. Contact Dermatitis, 39(5):252-6

15. Havet, M. and Loquillard, A. (1998). Verification clinique de la tolerance cutanee du produit EVICHC2982130 apres shampooing Fle 433833 apres application unique sous pansement occlusif - patch test. EVIC-CEBA, Laboratoire de Recherche et d'Experimentation, Vincennes, France. Unpublished report No. ECIC 98034 dated 02 October 1998 (Reference #55 in Submission 2004)

16 Guillot, J.P. and Gavaud-Kennedy, C. (1998). Etude clinique pour la verification de la bonne tolerance locale epicutane, apres application unique sur la peau du dos et sous patch occlusif (large) pendant 48 heures, chez 50 volontaires adultes sans, de deux sexes: patch test simple unique. Institut d'Expertise Clinique, Bessenay, France. Unpublished report No. ECIC 98019 dated 02 June 1998 (Reference #56 in Submission 2004)

17. Tronnier, H. (1986). Epikutantest - Genamin KDM-F (Behenyltrimethylammoniumchlorid). Hautklinik der Städtischen Kliniken, Dortmund, Germany. Unpublished report dated 12 December 1986 (Reference #57 in Submission 2004)

18. Zhai and Maibach (2001). Effects of skin occlusion on percutaneous absorption: an overview. Skin pharmacol Appl Skin Physiol, 14(1): 1-10

19. Zhai H, Maibach HI (2001). Skin occlusion and irritant and allergic contact dermatitis: an overview. Contact Dermatitis. 44(4): 206

20. OECD Guidelines for the Testing of Chemicals, available at: http://caliban.sourceoecd.org/vl=2326609/cl=13/nw=1/rpsv/home.htm
21. OECD (2004). OECD Guideline for the Testing of Chemicals (OECD TG 427), Skin absorption: *in vivo* method, adopted 13 April 2004
22. OECD (2004). OECD Guideline for the Testing of Chemicals (OECD TG 428), Skin absorption: *in vitro* method, adopted 13 April 2004
23. Scientific Committee on Consumer Products (2006). SCCP Opinion on Basic Criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients, updated March 2006. SCCP/0970/06, adopted by the SCCP during the 7th plenary on 28 March 2006
24. WHO (1999). Principles for assessment of risk to human health from exposure to chemicals. Environmental Health Criteria, vol. 210. World Health Organization, Geneva. Available at : http://www.inchem.org/documents/ehc/ehc/ehc210.htm
25. Scientific Committee on Consumer Products (2006). The SCCP's Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation. 6th revision, adopted by the SCCP during the 10th plenary meeting on 19 December 2006
26. Odio, M. and Friedlander, S.F. (2000). Diaper dermatitis and advances in diaper technology. Current Opinions in Pediatrics, 12(4):342-346
27. Yoshiike T., Aikawa Y., Sindhvananda J., Suto H., Nishimura K., Kawamoto T. and Ogawa H. (1993) skin barrier defect in atopic dermatitis: increased permeability of the stratum corneum using dimethyl sulfoxide and theophylline. J Derm Sci, 5(2): 92-6
28. Günther, C., Kecskes, S., Staks, T., Tauber, U. (1998). Percutaneous absorption of methylprednisolone aceponate following topical application of Avantan lotion on intact, inflamed and stripped skin of male volunteers. Skin Pharmacol. Appl. Skin Physiol. 11(1): 35-42
29. Wickett R.R., Visscher M.O. (V). (2006). Structure and function of the epidermal barrier. Am J Infect Control, 34(10 Suppl): S98-S110
30. Benson H.A. (2005). Transdermal drug delivery: penetration enhancement techniques. Curr Drug Deliv., 2(1): 23-33
31. Fang J.Y., Hung C.F., Chiu H.C., Wang J.J. and Chan T.F. (2003). Efficacy and irritancy of enhancers on the in-vitro and in-vivo percutaneous absorption of curcumin, J Pharm Pharmacol, 55(5): 593-601
32. Copoví A., Díez-Sales O., Herráez-Domínguez J.V. and Herráez-Domínguez M. (2006). Enhancing effect of alpha-hydroxyacids on "in vitro" permeation across the human skin of compounds with different lipophilicity. Int J Pharm, 314(1):31-36
33. Wilhelm K.P., Surber C., Maibach H.I. (1991) Effect of sodium lauryl sulfate-induced skin irritation on in vivo percutaneous penetration of four drugs. J Invest Dermatol, 97(5): 927-32
34 Frankild S., Andersen K.E., Nielsen G.D. (1995). Effect of sodium lauryl sulfate (SLS) on in vitro percutaneous penetration of water, hydrocortisone and nickel. Contact Dermatitis, 32(6): 338-45
35 Van der Merwe D., Riviere JE. (2005). Effect of vehicles and sodium lauryl sulphate on xenobiotic permeability and stratum corneum partitioning in porcine skin. Toxicology, 206(3): 325-35
36. Jakasa I., Verberk M.M., Bunge A.L., Kruse J. and Kezic S. (2006). Increased permeability for polyethylene glycols through skin compromised by sodium lauryl sulphate. Exp Dermatol, 5(10): 801-7
37. Isomaa B., Reuter J., Djupsund B.M. (1976). The subacute and chronic toxicity of cetyltrimethylammonium bromide (CTAB), a cationic surfactant, in the rat. Arch Toxicol, 35(2): 91-96 (Reference #35 in Submission 2004)
38. Bartnik F.G., Wingen F. (1976). Percutaneous absorption of dodecyltrimethylammonium bromide, a cationic surfactant, in the rat. Food Cosmet Toxicol, 17(6): 633-637 (Reference #32 in Submission 2004)
39. Ross J.H., Dong M.H. and Krieger M.I. (2000). Conservatism in pesticide exposure assessment. Regulatory Toxicology and Pharmacology, 31: 53-58

