Scientific Committee on Consumer Safety

SCCS

OPINION ON

ALKYL (C_{16}, C_{18}, C_{22}) TRIMETHYLAMMONIUM CHLORIDE

For other uses than as a preservative

COLIPA n° P72

The SCCS adopted this opinion at its 5th plenary
on 8 December 2009

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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.

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

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This opinion has been subject to a commenting period of four weeks after its initial publication. All 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.

Keywords: SCCS, scientific opinion, preservative, alkyl (C_{16}, C_{18},C_{22}) trimethylammonium chloride, P72, CAS 112-02-7; 112-03-8; 17301-53-0, EINECS 203-928-6; 203-929-1; 241-327-0

Opinion to be cited as: Opinion of the SCCS on Alkyl (C_{16}, C_{18}, C_{22}) trimethylammonium chloride, other uses than as a preservative, 8 December 2009

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1. BACKGROUND

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

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

Alkyl (C12-22) trimethyl ammonium, bromide and chloride, (COLIPA[1] P72) bears the symbol (+) and can therefore be used in cosmetics at higher concentrations, as long as it is not employed as preservative. Alkyl (C12-22) trimethyl ammonium, bromide and chloride is currently authorized as a preservative up to a maximum concentration of 0.1% (Annex VI, Part 1, No. 44).
In its opinion of 17 February 1999 concerning the restrictions on materials listed in Annex VI of Directive 76/768/EEC on cosmetic products, the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) stated that those substances indicated by (+) in Annex VI, when incorporated into cosmetic formulations for non-preservative functions, should be subjected to the same restrictions in usage levels and warnings as when used for preservative effects.

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

In 2004, the European Commission received a submission from industry (Submission I) proposing that Alkyl (C_{16}, C_{18}, C_{22}) trimethylammonium chloride can be used for non-preservative purposes as specified in the safety dossier:

cetrimonium chloride (C_{16}), steartrimonium chloride (C_{18}):

Rinse-off hair care products up to	2.5%
Leave-on hair care products up to	1.0%
Leave-on facial cream products up to	0.5%

behentrimonium chloride (C_{22}):

Rinse-off hair care products up to	5.0%
Leave on hair care and facial cream products up to	3.0%

In 2006, the SCCP adopted opinion SCCP/0917/05, which, after some pertinent comments from the applicant, was reviewed and updated by SCCP/1087/07. The Committee concluded as follows:

"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. Nevertheless, this value does not apply in cases where the final formulation (finished cosmetic products) containing cetrimonium chloride, steartrimonium chloride and/or behentrimonium chloride is irritating to the skin and thus may increase the dermal absorption of the ingredient(s). Moreover, single human patch tests clearly showed that all combinations of quaternary ammonium compound concentrations and formulations lead to diverging results. Considering the fact

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

that quaternary ammonium compounds are known to be irritating, combination effects should also be taken into account.

Therefore the following concentration limits should apply for rinse-off cosmetic products:

- the sum of the *cetrimonium* and *steartrimonium chloride* concentrations should not exceed 0.5%, and
- the total sum of *behentrimonium*, *cetrimonium* and/or *steartrimonium chloride* should not exceed a maximum level of 3%.

The submission requests evaluation of the use of these quaternary ammonium derivatives in leave-on (face)-cream products. This dossier only allows the safety evaluation of quaternary ammonium compounds in leave-on cosmetic products when present in a maximum level of 0.1% (preservative or non-preservative). The safety evaluation of the leave-on (face)-cream products containing these substances needs to be assessed on a case by case basis."

Recently, a further submission was received. The applicant provided argumentation and market surveillance data with the aim to support the safety of these preservatives at the use concentrations originally applied for.

2. TERMS OF REFERENCE

1. *On the basis of the data provided, does the SCCS consider that Alkyl (C_{16}, C_{18}, C_{22}) trimethylammonium chloride is safe for consumers, when used in cosmetic products for non-preservative purposes in the concentrations specified above?*

2. *Does the SCCS have any further scientific concerns in relation to its use in the cosmetic products?*

3. OPINION

The present opinion combines the major part of SCCP/1087/07 with the newly introduced information, thus presenting a full overview of the data introduced for alkyl (C_{16}, C_{18}, C_{22}) trimethylammonium chloride and the final SCCS conclusion, taking into account the full data package.

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS (TAKEN FROM SCCP/1087/07)

3.1.1 Chemical identity

3.1.1.1 Primary names and/or INCI names

a) Cetrimonium chloride
b) Steartrimonium chloride
c) Behentrimonium chloride

3.1.1.2 Chemical names

a) C_{16}-alkyltrimethylammonium chloride
Cetyltrimethylammonium chloride
Cetyl trimethyl ammonium chloride
N-hexadecyltrimethylammonium chloride
1-hexadecanaminium, N,N,N-trimethyl-, chloride
N,N,N-trimethyl-1-hexadecanaminium chloride

b) C_{18}-alkyltrimethylammonium chloride
Trimethyloctadecylammoniumchloride (ECB)
Stearyltrimethylammonium chloride
Stearyl trimethyl ammonium chloride
N-octadecyltrimethylammonium chloride
1-octadecanaminium, N,N,N-trimethyl-, chloride
N,N,N-trimethyl-1-octadecanaminium chloride

c) C_{22}-alkyltrimethylammonium chloride
Docosyltrimethylammonium chloride (ECB)
Behenyltrimethylammonium chloride
Behenyl trimethyl ammonium chloride
1-docosanaminium, N,N,N-trimethyl-, chloride
N,N,N-trimethyl-1-docosanaminium chloride

3.1.1.3 Trade names and abbreviations

a) Arquad 16-29
Arquad 16-25LO
Dehyquart A-CA
Genamin CTAC
Incroquat CTC 30
Quartamin 60W25
Varisoft 300

b) Arquad 18-50
Genamin STAC
Quartamin 86W
Quartamin TH-V

c) Genamin KDMP
Incroquat Behenyl TMC 25
Incroquat Behenyl TMC 85
Incroquat Behenyl TMC/P
Quartamin AB
Varisoft BT 85

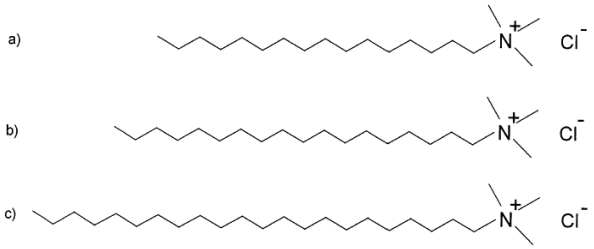
COLIPA P 72 [covers a), b) and c)]

3.1.1.4 CAS / EC number

CAS: a) 112-02-7
b) 112-03-8
c) 17301-53-0

EC: a) 203-928-6
b) 203-929-1
c) 241-327-0

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

Molecular formula: a) $C_{19}H_{42}ClN$
b) $C_{21}H_{46}ClN$
c) $C_{25}H_{54}ClN$

3.1.2 Physical form

3.1.2.1 Cetrimonium chloride

Liquid (Quartamin 60W25: 24-26% *cetrimonium chloride* in water)
Ref.: 5

Liquid (Genamin CTAC: 28-30% *cetrimonium chloride* in water)
Ref.: 62

Liquid (Dehyquart A-CA: 24-26% *cetrimonium chloride* in water)
Ref.: 63

3.1.2.2 Steartrimonium chloride

Solid (Genamin STAC: 78-82% *steartrimonium chloride*
+ 18.5-19.5% isopropanol and 0-2% water)
Ref.: 64

Liquid (Quartamin 86W: 26.5-29.5% *steartrimonium chloride* in water)

Ref.: 65

3.1.2.3 Behentrimonium chloride

Solid (Genamin KDMP: 77-83% *behentrimonium chloride* + 17-23% isopropanol and 0-3% water)

Ref.: 66

3.1.3 Molecular weight

a) 320.00 g/mol
b) 348.05 g/mol
c) 404.16 g/mol

3.1.4 Purity, composition and substance codes

3.1.4.1 Cetrimonium chloride

A. Genamin CTAC

General: 28-30% *cetrimonium chloride* in water
Batches used: E06112547 (1983) no purity stated
E06178641 (1993) 28.7% *cetrimonium chloride* (DIN ISO 2871)

Ref.: 62

B. Quartamin 60W25

General: 24-26% *cetrimonium chloride* in water
Batch used: 3-4 no purity stated

Ref.: 5

C. Dehyquart A-CA

General: 24-26% *cetrimonium chloride* in water
Batch used: None stated

Ref.: 63

3.1.8.2 Steartrimonium chloride

A. Genamin STAC

General: 78-82% *steartrimonium chloride*
18.5-19.5% isopropanol
≤ 2.0% water
≤ 2.5% free amine and amine hydrochloride

Batches used:
- E061859561 (1994) 79.8% *steartrimonium chloride* (DIN ISO 2871)
18.8% isopropanol (calculated)
0.6% water (DIN 51777)
≤ 2.5% free amine and amine hydrochloride

- 1061969521 (1995) 79.2% *steartrimonium chloride* (DIN ISO 2871)
19.1% isopropanol (calculated)
0.9% water (DIN 51777)
≤ 2.5% free amine and amine hydrochloride