40. Van Ravenzwaay B. and Leibold E. (2004). A comparison between *in vitro* rat and human and *in vivo* rat skin absorption studies. Human and Experimental Toxicology, 23:421-430.

Submission 2010

1. Scientific Committee on Consumer Safety SCCS, Opinion On Alkyl (C16, C18, C22) Trimethylammonium Chloride For Other Uses Than As A Preservative SCCS/1246/09, 8 December 2009
2. Evaluation Of Eye Irritation Potential Of GTS72278 In Vitro Using The Isolated Chicken Eye Test, P&G Study # 201240-66691, November 2010
3. Determination Of Skin Irritation Potential Using The Episkin Reconstituted Human Epidermis Model, P&G Study # 201240-66693, October 2010
4. In vitro penetration of soytrimonium chloride from two hair dye formulations through dermatomed human skin. P&G study 201456-66918, December 2010
5. U.S. Environmental Protection Agency, High Production Volume (HPV) Challenge, Robust Summaries And Test Plans: Fatty Nitrogen Derived Cationics: Available at: http://www.epa.gov/hpv/pubs/summaries/fatnitro/c13407tc.htm
6. Parkinson A. (1996), Biotransformation of Xenobiotics. In Klaassen CD et al. (Eds,) Casarett and Doull's Toxicology: The Basic Science of Poisons, Fifth Edition, 113-186, New York, McGraw-Hill
7. Reh R. et al (2008), Involvement of Stearoyl-CoA Desaturase in the reduction of Amidoxime Prodrugs, Xenobiotica, 38 (9), 1177-1190
8. Rise P. et al (2005), Differential Modulation By Simvastatin Of The Metabolic Pathways In The n-9, n-6 and n-3 Fatty Acid Series In Human Monocytic And Hepatocytic Cell Lines, Biochemical Pharmacology, 69, 1095-1100
9. Obukowicz MG. et al (1998), Novel, Selective Δ6 or Δ5 Fatty Acid Desaturase Inhibitors As Antiinflammatory Agents in Mice, Journal of Pharmacology and Experimental Therapeutics, 287 (1), 157-166
10. Bruce JS, Salter AM. (1996), Metabolic fate Of Oleic Acid, Palmitic Acid And Stearic Acid In Cultured Hamster Hepatocytes, Journal of Biochemistry, 316, 847-852
11. Duckett SK. et al (2002), Effect Of High-Oil Corn Or Added Corn Oil On Ruminal Biohydrogenation Of Fatty Acids And Conjugated Linoleic Acid Formation In Beef Steers Fed Finishing Diets, Journal of Animal Science, 80 (12), 3353-3360
12. ECVAM (2007), ESAC Statement On The Conclusions Of The ICCVAM Retrospective Study On Organotypic In Vitro Assays As Screening Tests To Identify Potential Ocular Corrosives And Severe Irritants As Determined By US EPA, EU (R41) And UN GHS Classifications In A Tiered Testing Strategy, As Part Of A Weight Of Evidence Approach. Available from: http://ecvam.jrc.it
13. Prinsen MK. (1996), The Chicken Enucleated Eye Test (CEET): A Practical (Pre)Screen For The Assessment Of Eye Irritation/Corrosion Potential Of Test Materials, Fd. Chem. Tox., 34 (2), 291-296
14. Prinsen MK and Koeter HBWM. (1993), Justification Of The Enucleated Eye Test With Eyes Of Slaughterhouse Animals As An Alternative To The Draize Eye Irritation Test With Rabbits, Fd. Chem. Tox., 31 (1) 69-76
15. Schutte K. et al (2009) The Isolated Chicken Eye Test As A Suitable In Vitro Method For Determining The Eye Irritation Potential Of Household Cleaning Products, Regulatory Toxicology and Pharmacology, 54, 272-81
16. ECVAM (2007), Statement On The Validity Of In Vitro Tests For Skin Irritation. Available from: http://ecvam.jrc.it
17. Validation of the LC-MS Method for the Quantitation of Active Soytrimonium Chloride From GTS72278 In Various Sample Matrices Generated As Part Of An In Vitro Penetration Study, P&G Study # 201456-67440, Intertek ASG, Manchester, UK. Study No. 1319502,December 2010