Ref.: 64

B. Quartamin 86W

General: 26.5-29.5% *steartrimonium chloride* in water
Batch used: 1081 No purity stated

Ref.: 65

3.1.8.3 Behentrimonium chloride

Genamin KDMP

General: 77-83% *behentrimonium chloride*
17-23% isopropanol
≤ 3.0% water
≤ 2.0% free amine and amine hydrochloride

Batches used:
- E06186598 (1994): 78.9% *behentrimonium chloride* (DIN ISO 2871)
18.7% isopropanol (calculated)
1.9% water (DIN 51777)
0.5% free amine and amine hydrochloride
- 0040242: purity not stated
- 20010400390104: purity not stated

Ref.: 66

3.1.5 Impurities / accompanying contaminants

As stated above: free amine and amine hydrochloride: ≤ 2.5%

3.1.6 Solubility

3.1.6.1 Cetrimonium chloride

Water: soluble (Dehyquart A-CA: 24-26% *cetrimonium chloride* in water)
Ethanol: very slightly soluble (Dehyquart A-CA: 24-26% *cetrimonium chloride* in water)

Ref.: 63

3.1.6.2 Steartrimonium chloride

Water:	> 10 g/l	(Genamin STAC: 78-82% *steartrimonium chloride* + 18.5-19.5% isopropanol and 0-2% water)
Ethanol:	> 10 g/l	(Genamin STAC: 78-82% *steartrimonium chloride* + 18.5-19.5% isopropanol and 0-2% water)
Isopropanol:	> 10 g/l	(Genamin STAC: 78-82% *steartrimonium chloride* + 18.5-19.5% isopropanol and 0-2% water)

Ref.: 64

3.1.6.3 Behentrimonium chloride

Water:	partially soluble	(Genamin KDMP: 77-83% *behentrimonium chloride* + 17-23% isopropanol and 0-3% water)
Ethanol:	> 10 g/l	(Genamin KDMP: 77-83% *behentrimonium chloride* + 17-23% isopropanol and 0-3% water)

Isopropanol:	> 10 g/l	(Genamin KDMP: 77-83% *behentrimonium Chloride* + 17-23% isopropanol and 0-3% water)

Ref.: 66

3.1.7 Partition coefficient (Log P_{ow})

According to the submission, 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

3.1.8 Additional physical and chemical specifications

3.1.8.1 Cetrimonium chloride

Density (20°C):	0.975 g/ml	(Quartamin 60W25: 24-26% *cetrimonium chloride* in water)
Viscosity (20°C):	≤ 50cPs	
pH (6.0-6.5% aqueous solution):	5.0 - 6.5	(25% Quartamin 60W25 in water)

Ref.: 5

Density (20°C):	0.97 g/cm³	(Genamin CTAC: 28-30% *cetrimonium chloride* in water)
Viscosity (20°C)	≤ 100 mPa	
pH (0.3% aqueous solution):	5.0 - 7.0	(1% Genamin CTAC in water)

Ref.: 62

pH (24-26% aqueous solution):	5.5 - 7.5	(Dehyquart A-CA: 24-26% *cetrimonium chloride* in water, tested as such)

Ref.: 63

3.1.8.2 Steartrimonium chloride

Melting point:	60-70°C	(Genamin STAC: 78-82% *steartrimonium chloride* + 18.5-19.5% isopropanol and 0-2% water)
Viscosity (20°C)	≤ 100mPa	
pH (0.8% aqueous solution):	4.0 - 6.0	(1% Genamin STAC in water)

Ref.: 64

pH (6.6-7.4% aqueous solution):	5.0 - 7.0	(25% Quartamin 86W (26.5-29.5% *steartrimonium chloride*) in water))

Ref.: 65

3.1.8.3 Behentrimonium chloride

Physical form:	solid	(Genamin KDMP: 77-83% *behentrimonium Chloride* + 17-23% isopropanol and 0-3% water)
pH (0.8% aqueous solution):	5.0 - 7.0	(1% Genamin KDMP in water)
Solubility (water)	partially soluble	
Solubility (ethanol)	> 10 g/l	
Solubility (isopropanol)	> 10 g/l	

Ref.: 66

3.1.9 Stability

No data submitted

General comments with regard to section 3.1

The identification of the test substances has not been reported appropriately. Although it is recognized that the quaternary ammonium compounds under investigation have a long history of use, the following shortcomings are noted:

- Sections 3.1.1 - 3.1.6 are unreferenced

- Three references that are used for the physico-chemical specifications do not concern the quaternary ammonium compounds under study, but their corresponding tertiary amine compounds. More specifically, the Material Safety Data Sheets for Genamin 16 R 302 D, Genamin 18 R 302 D and Genamin 20/22 R 302 D instead of the ones for Genamin CTAC, Genamin STAC and Genamin KDMP are included.

- Stability data are lacking.

- Annex 1 of the original submission was included to provide detailed descriptions of purity, composition and substance codes for batches used in toxicity studies. This annex has been incorporated in the current opinion under section 3.1.4. It lists batch n°1081 for Quartamin 86W, a batch that has not been used in any of the presented studies. On the other hand, batch nr. 1841 (*Steartrimonium chloride*) which has been used in an acute oral toxicity rat study (see 3.3.1.1.E), in a skin and eye irritation rabbit study (see 3.3.2.1.C and 3.3.2.2.D), in a skin sensitisation assay in the guinea pig (see 3.3.3.C) and in an bacterial reverse mutation assay (see 3.3.6.1.B), was not described in Annex 1.

3.2 FUNCTION AND USES

Cetrimonium chloride, steartrimonium chloride, and *behentrimonium chloride* are currently listed on Annex VI of the Cosmetics Directive 76/768/EEC under the entry "Alkyl (C_{12}-C_{22}) trimethylammonium, bromide and chloride (+)" permitted to be used as preservative up to 0.1% in finished products and at higher levels (as indicated by a "+" sign) for other specific purposes. Based on the toxicological profile and the risk assessment of *cetrimonium chloride, steartrimonium chloride,* and *behentrimonium chloride* for cosmetic uses presented hereafter.

The applicant proposes that Alkyl (C_{16}, C_{18}, C_{22}) trimethylammonium chloride can be used for non-preservative purposes in the following concentrations:

cetrimonium chloride (C_{16}), steartrimonium chloride (C_{18}):

Rinse-off hair care products up to	2.5%
Leave-on hair care products up to	1.0%
Leave-on facial cream products up to	0.5%

behentrimonium chloride (C_{22}):

Rinse-off hair care products up to	5.0%
Leave on hair care and facial cream products up to	3.0%

According to the submission, the proposed adaptations reflect the other uses of alkyl (C_{16}, C_{18}, C_{22}) trimethylammonium chlorides in currently marketed cosmetic products.

3.3 TOXICOLOGICAL EVALUATION

3.3.1 Acute toxicity (taken from SCCP/1087/07)

3.3.1.1. Acute oral toxicity

A. *Cetrimonium chloride* - Acute oral toxicity in the mouse

In a study from 1978, 5 male and 5 female mice were administered 300, 400, 500 or 600 mg/kg of *cetrimonium chloride* by oral gavage. The LD_{50} of the substance was reported to be between 400 and 600 mg/kg.

Ref.: 1

B. *Cetrimonium chloride* - Acute oral toxicity in the rat - study 1

Guideline:	OECD TG 401 (1981), Annex V to Dir. 67/548/EEC, Method B.1: Acute oral toxicity
Date of test:	Jun-Jul 1984
Species/strain:	Wistar rat
Group size:	5 rats/sex/dose
Test substance:	Genamin CTAC (28-30% *cetrimonium chloride* in water)
Batch:	E06112547 (04.10.1983)
Purity:	Not stated
Dosages:	630 (only females), 1000, 1600, 2500, 3150 (only males) and 4000 (only males) mg/kg bw
Observation period:	14 days
GLP/QAU:	In compliance

The test substance was applied by oral gavage at dosages of 630, 1000, 1600, 2500, 3150, and 4000 mg/kg bw to groups of 5 male and/or 5 female rats. The lowest dose was administered to 5 females only. The two highest doses were administered to male rats only. The animals were checked daily for mortality and clinical signs. Body weights were recorded at start and on days 7 and 14. Animals were observed for 14 days. Animals that died during the test and all surviving animals at the end of the observation period were submitted to gross necropsy.

Results

Dosage (mg/kg bw)	Lethality in male rats (No. of deaths / total)	Lethality in female rats (No. of deaths / total)
630	not tested	1/5
1000	1/5	1/5
1600	0/5	2/5
2500	0/5	4/5
3150	3/5	not tested
4000	5/5	not tested

During the first days after administration, animals of all treated groups showed decreased motor activity, squatting posture, sunken flanks, half-closed eyes, piloerection, pale skin, laboured irregular respiration, miosis and diarrhoea. All clinical signs had completely ceased by day 10.

Except for one male of the 1000-mg/kg bw group that was found dead on day 13, all deaths occurred within the first 5 days. The LD_{50} values were calculated to be 2970 and 1550 mg/kg bw for male and female rats, respectively.