17a. Addendum 1: Validation of the LC-MS Method for the Quantitation of Active Soytrimonium Chloride From GTS72278 In Various Sample Matrices Generated As Part Of

An In Vitro Penetration Study, P&G Study # 201456-67440, Intertek ASG, Manchester, UK. Study No. 1319502, March 2012 (**submitted May 2012**)

Additional references

18. Submission to the Scientific Committee on Consumer Safety. Alkyl (C16, C18, C22) Trimethylammonium Chloride – Addition of Soytrimonium chloride for Uses other than as a Preservative. Supplemental Dossier Addressing the Non-preservative Use of Soytrimonium Chloride in Hair Colourant Products. December 2010
19. OECD Testing Guideline 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method (adopted July 2010).
20. Scientific Committee on Consumer Safety SCCS, Basic Criteria for the in vitro assessment of dermal absorption of cosmetic ingredients. SCCS/1358/10, 22 June 2010
21. Hill MJ (ed.) 1988): Nitrosamines – Toxicology and Microbiology. VCH, Weinheim (Germany).
22. Preussmann R (ed.) 1983: Das Nitrosaminproblem, pp. 189 ff. Verlag Chemie, Weinheim (in German)
23. Mirvish S (1970). Kinetics of dimethylamine nitrosation in relation to nitrosamine carcinogenesis. J. Nat. Cancer Inst., 44, 633-639**.**
24 Maduagwu EN (1985) Nitrosation of quaternary ammonium compounds in vivo in the Wistar rat. Xenobiotica 15(12):1061-1068.
25. Maduagwu EN (1988) Intervention of nitrite in the metabolism of quaternary ammonium compounds. Drug Metab Dispos May-Jun;16(3):512-514.
26. Okun,J.D, Archer,M.C. Kinetics of nitrosamine formation in the presence of micelle-forming surfactants. J Natl Cancer Inst.1977,58(2) : 409-11)
27. Scientific Committee on Consumer Safety (2012) Opinion on Nitrosamines and Secondary Amines in Cosmetic Products, SCCS/1458/11, 27 March 2012
28. Scientific Committee on Consumer Safety (2011), pre-consultation Opinion on Fragrance allergens in cosmetic products, SCCS/1459/11, 26-27 December 2011, http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_073.pdf
29. OECD (2007): Guidance on grouping of chemicals. Series on testing and assessment No. 80. Paris.
30. ECHA (2008): Guidance on information requirements and chemical safety assessment. Chapter R.6: QSARs and grouping of chemicals. Guidance for the implementation of REACH. Helsinki.
31. 31. Thorup I (2000). Evaluation of health hazards by exposure to quaternary ammonium compounds (cationic surfactants) and estimation of a limit value in air. In: Environmental Project No. 555 2000, Danish Environmental Protection Agency, Danish Ministry of Environment and Energy. http://www2.mst.dk/Udgiv/publications/2000/87-7944-210-2/pdf/87-7944-211-0.pdf
32. Drotman R B (1977). Metabolism of Cutaneously Applied Surfactants. pp. 95-109. In Cutaneous Toxicity. Academic Press Inc., New York, NY (cited in ref. 1, Submission 2004)