Animals that had died during the study showed bleeding of the stomach mucosa, inflated stomach, white and/or partially detached stomach mucosa, hyaline aspect of the small intestine, reddened small intestinal mucosa, dark stained adrenals and lung haemorrhage. No macroscopic alterations were observed in rats that had survived until the end of the observation period.

Conclusion
The study authors conclude that the LD_{50}-value for *Cetrimonium chloride* is 2410 mg/kg day for male and female rats combined.

Ref.: 6

C. *Cetrimonium chloride* - Acute oral toxicity in the rat - study 2

Guideline:	OECD TG 420, Annex V to Dir. 67/548/EEC, Method B.1 bis: Acute oral toxicity - fixed dose procedure
Date of test:	Mar-Apr 1997
Species/strain:	Sprague Dawley rat
Group size:	preliminary study: 1 female; main study: 5 rats/sex/dosage
Test substance:	Quartamin 60W25 (24-26% *cetrimonium chloride* in water)
Batch:	3-4
Purity:	Not stated
Dosages:	Preliminary study: 2000 mg/kg bw; main study: 500 & 2000 mg/kg bw
Observation period:	14 days
GLP/QAU:	In compliance

In a preliminary study, Quartamin 60W25 was administered orally to one female rat at 2000 mg/kg bw. The rat was observed twice daily for 14 days.

In the main study, the test substance was applied once by oral gavage at dosages of 500 and 2000 mg/kg bw to groups of 5 male and 5 female rats. The animals were checked twice daily for mortality and clinical signs for 14 days. Body weight was recorded at start and on days 1, 2, 3, 7 and 14. Animals that died during the test and all surviving animals at the end of the observation period were submitted to gross necropsy.

Results

Dosage (mg/kg bw)	Lethality in male rats (No. of deaths / total)	Lethality in female rats (No. of deaths / total)
500	0/5	0/5
2000	3/5	2/5

All deaths occurred within the first 2 days.

The following clinical signs occurred at 2000 mg/kg bw during the first days after administration: decreased motor activity, ataxia, hunched back, half-closed eyes, piloerection, salivation, and diarrhoea. All symptoms had completely disappeared by day 6. Neither clinical signs nor any effect on body weight development were observed at 500 mg/kg bw, while at 2000 mg/kg bw, body weight decreased in the majority of the animals during the first three days after treatment.
Finally, no macroscopic alterations were observed in rats that had received 500 or 2000 mg/kg bw.

Conclusion
The study authors conclude that since no lethality occurred at 500 mg/kg bw and no significant clinical signs were observed at this dosage level, Quartamin 60W25 can be considered to be moderately toxic to non-toxic.

Ref.: 7

D. *Steartrimonium chloride* - Acute oral toxicity in the mouse

In a study from 1989, 5 male and 5 female mice were administered *steartrimonium chloride* by oral gavage (dosages not stated). The LD_{50} of the substance was reported to be 633 mg/kg bw for the males and 536 mg/kg bw for the females.

Ref.: 1

E. *Steartrimonium chloride* - Acute oral toxicity in the rat - study 1

Guideline:	OECD TG 401, Annex V to Dir. 67/548/EEC, Method B.1: Acute oral toxicity
Date of test:	Feb-Apr 1996
Species/strain:	Sprague Dawley rat
Group size:	5 rats/sex/dosage
Test substance:	Quartamin 86W (unknown percentage of *steartrimonium chloride*)
Batch No.:	1841 (batch nr. not documented)
Purity:	Not stated
Dosages:	2000 mg/kg bw (limit test)
Observation period:	14 days
GLP/QAU:	In compliance (only report was audited, not the study itself)

Quartamin 86W was applied once by oral gavage at a dosage of 2000 mg/kg bw to 5 male and 5 female rats. The animals were checked daily for mortality and clinical signs for 14 days. Body weights were recorded at start and on days 7 and 14. Animals that died during the test and all surviving animals at the end of the observation period were submitted to gross necropsy.

Results
In a preliminary study with 2000 mg/kg bw one female died after 6 days. In the main study, no lethality was noted. No clinical signs were observed at 2000 mg/kg. Neither treatment-related effects on body weight gain, nor macroscopic alterations were observed.

Conclusion
The study authors conclude that since no lethality occurred at 2000 mg/kg bw and no significant clinical signs were observed at this dosage level, Quartamin 86W was considered to be non-toxic.

Ref.: 8

F. *Steartrimonium chloride* - Acute oral toxicity in the rat - study 2

Guideline:	OECD TG 401, Annex V to Dir. 67/548/EEC, Method B.1: Acute oral toxicity
Date of test:	Jan-Feb 1996
Species/strain:	Wistar rat
Group size:	5 rats/sex/dosage
Test substance:	Genamin STAC (79.2% *steartrimonium chloride*, 19.1% isopropanol and 0.9% water)
Batch No.:	1061969521
Purity:	79.2% (DIN ISO 2871)
Dosages:	630 (only females), 800, 1250 (only males) and 2000 mg/kg bw
Observation period:	14 days
GLP/QAU:	In compliance (only report was audited, not the study itself)

Genamin STAC was applied once by oral gavage at dosages of 800 and 2000 mg/kg bw to groups of 5 male and 5 female rats and at 630 and 1250 mg/kg bw to 5 females. The animals were checked twice daily for mortality and clinical signs for 14 days. Body weights

were recorded at study start and on days 7 and 14. Animals that died during the test and all surviving animals at the end of the observation period were submitted to gross necropsy.

Results

Dosage (mg/kg bw)	Lethality in male rats (No. of deaths / total)	Lethality in female rats (No. of deaths / total)
630	not tested	2/5
800	0/5	3/5
1250	not tested	5/5
2000	5/5	5/5

The following clinical signs occurred at all dose levels during the first days after administration: squatting posture, sunken flanks, bristled coat, stilted and uncoordinated gait, half-closed eyes, irregular respiration, swollen abdomen, and diarrhoea. Additionally, ataxia was observed in animals of the 2000 mg/kg bw group. All symptoms had completely disappeared by day 8. Body weight development was not impaired. Animals that had died during the study showed inflated stomach, detachment of the stomach mucosa and petechial bleedings, small intestinal containing yellowish mucous or clear fluid, detachment of intestinal mucosa, mucosal bleeding. No macroscopic alterations were observed in rats that survived until the end of the observation period.
Except for one male and one female that were found dead on days 4 and 7, respectively, all deaths occurred within the first day.

Conclusion
The study authors calculated a LD_{50} value of 702.5 mg/kg bw for female rats using the probit method, whereas the LD_{50} value in males could not be calculated.

Ref.: 9

G. *Behentrimonium chloride* - Acute oral toxicity

No data submitted

3.3.1.2. Acute dermal toxicity

A. *Cetrimonium chloride* - Acute dermal toxicity in the rabbit

A study report from 1977 describes an acute dermal toxicity test with *cetrimonium chloride* in 6 New Zealand white albino rabbits. The only dosage tested (4.3 ml/kg bw) caused death in 50% of the animals, and therefore is considered the LD_{50}-value of *cetrimonium chloride*.

Ref.: 1

B. *Steartrimonium chloride* - Acute dermal toxicity

No data submitted

C. *Behentrimonium chloride* - Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity (taken from SCCP/1087/07)

3.3.2.1 Skin irritation - rabbit

A. *Cetrimonium chloride* - skin irritation rabbit - study 1

Guideline:	OECD TG 404, Annex V to Dir. 67/548/EEC, Method B.4: Acute Dermal 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:	0.5 ml of test substance on 6 cm²
Observation period:	21 days
GLP/QAU:	not available (test performed before EU GLP-Guidelines)

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

B. *Cetrimonium chloride* - skin irritation rabbit - study 2

Guideline:	OECD TG 404, Annex V to Dir. 67/548/EEC, Method B.4: Acute Dermal 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:	0.5 ml of test substance on 6 cm²
Observation period:	14 days
GLP/QAU:	In compliance

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

C. *Steartrimonium chloride* - skin irritation rabbit - study 1

Guideline:	OECD TG 404, Annex V to Dir. 67/548/EEC, Method B.4: Acute Dermal Irritation/Corrosion
Date of test:	Sep 1996
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 nr. 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

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

D. *Steartrimonium chloride* - skin irritation rabbit - study 2

Guideline:	OECD TG 404, Annex V to Dir. 67/548/EEC, Method B.4: Acute Dermal Irritation/Corrosion
Date of test:	Jan-Feb 1996
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

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

E. *Behentrimonium chloride* - skin irritation rabbit - study 1

Guideline:	OECD TG 404, Annex V to Dir. 67/548/EEC, Method B.4: Acute Dermal Irritation/Corrosion
Date of test:	May-Jun 2001
Species/strain:	New Zealand white albino rabbit
Group size:	3 males exposed for 3 minutes
Test substance:	Formulation (77-83% *Behentrimonium chloride*, 17-23% isopropanol, ≤ 3% water, ≤ 2% free amine and amine HCl), tested at 10% in a 0.5% methylcellulose aqueous solution
Batch:	0040242 (corresponds to Genamin KDMP)
Purity:	Not stated
Dose:	0.5 ml of test substance
Observation period:	5 days
GLP/QAU:	In compliance

A gauze pad with 0.5 ml test solution was placed on the shaved skin of three male rabbits and covered with semi-occlusive dressing for 3 minutes. After the application time, the patch was removed and the area was wiped with a dry gauze pad. Skin reactions were evaluated 1, 24, 48, and 72 hours and 5 days after patch removal.

Results
No mortality or other clinical effects were observed. At one hour all three rabbits showed grade 1 erythema that had ceased completely in all animals at 24 hours. No erythema was observed at 48 hours and 5 days. No oedema was noted at any time points. The mean score values of the 24, 48 and 72 hour readings were 0.4 for erythema and 0.0 for oedema.

Conclusion
The study authors conclude that a 10% aqueous solution of the test substance produced some irritation when applied for 3 minutes.

Ref.: 14

F. *Behentrimonium chloride* - skin irritation rabbit - study 2

Guideline:	OECD TG 404, Annex V to Dir. 67/548/EEC, Method B.4: Acute Dermal Irritation/Corrosion
Date of test:	Dec 2001
Species/strain:	New Zealand white albino rabbit
Group size:	3 males exposed for 3 minutes
Test substance:	Formulation (77-83% *Behentrimonium chloride*, 17-23% isopropanol, ≤ 3% water, ≤ 2% free amine and amine HCl), tested at 6.25% in a 0.5% methylcellulose aqueous solution
Batch:	0040242 (corresponds to Genamin KDMP)
Purity:	Not stated
Dose:	0.5 ml of test substance
Observation period:	5 days
GLP/QAU:	In compliance

A gauze pad with 0.5 ml test solution was placed on the shaved skin of three male rabbits and covered with semi-occlusive dressing for 3 minutes. After the application time, the patch was removed and the area was wiped with a dry gauze pad. Skin reactions were evaluated 1, 24, 48, and 72 hours after patch removal.

Results
No mortality or other clinical effects were observed. No erythema or oedema was noted at any time point. The mean score values of the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Conclusion
The study authors conclude that a 6.25% aqueous solution of the test substance showed no clinical evidence of skin irritation when applied for 3 minutes.

Ref.: 15

3.3.2.2 Mucous membrane irritation - rabbit

A. *Cetrimonium chloride* - eye irritation rabbit - study 1

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

B. *Cetrimonium chloride* - eye irritation rabbit - study 2

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

C. *Cetrimonium chloride* - eye irritation rabbit - study 3

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

D. *Steartrimonium chloride* - eye irritation rabbit

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 nr. 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

E. *Behentrimonium chloride* - eye irritation rabbit - study 1

Guideline:	OECD TG 405, Annex V to Dir. 67/548/EEC, Method B.5: Acute Eye Irritation/Corrosion
Date of test:	Jun-Jul 2001
Species/strain:	New Zealand white albino rabbit
Group size:	3 males exposed for 3 minutes
Test substance:	Formulation (77-83% *Behentrimonium chloride*, 17-23% isopropanol, ≤ 3% water, ≤ 2% free amine and amine HCl), tested at 10% in a 0.5% methylcellulose aqueous solution
Batch:	010400390104 (corresponds to Genamin KDMP)
Dose:	0.1 ml of test substance
Observation period:	22 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. Thirty seconds after treatment, both eyes were rinsed for 30 seconds with sterile isotonic saline solution. 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 daily from day 5 to day 22 after the instillation. At 48 hours and thereafter, additional examinations using fluorescein solution were performed.

Results
Corneal opacity was observed in 1 of 3 rabbits with grade 2 at 24 and 48 hours (with1/4 to 1/2 of the corneal area affected) and grade 1 at all later time points including day 22. Grade 1 iritis was found at 1 hour in one rabbit and between 24 hours and day 7, but not thereafter, in another rabbit. Conjunctival irritation was evident as grade 2-3 redness between 1 and 72 hours; the reaction declined and had ceased completely in the two rabbits after 7 and 11 days, while grade 1 redness persisted until day 22 in the third rabbit. Grade 1-3 swelling was reported between 1 and 72 hours; the reaction declined and had ceased completely in the two rabbits after 5 and 9 days, while grade 1 swelling persisted until day 22 in the third rabbit (the same animal also showed persisting redness). The mean score values at 24, 48 and 72 hours were 0.6 for opacity, 0.3 for iritis, 2.3 for conjunctival redness and 2.2 for conjunctival chemosis.

Conclusion
The study authors conclude that the 10% *Behentrimonium chloride* solution caused irreversible ocular damage (conjunctival irritation that persisted throughout the test period until day 22 after instillation).

Ref.: 20

F. *Behentrimonium chloride* - eye irritation rabbit - study 2

Guideline:	OECD TG 405, Annex V to Dir. 67/548/EEC, Method B.5: Acute Eye Irritation/Corrosion
Date of test:	Dec 2001
Species/strain:	New Zealand white albino rabbit
Group size:	3 males
Test substance:	Formulation (77-83% *Behentrimonium chloride*, 17-23% isopropanol, ≤ 3% water, ≤ 2% free amine and amine HCl), tested at 6.25% in a 0.5% methylcellulose aqueous solution
Batch:	010400390104 (corresponds to Genamin KDMP)
Purity:	Not stated
Dose:	0.1 ml of test substance
Observation period:	18 days

GLP/QAU: In compliance

A volume of 0.1 ml of the test article was placed into the conjunctival sac of the left eye of each animal. The untreated right eye served as control. Thirty seconds after treatment, both eyes were rinsed for 30 seconds with sterile isotonic saline solution. 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 daily from day 5 to day 18 after the instillation. At 48 hours and thereafter, additional examinations using fluorescein solution were performed.

Results
Corneal opacity was observed in all three rabbits with grade 2 at 24 hours (with 1/4 to 1/2 of the corneal area affected), but not thereafter. Grade 1 iritis was found at 24 hours in all three rabbits and later only in one rabbit until 72 hours. Conjunctival irritation was evident as grade 2-3 redness between 1 and 72 hours; the reaction declined and had ceased completely in the three rabbits after 7 and/or 15 days. Grade 1-3 swelling was reported between 1 and 72 hours; the reaction declined and had ceased completely in the three rabbits after 5, 6 and 18 days. The mean score values at 24, 48 and 72 hours were 0.7 for opacity, 0.6 for iritis, 2.6 for conjunctival redness and 2.3 for conjunctival chemosis.

Conclusion
The study authors conclude that the 6.25% *behentrimonium chloride* solution caused conjunctival irritation that persisted for up to 15 days.

Ref.: 21

G. *Behentrimonium chloride* - eye irritation rabbit - study 3

Guideline:	OECD TG 405, Annex V to Dir. 67/548/EEC, Method B.5: Acute Eye Irritation/Corrosion
Date of test:	Dec 2001
Species/strain:	New Zealand white albino rabbit
Group size:	3 males
Test substance:	Formulation (77-83% *Behentrimonium chloride*, 17-23% isopropanol, ≤ 3% water, ≤ 2% free amine and amine HCl), tested at 3.75% in a 0.5% methylcellulose aqueous solution
Batch:	010400390104 (corresponds to Genamin KDMP)
Purity:	Not stated
Dose:	0.1 ml of test substance
Observation period:	4 days
GLP/QAU:	In compliance

A volume of 0.1 ml of the test article was placed into the conjunctival sac of the left eye of each animal. The untreated right eye served as control. Thirty seconds after treatment, both eyes were rinsed for 30 seconds with sterile isotonic saline solution. 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. At 24 hours, examinations using fluorescein solution were performed.

Results
Neither corneal opacity nor iritis was observed at any time point. Grade 1 iritis was found at 24 hours in all three rabbits and later only in one rabbit until 72 hours. Conjunctival irritation was evident as grade 1-2 redness at 1 hour (3/3 animals), 24 hours (2/3) and 48 hours (1/3), but not thereafter. Grade 1-2 swelling was reported at 1 hour (3/3) and 24 hours (2/3), but not at later time points. The mean score values at 24, 48 and 72 hours were 0.0 for opacity, 0.0 for iritis, 0.3 for conjunctival redness and 0.2 for conjunctival chemosis.

Conclusion
The study authors conclude that the 3.75% *Behentrimonium chloride* solution produced some eye irritation.

Ref.: 22

General comment with regard to eye irritation
This class of compounds is well-known as being ocular irritants. It is therefore surprising that so many *in vivo* studies have been undertaken, some of these being very recent.

3.3.3 Skin sensitisation (taken from SCCP/1087/07)

A. *Cetrimonium chloride* - sensitisation, Maximisation test

Guideline:	OECD TG 406, Annex V to Dir. 67/548/EEC, Method B.6: Skin sensitisation - Magnusson Kligman Maximisation Test
Date of test:	Mar-May 1997
Species/strain:	Dunkin Hartley albino guinea pig
Group size:	Preliminary study: 9 males and 10 females Main study: 10 animals/sex in the treatment group and 5 animals/sex in the two control groups
Test substance:	Quartamin 60W25 (24-26% *cetrimonium chloride* in water)
Batch:	3-4
Purity:	Not stated
Dosages:	Induction: intradermal injections of 0.125% Quartamin 60W25, Challenge: topical application of 0.5% Quartamin 60W25 on 8 cm^2
Observation period:	30 days
GLP/QAU:	In compliance

The preliminary study showed that topical application of a 1% Quartamin 60W25 led to erythema formation, while no alterations were observed in the areas treated with the test substance diluted to 0.5%, 0.3% or 0.1%. Therefore 0.5% was chosen as the challenge concentration. With regard to the intradermal injections, the lowest concentration tested in the preliminary study (0.125%) induced clear erythema formation and was chosen as induction concentration.

In the main study, the test group consisted of 10 male and 10 female Guinea pigs and the two negative control groups of 5 male and 5 female Guinea pigs. Induction commenced (day 0) with pairs of three intradermal injections, of a) FCA, b) test substance (0.125%) in saline, and c) test substance (0.125%) in a 1: 1 mixture of FCA and saline. A 2 x 4 cm shaven area on the supracapular region of each animal, on either side of the mid-dorsal line was treated. The control group received injections of the respective vehicles without test substance. One week later, the induction process was completed with a single topical application of the test substance (3% in distilled water) on a 2 x 4 cm filter paper patch onto the test area. The patch was covered by an occlusive bandage for 48 hours. The control groups received only the vehicle without test substance. After removal of the patches, the skin area was washed with warm water. On day 21, the previously shaven left side of treated animals and the first control group animals was treated topically with 0.5 ml test substance (0.5%) in distilled water; 0.5 ml vehicle alone was applied to the right side. The sites were covered by an occlusive bandage for 24 hours. After patch removal, the zone was washed with warm water. On day 28, a second challenge was carried out identically to the first one. The test substance was applied to the right side of the treated animals and the second control group animals. Each animal was observed at least twice a day for clinical signs.

Body weights were recorded at the start of treatment and on completing the challenge phase observations. The skin reactions were evaluated blind 24 and 48 hours after removal

of the patches. Microscopic examination of skin reactions were done, when a cutaneous reaction was found after the second challenge treatment.

Results
No deaths, clinical signs or alterations in body weights were observed in the treatment group. After the first challenge, 3 males of the treatment group and 1 female of the control group presented mild erythema (grade 1) on the side treated with test substance 24 hours after treatment; two of the males and the one female also showed mild erythema (grade 1) on the vehicle-treated side. After 48 hours, mild erythema persisted in one male on the vehicle-treated side. After the second challenge, mild erythema (grade 1) was observed in 3 males and 1 female of the treatment group and 3 males and 1 female of the control group on the test substance treated side at 24 hours; one male of the treatment group and 2 males of the control group showed mild erythema (grade 1) also on the vehicle-treated side. Histopathology was performed on all skin areas showing erythema. At 48 hours, no skin reactions were found in additional skin areas of either the treatment or the control groups. No histological alterations were found in the samples studied.

Conclusion
The study authors conclude that Quartamin 60W25 (24-26% *cetrimonium chloride* in water) produced no skin sensitisation under conditions of the study. The observed skin reactions occurred at similar incidence and severity in the animals of both the treatment and control groups and similar reactions were found in vehicle-treated animals of both groups.

Ref.: 23

Comments
- The results of the intradermal injection preliminary study are lacking. Since there were still moderate effects in the lowest dose tested, they would be useful. Potentially the induction dose is too high.
- No individual historical positive control group data are available.
- The individual scores after induction are not stated. They become important due to the fact that unexpected irritative effects in the control group cast doubt on the reliability of the presented study. Therefore the results of this study cannot be used for hazard assessment.

B. *Cetrimonium chloride* - sensitisation, Buehler test

Guideline:	OECD TG 406, Annex V to Dir. 67/548/EEC, Method B.6: Skin sensitisation - Buehler test
Date of test:	Sep-Nov 1994
Species/strain:	Pirbright albino guinea pig
Group size:	Preliminary study: 6 animals Main study: 30 females in the treatment group and 10 animals in the control group
Test substance:	Genamin CTAC (28.7% *cetrimonium chloride* in water)
Batch:	E06178641 (26.07.1993)
Purity:	28.7% (DIN ISO 2871)
Dosages:	Induction: topical application of 4% Genamin CTAC on 4 cm² Challenge: topical application of 1% Genamin CTAC on 4 cm²
Observation period:	30 days
GLP/QAU:	not available

The study authors reported that, in a preliminary study, concentrations of 100 and 20% of the test substance caused moderate erythema and slight oedema, while 4% caused slight, well-defined erythema and was subsequently used for induction. No irritation was observed after application of 1.0 and 0.2%, wherefore 1% was used as challenge concentration.

In the main study, the test group consisted of 30 female Guinea pigs and the control group of 10 female Guinea pigs. On day 1, a 4% solution of the test substance in water was prepared and applied on a 2 x 2 cm cellulose patch to the clipped skin of the left flank. The patch was covered with an occlusive dressing for 6 hours and removed afterwards. This treatment was repeated on days 8 and 15. During the induction phase, the skin sites were examined for local effects 24 hours after each treatment. On day 29 (challenge exposure) a 1% solution of the test substance in water was applied on a 2 x 2 cm patch to the clipped skin of the right flank and covered with an occlusive dressing for 6 hours. The dressing was removed 6 hours after the application. Twenty-four and 48 hours after removal of the patches the skin reactions were scored. All animals were observed daily for signs of systemic toxicity. Body weights were recorded on days 1 and 31.

Results
During the study, no clinical effects were observed. Body weight development of the treated group was not different from that of the control group. During the induction phase, treated animals showed slight to well-defined erythema and very slight oedema at the treated skin area. After challenge, skin reactions were observed neither in the treated group nor in the control group.

Conclusion
The study authors conclude that Genamin CTAC (28.7% *cetrimonium chloride* in water) when diluted to 4% (induction) and 1% (challenge) is non-sensitising.

Ref.: 24

Comments
- The individual results of the preliminary study are lacking.
- No individual historical positive control group data are available.

C. *Steartrimonium chloride* - sensitisation, Maximisation test

Guideline:	OECD TG 406, Annex V to Dir. 67/548/EEC, Method B.6: Skin sensitisation - Magnusson Kligman Maximisation Test	
Date of test:	Mar-May 1996	
Species/strain:	Dunkin Hartley albino guinea pig	
Group size:	Preliminary study: 10 males Main study: 10 males in the treatment group and 5 males in the control group	
Test substance:	Quartamin 86W (unknown percentage of *steartrimonium chloride*)	
Batch:	1841 (batch nr. not documented)	
Purity:	Not stated	
Dosages:	Induction:	intradermal application of 0.1% Quartamin 86W in distilled water topical application of 5% Quartamin 86W in distilled water on 8 cm²
	Challenge:	topical application of 10 and 5% Quartamin 86W in distilled water on 8 cm²
Observation period:	30 days	
GLP/QAU:	In compliance	

In the preliminary study, intradermal injection of the lowest tested concentration of 0.1% Quartamin 86W in distilled water resulted in moderate erythema at 24 hours that gradually declined to grade 2 after 48 hours, grade 1-2 after 72 hours and grade 0-1 after 7 days. The experimenters chose to use this concentration for intradermal induction. In two animals previously injected with FCA, a topically applied concentration of 5% resulted in grade 1-2 erythema after 24 hours with desquamation after 48 hours. 5% was defined as the lowest concentration causing mild to moderate irritation under 48h occlusive patch and

thus used for topical induction. Finally, the concentration not resulting in a primary skin irritation after application under occlusion for 24 hours appeared to be 10% Quartamin 86W in distilled water wherefore 10 and 5% were chosen as challenge concentrations.

In the main study, the test group consisted of 10 male Guinea pigs and a negative control group of 5 males. Induction commenced (day 0) with three double intradermal injections of FCA, test substance (0.1%) in water, and test substance (0.1%) in a 1:1 mixture of FCA:water, on the shaven test area of the shoulder region, on either side of the mid-dorsal line. The control group received injections of the respective vehicles without test substance. One week later, the induction process was completed with a single topical application of the test substance (5% in water) on a 2 x 4 cm filter paper patch onto the skin test area. The patch was covered by an occlusive bandage for 48 hours. The control groups received only the vehicle without test substance. On day 21, the previously shaven skin of treated animals and the control group animals was treated topically with patches of 10% test substance on the right side and 5% test substance on the left side. The sites were covered by an occlusive bandage for 24 hours. After patch removal, the zone was swabbed with water-soaked cotton wool. Body weights were recorded at the start of treatment and on completing the challenge phase observations. The skin reactions were evaluated blind 24 and 48 hours after removal of the patches.

Results
No deaths, clinical signs or alterations in body weights in the treatment group were observed. Well-defined or moderate to severe erythema was noted at the intradermal induction sites of all test group animals at 24 and 48 hours. Very slight erythema was noted at the intradermal induction sites of all control group animals at 24 hours and persisted in one animal at the 48 hour observation. After the topical induction, slight to well-defined erythema was noted at the induction sites of all test group animals at 24 hours. No skin reactions were noted in the control group animals. After challenge with 10 and 5% test substance, no skin reactions were noted at the challenge sites of the test or control group animals.

Conclusion
The study authors conclude that Quartamin 86W (unknown percentage of *steartrimonium chloride*) produced no skin sensitisation under the conditions of the test.

Ref.: 25

Comment
No individual historical positive control group data are available.

D. *Steartrimonium chloride* - sensitisation, Buehler test

Guideline:	OECD TG 406, Annex V to Dir. 67/548/EEC, Method B.6: Skin sensitisation - Buehler test
Date of test:	Feb-Mar 1995
Species/strain:	Pirbright albino guinea pig
Group size:	Preliminary study: 6 animals Main study: 20 animals in the treatment group and 10 animals in the control group
Test substance:	Genamin STAC (79.8% *steartrimonium chloride*, 18.8% isopropanol and 0.6% water)
Batch:	E061859561 (31.05.1994)
Purity:	79.8% (DIN ISO 2871)
Dosages:	Induction: topical application of 4% Genamin STAC in ethanol:water (80:20) on 4 cm²
	Challenge: topical application of 1% Genamin STAC in isopropanol on 4 cm²

Observation period: 31 days
GLP/QAU: In compliance

The study report mentions that the preliminary study results have identified 4% Genamin STAC in ethanol:water 80:20 as a concentration causing slight, well-defined erythema and very slight oedema and therefore suitable for induction. No irritation is reported after application of 1.0, 0.2 and 0.04%, wherefore 1% was used as challenge concentration.
In the main study, the test group consisted of 20 female Guinea pigs and the control group of 10 female Guinea pigs. On day 1, a 4% solution of the test substance in ethanol was prepared and applied on a 2 x 2 cm cellulose patch to the clipped skin of the left flank. The patch was covered with an occlusive dressing for 6 hours and was removed afterwards. This treatment was repeated on days 8 and 15. During the induction phase, the skin sites were examined for local effects 24 hours after each treatment. On day 29 (challenge exposure) a 1% solution of the test substance in isopropanol was applied on a 2 x 2 cm patch to the clipped skin of the right flank and covered with an occlusive dressing for 6 hours. 24 and 48 hours after removal of the patches the skin reactions were scored. All animals were observed daily for signs of systemic toxicity. Body weights were recorded on days 1 and 31.

Results
During the study, no clinical effects were observed. Body weight development of the treated group was not different from that of the control group. During the induction phase, treated animals showed slight, well-defined to severe erythema and very slight to well-defined oedema at the treated skin area. After challenge, skin reactions were observed neither in the treated group nor in the control group.

Conclusion
The study authors conclude that Genamin STAC (79.8% *steartrimonium chloride*, 18.8% isopropanol and 0.6% water) is non-sensitising under the conditions of the test.

Ref.: 26

Comments
- The individual results of the preliminary study are lacking.
- No individual historical positive control group data are available.
- No rationale is given for the use of different vehicles in the induction (ethanol:water 80:20) and the challenge (isopropanol) phase.

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

Guideline:	OECD TG 406, Annex V to Dir. 67/548/EEC, Method B.6: Skin sensitisation - Buehler test
Date of test:	Mar-Apr 1995
Species/strain:	Pirbright albino guinea pig
Group size:	Preliminary study: 6 animals Main study: 20 animals in the treatment group and 10 animals in the control group
Test substance:	Genamin KDMP (78.9% *Behentrimonium chloride*, 18.7% isopropanol and 1.9% water)
Batch:	E06186598 (07.06.1994)
Purity:	78.9% (DIN ISO 2871)
Dosages:	Induction: topical application of 20% Genamin KDMP in ethanol:water (80:20) on 4 cm²
	Challenge: topical application of 0.8% Genamin KDMP in isopropanol on 4 cm²
Observation period:	31 days
GLP/QAU:	In compliance

The study report mentions that the preliminary study results have identified 20% Genamin KDMP in ethanol:water 80:20 as a concentration causing moderate erythema and very slight oedema, while 4% produced slight erythema in one of the two tested guinea pigs. Therefore 20% was considered suitable for induction. No irritation is reported after application of 0.8%, wherefore the latter was chosen as challenge concentration.

In the main study, the test group consisted of 20 female Guinea pigs and the control group of 10 female Guinea pigs. On day 1, a 20% solution of the test substance in ethanol was prepared and applied on a 2 x 2 cm cellulose patch to the clipped skin of the left flank. The patch was covered with an occlusive dressing for 6 hours and was removed afterwards. This treatment was repeated on days 8 and 15. During the induction phase, the skin sites were examined for local effects 24 hours after each treatment. On day 29 (challenge exposure) a 0.8% solution of the test substance in isopropanol was applied on a 2 x 2 cm patch to the clipped skin of the right flank and covered with an occlusive dressing for 6 hours. 24 and 48 hours after removal of the patches the skin reactions were scored. All animals were observed daily for signs of systemic toxicity. Body weights were recorded on days 1 and 31.

Results
During the study, no clinical effects were observed. Body weight development of the treated group was not different from that of the control group. During the induction phase, treated animals showed slight to well-defined erythema and very slight oedema at the treated skin area. After challenge, skin reactions were observed neither in the treated group nor in the control group.

Conclusion
The study authors conclude that Genamin KDMP (78.9% *Behentrimonium chloride*, 18.7% isopropanol and 1.9% water) is non-sensitising under the conditions of the test.

Ref.: 27

Comments
- The individual results of the preliminary study are lacking.
- No individual historical positive control group data are available.
- No rationale is given for the use of different vehicles in the induction (ethanol:water 80:20) and the challenge (isopropanol) phase.

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

Guideline:	OECD TG 406, Annex V to Dir. 67/548/EEC, Method B.6: Skin sensitisation - Buehler test
Date of test:	Nov 2000 – Jan 2001
Species/strain:	Dunkin Hartley guinea pig
Group size:	Preliminary study: 4 animals Main study: 10 animals/sex in the treatment group and 5 animals/sex in the control group
Test substance:	Genamin KDMP (77-83% *Behentrimonium chloride*, 17-23% isopropanol, ≤ 3% water and ≤ 2% free amine and amine HCl)
Batch:	0040242
Purity:	Not stated
Dosages:	Induction: topical application of 10% Genamin KDMP in corn oil on 8 cm² Challenge: topical application of 0.5% Genamin KDMP in corn oil on 8 cm²
Observation period:	46 days
GLP/QAU:	In compliance

In the preliminary study, a concentration of 10% of the test substance in corn oil applied on a filter paper patch caused grade 1 erythema after 24 hours, while a concentration of 5% did not cause any skin reaction. Therefore 10% was used for the dermal induction. A concentration of 10% in corn oil applied on a Finn chamber, caused grade 2 erythema after 24 and 48 hours. Concentrations of 5 and 1% caused grade 1 erythema after 24 and 48 hours, while 0.5% caused no skin reactions. Therefore a concentration of 0.5% was used for the challenge.
In the main study, the test group consisted of 10 male and 10 female Guinea pigs and the control group of 5 male and 5 females. On day 1, a 10% solution of the test substance in corn oil was applied on a 8cm² filter paper patch to the clipped skin of the left flank. The patch was covered with an occlusive dressing for 6 hours and was removed afterwards. This treatment was repeated on days 8 and 15. During the induction phase, the skin sites were examined for local effects 24 hours after each treatment. On day 29 (challenge exposure) a 0.5% solution of the test substance in corn oil was loaded into a Finn chamber and this was applied to the clipped skin of the right flank and covered with an occlusive dressing for 6 hours. As equivocal cutaneous reactions were noted, a second challenge was performed on day 43. This time, the test substance was applied to the left side and the vehicle to the right side. Twenty-four, 48 and 72 hours after removal of the patches the skin reactions were scored. All animals were observed daily for signs of systemic toxicity. Body weights were recorded on days 1, 32 and 46.

Results
In the main experiment, one male animal of the treatment group died spontaneously on day 14; the death was considered as unrelated to treatment. During the whole study, no clinical effects were observed. Body weight development of the treated group was not different from that of the control group. During the induction phase, a few of the treated animals showed slight to well-defined erythema (grade 1 or 2) at the treated skin area. After the challenge, no skin reactions were observed the control group. In the treatment group, grade 1 erythema was noted in 3/19 animals at 24 hours, 5/19 at 48 hours and 2/19 at 72 hours. After re-challenge, no skin reactions were found.

Conclusion
The study authors conclude that since no skin reactions were observed in the re-challenge, the skin reactions observed after the first challenge can be attributed to the irritant properties of the test substance. Therefore they consider Genamin KDMP (77-83% *Behentrimonium chloride*, 17-23% isopropanol, ≤ 3% water and ≤ 2% free amine and amine HCl) as non-sensitising under conditions of this assay.

Ref.: 28

General comments with regard to skin sensitisation

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), 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 skin sensitisers.

3.3.4 Dermal / percutaneous absorption (taken from SCCP/1087/07)

3.3.4.1 *In vitro* dermal / percutaneous absorption

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

Guideline:	Draft OECD TG 428: Percutaneous Absorption: *in vitro* Method (1994)
Date of test:	Jul - Aug 1997
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:	Presumably a 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/cm²), rinsed off with shampoo & water after 30 min
Duration of study:	72 hours
GLP/QAU:	In compliance

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 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

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 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 were 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.

- The contact time of 30 minutes may be relevant for hair care products, but is not adequate for the requested leave-on application.

B. *Steartrimonium chloride* - dermal absorption *in vitro*

No data submitted

C. *Behentrimonium chloride* - dermal absorption *in vitro*

No data submitted

3.3.4.2 *In vivo* dermal / percutaneous absorption - rat

A. *Cetrimonium chloride* - dermal absorption *in vivo* - rat

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). 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

B. *Steartrimonium chloride* - dermal absorption *in vivo*

No data submitted

C. *Behentrimonium chloride* - dermal absorption *in vivo*

No data submitted

General comments on dermal absorption

The *in vivo* study described under section A.3 is considered relevant for the following reasons:

- The test was performed in a leave-on setting with an analogous compound to the molecules under study.
- The analogous compound (laurtrimonium bromide) has a lower molecular weight, which means that its dermal absorption is likely to be higher than the value for the compounds under study.
- The *in vivo* study was performed in the rat, which is known to display a dermal absorption of about ten times higher than human skin.

Therefore the setting of this *in vivo* study can be considered as a worst case and the dermal absorption value can be used for calculation of the Margin of Safety.

3.3.5 Repeated dose toxicity (taken from SCCP/1087/07)

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

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
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:
- minor increase in serum ALT activity in males and females (within 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
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 a possible sign of some systemic toxicity.

The dosage of 100 mg/kg bw/day was considered to be the no-observed-effect-level (NOEL) of Dehyquart A-CA (24-26% *cetrimonium chloride* in water) 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)

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

No data submitted

D. *Behentrimonium 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

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. 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

Comment
For the calculation of the MoS, the NOAEL of 10 mg/kg bw/day, as deduced from this study, will be used.

B. *Steartrimonium chloride* - chronic toxicity

No data submitted

C. *Behentrimonium chloride* - chronic toxicity

No data submitted

3.3.6 Mutagenicity / genotoxicity (taken from SCCP/1087/07)

3.3.6.1 Mutagenicity/Genotoxicity *in vitro*

A. *Cetrimonium chloride* - Bacterial reverse mutation assay

A US National Toxicology Programme report mentions two references of 1980 and 1984 describing bacterial reverse mutation assays with *cetrimonium chloride* in *Salmonella typhimurium* TA98 and TA100, with and without S9 activation. *Cetrimonium chloride* showed to be negative under the test conditions with a cytotoxic concentration of 5.0 µg/plate without metabolic activation.

Ref.: 1

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

Date of study:	Mar-May 1996	
Guideline:	OECD TG 471, Annex V to Dir. 67/548/EEC, Method B.13/14: Mutagenicity: reverse mutation test using bacteria	
Species/strain:	Salmonella typhimurium, TA98, TA100, TA1535, TA1537, TA 1538	
Replicates:	3	
Test substance:	Quartamin 86W (unknown percentage of *steartrimonium chloride*)	
Batch:	1841 (batch nr. not documented)	
Concentrations:	Preliminary study 1:	0 - 50 - 500 - 1500 - 5000 µg /plate
	Preliminary study 2:	- S9-mix: 0 - 0.5 - 1.5 - 5- -15 - 50 µg/plate
		+ S9-mix: 0 - 5 - 15 - 50 - 150 - 500 µg/plate
	Main study:	- S9-mix: 0 - 0.15 - 0.5 - 1.5 - 5- -15 - 50 - 150 – 500 µg/plate

	+ S9-mix: 0 - 0.5 - 1.5 - 5 - 15 - 50 - 150 - 500 µg/plate
GLP/QAU:	In compliance

The strains were exposed to the test material dissolved in acetone on plates containing histidine deficient agar in the presence and absence of rat liver metabolic activating system (S9-mix prepared from livers of male Sprague-Dawley rats that had received a single intraperitoneal injection of Aroclor 1254 five days before). The concentrations tested ranged from 0.15 to 500 µg/plate. Acetone alone served as negative control. As a positive standard requiring metabolic activation 2-aminoanthracene was used. N-ethyl-N'-nitro-N-nitrosoguanidine (for TA100 and TA1535), 9-aminoacridine (for TA1537), 4-nitroquinoline-1-oxide (for TA98) and 4-nitro-o-phenylenediamine (TA1538) were used as positive standards without metabolic activation. Two independent experiments were performed. Toxicity testing was done in a pre-test using strain TA100 and test substance concentrations of 50 to 5000 µg/plate with and without S9-mix. Due to toxicity at all dose levels, the pre-test was repeated with concentrations of 0.5, 1.5, 15 and 50 µg/plate.

Results
In the pre-test, the test substance caused an incomplete bacterial lawn at 15 µg/plate without S9-mix and at 150 µg/plate with S9-mix. In the two main experiments, the test substance proved toxic to all Salmonella strains at 50 µg/plate or higher in the absence and presence of S9-mix. The test substance did not induce a biologically significant increase of the mean number of revertant colonies compared to the controls, neither in the absence nor in the presence of a metabolic activation system.

Conclusion
It was concluded that Quartamin 86W (unknown percentage of *steartrimonium chloride*) had no mutagenic activity on any of the bacterial tester strains used either with or without S9-mix at up to 15 µg active substance/plate.

Ref.: 36

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

A US National Toxicology Programme report mentions a reference of 1983 describing a bacterial reverse mutation assay with *steartrimonium chloride* in *Salmonella typhimurium* TA98 and TA100, with and without S9 activation. *Steartrimonium chloride* showed to be negative under the conditions of test.

Ref.: 1

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

Date of study:	Dec 1994
Guideline:	OECD TG 471, Annex V to Dir. 67/548/EEC, Method B.13/14: Mutagenicity: reverse mutation test using bacteria
Species/strain:	Salmonella typhimurium, TA98, TA100, TA1535, TA1537
Replicates:	3
Test substance:	Genamin STAC (79.8% *steartrimonium chloride*, 18.8% isopropanol and 0.6% water)
Batch:	E061859561 (31.05.1994)
Purity:	79.8% (DIN ISO 2871)
Concentrations:	0.8, 4, 20, 100, 500, 2500 and 5000 µg/plate with and without metabolic activation (rat S9-mix)
GLP/QAU:	In compliance

The Salmonella typhimurium strains were exposed to the test material dissolved in ethanol on plates containing histidine deficient agar in the presence and absence of rat liver metabolic activating system (S9-mix prepared from livers of male Sprague-Dawley rats that

had received a single intraperitoneal injection of Aroclor 1254 five days before). The concentrations tested ranged from 0.8 to 5000 µg/plate. Ethanol alone served as negative control. As a positive standard requiring metabolic activation 2-aminoanthracene was used. Sodium azide (for TA100, TA1535), 9-aminoacridine (for TA1537) and 2-nitrofluorene (for TA98) were used as positive standards without metabolic activation.

Results
The test compound proved toxic in the absence and presence of S9-mix at 100 µg/plate (incomplete or no bacterial lawn) and higher concentrations. The test substance did not induce a biologically significant increase of the mean number of revertant colonies compared to the controls, neither in the absence nor in the presence of a metabolic activation system.

Conclusion
The study authors concluded that Genamin STAC (79.8% *steartrimonium chloride*, 18.8% isopropanol and 0.6% water) had no mutagenic activity on any of the bacterial tester strains used either with or without S9-mix at up to 20 or 100 µg/plate respectively.

Ref.: 37

E. *Behentrimonium chloride* - Bacterial reverse mutation assay

Date of study:	Jan 1995
Guideline:	OECD TG 471, Annex V to Dir. 67/548/EEC, Method B.13/14: Mutagenicity: reverse mutation test using bacteria
Species/strain:	Salmonella typhimurium, TA98, TA100, TA1535, TA1537
Replicates:	3
Test substance:	Genamin KDMP (78.9% *Behentrimonium chloride*, 18.7% isopropanol and 1.9% water)
Batch:	E06186598 (07.06.1994)
Purity:	78.9% (DIN ISO 2871)
Concentrations:	0.8, 4, 20, 100, 500, 2500 and 5000 µg/plate with and without metabolic activation (rat S9-mix)
GLP/QAU:	In compliance

The strains were exposed to the test material dissolved in ethanol on plates containing histidine deficient agar in the presence and absence of rat liver metabolic activating system (S9-mix prepared from livers of male Sprague-Dawley rats that had received a single intraperitoneal injection of Aroclor 1254 five days before). The concentrations tested ranged from 4 to 5000 µg/plate. Ethanol alone served as negative control. As a positive standard requiring metabolic activation 2-aminoanthracene was used. Sodium azide (for TA100, TA1535), 9-aminoacridine (for TA1537) and 2-nitrofluorene (for TA98) were used as positive standards without metabolic activation.

Results
The test compound proved toxic in the absence of S9-mix at 500 µg/plate (incomplete bacterial lawn) and at higher concentrations (no bacterial lawn); in the presence of S9-mix, incomplete or no bacterial lawn was found at 2500 and 5000 µg/plate. The test substance did not induce a biologically significant increase of the mean number of revertant colonies compared to the controls, neither in the absence nor in the presence of a metabolic activation system.

Conclusion
The study authors concluded that Genamin KDMP (78.9% *Behentrimonium chloride*, 18.7% isopropanol and 1.9% water) had no mutagenic activity on any of the bacterial tester strains used either with or without S9-mix at up to 500 µg/plate (with S9-mix) or 100 µg/plate (without S9-mix).

Ref.: 38

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

Date of study:	Apr-Jul 1989
Guideline:	OECD TG 473, Annex V to Dir. 67/548/EEC, Method B.10: Mutagenicity: *in vitro* mammalian chromosome aberration test
Species/strain:	V79 Chinese hamster cells
Replicates:	2
Test substance:	24-26% *cetrimonium chloride* in water
Batch:	3118322 (18.01.1989)
Purity:	Not stated
Concentrations:	- S9-mix: 0.1, 0.3, 0.6, 1.0, 3.0 and 6.0 µg/ml + S9-mix: 0.1, 0.5, 1.0, 3.0, 6.0 and 10.0 µg/ml
GLP/QAU:	In compliance (protocol and study report QAU inspected, no inspections during study)

Logarithmically growing cells were incubated with the test substance in serum-free culture medium at concentrations of 0.1 to 6 µg/ml without S9-mix and at 0.1 to 10 µg/ml with S9-mix (from Aroclor 1254 induced rats) for 4 hours. Cells were subsequently washed in glucose-containing saline and cultured in normal medium for 7, 18 and 28 hours. Ethylmethanesulfonate and cyclophosphamide were used as positive controls in 18-hour cultures without and with S9-mix, respectively. Two hours (7 hour interval) or 2.5 hours (18 and 28 hours intervals) before the end of the incubation period, colcemid was added to the cultures. The cells were put onto glass slides, treated with hypotonic potassium chloride solution, fixed in methanol and acetic acid and stained with Giemsa solution. In each experimental group two parallel cultures were set up. Per culture 100 metaphases were scored for structural chromosomal aberrations (breaks, fragments, deletions, exchanges and chromosomal disintegrations). Chromosomal gaps were recorded separately.

Results
In concentration-finding pre-tests, cytotoxic effects were observed at 1 µg/ml without S9-mix and at 6 µg/ml with S9-mix as a colony-forming ability below 20% of controls. Evaluated dose levels were 1.0 µg/ml without S9-mix and 10.0 µg/ml with S9-mix for 7 hours; 0.3, 1.0 and 3.0 µg/ml without S9-mix and 1.0, 3.0 and 10.0 µg/ml with S9-mix for 18 hours; and 3.0 µg/ml without S9-mix and 10.0 µg/ml with S9-mix for 28 hours. There were no biologically relevant and statistically significant increases in cells with structural aberrations after treatment with the test substance at any fixation interval either with or without metabolic activation. The reference mutagens used as positive controls showed distinct increases in cells with structural chromosome aberrations.

Conclusion
The study authors concluded that *cetrimonium chloride* (24-26% in water) did not induce chromosomal aberrations in V79 cells either with or without S9-mix at up to 3.0 and 10.0 µg/ml respectively.

Ref.: 39

Comment
100 instead of 200 metaphases were scored per tested concentration.

G. *Cetrimonium chloride - in vitro* cell transformation assay

A publication of 1979 describes how cryopreserved 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

3.3.7 Carcinogenicity

No data submitted

3.3.8 Reproductive toxicity (taken from SCCP/1087/07)

3.3.8.1 2-Generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

A. *Cetrimonium chloride,* dermal administration, rabbit

20 mated female New Zealand albino white rabbits per group are reported to be exposed for days 7 to 18 of gestation to *cetrimonium chloride* at dermal dosage levels of 0, 10, 20 and 40 mg/kg bw/day. Prior to initial treatment, the dorsal area of each animal was shaved and any skin lesions were documented. After the 2-hour exposure period, the application site was rinsed with water and dried. Animals were observed twice daily for signs of toxicity, including skin irritation from days 7 through 29. Body weights were taken on gestation days 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 29. Individual food consumption was measured daily. A gross necropsy was conducted on animals that died in an attempt to determine the cause of death. Foetuses less than 28 days old were fixed in buffered neutral formalin and those 28 days or older were cleared and stained. All surviving dams were sacrificed at study termination on gestation day 29 using sodium pentobarbital. An examination of the uterus (including the number and location of live and dead foetuses, early and late resorptions, and implantation sites), and ovaries (including the number of corpora lutea), was conducted. Following removal of the foetuses, the abdominal and thoracic cavities and organs of the dams were examined.

Uteri from females that appeared non-gravid were placed in 10% ammonium sulfide solution for confirmation of pregnancy. At sacrifice foetuses were identified, weighed, and examined externally for defects. Gross dissection and examination of viscera, and internal sex determination also were conducted on each foetus. Finally, an examination of the skeleton for anomalies and ossification variations was conducted after clearing and alizarin red staining of the foetuses.

Two control, one intermediate and one high dose pregnant females died during the study. The cause of death could not be determined. Two of the animals that died aborted prior to death (one control and one intermediate dose group animal). Two additional abortions occurred, one each in the intermediate and high dose groups. None of these deaths or abortions were considered related to test substance toxicity.

Skin irritation was observed at all doses with dose-related severity and duration, and included erythema, oedema, desquamation, atonia and coriaceousness. Marked to moderate irritation was observed primarily in the mid and high dose groups. No treatment-related maternal body weight or food intake effects were noted. A slight increase in congested lungs was observed in the high dose group at necropsy.

The incidence of foetal malformation and genetic and developmental variation in the treated groups was comparable to that of the control group. No other treatment-related effects were noted.

Under the test conditions used, *cetrimonium chloride* was found to be non-foetotoxic and non-teratogenic. The NOEL for maternal systemic toxicity and embryo-foetal toxicity appeared to be 40 mg *cetrimonium chloride*/kg bw/day.

Ref.: 41 (= pp.222-224 of Ref.1)

B. *Steartrimonium chloride* - dermal administration, rat

20 mated female Sprague Dawley rats per group are reported to be exposed for days 6 to 15 of gestation to *steartrimonium chloride* at dosage levels of 4.5, 7.5 and 12.5 mg/kg bw/day. The test substance was applied with a syringe and gently massaged into the shaved area (4 x 4 cm) of skin in the scapula region for not more than one minute. The test substance was left on the skin and was neither removed by washing nor occluded. The 20 mated female rats per group resulted in 10 to 20 pregnant dams per group that provided between 192 and 259 live foetuses per group for examination. All animals were observed for signs of systemic and local reactions. Body weights, food and water consumption were recorded at regular intervals throughout the study. On day 20 of gestation, dams were killed, litter values determined and foetuses subsequently examined for visceral and skeletal abnormalities.

There were no systemic signs of toxicity in the dams, no deaths or treatment-related macroscopic pathology changes in internal organs were noted. A dose-related local reaction was recorded in terms of incidence and severity of erythema and oedema. Local reactions were evident on the day of the first administration, reaching a peak around the mid-point of the dosing period; thereafter, local adverse effects stabilised or declined. There was no marked or consistent treatment-related difference in weight gain, although marginally lower weight gains during the dosing period were observed in all treated groups when compared to control means. There was no marked effect on food or water consumption.

Litter values assessed by litter size, post-implantation loss, litter and mean foetal weights and the embryotoxic and foetal development were unaffected by treatment. There were no significant differences from concurrent control values in respect of the incidence of malformed or anomalous young or of litters containing affected young. Types of malformations or anomalies observed were within the range of historical control values for this strain.

Skin changes at the application sites were considered to be a result of local irritation and not indicative of systemic toxicity.

Under the test conditions used, *steartrimonium chloride* was found to be non-foetotoxic and non-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

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

C. *Behentrimonium chloride*

No data submitted

3.3.9 Toxicokinetics (taken from SCCP/1087/07)

A. *Cetrimonium chloride*

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* shows to be poorly absorbed in the gastro-intestinal tract and not readily metabolized in the rat body.

Ref.: 43

A publication of 1979 reports on the elimination and tissue distribution of ^{14}C-labelled *laurtrimonium bromide* 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

B. *Steartrimonium chloride*

No data submitted

C. *Behentrimonium chloride*

No data submitted

3.3.10 Photo-induced toxicity

No data submitted

3.3.11 Human data

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

A. *Cetrimonium chloride* - single patch test 1

Date of study:	Aug 1998
Method:	Epicutaneous patch test
Subjects:	Healthy male and female volunteers, age 17-72 years (mean 31 years) without skin diseases
Group size:	19 male and 33 female subjects
Test substance:	Rinse-off formulation (Wellazid 7/0609/05/14/02): main components: 86.4% water 4.7% glyceryl stearate

	3.6% stearyl alcohol 3.5% *cetrimonium chloride*
Dosages:	20 µl per chamber of Finn-Chambers (8 mm diameter)
Exposure time:	24 hours
Scoring system:	**0** no apparent cutaneous involvement **0.5** greater than 0, less than 1 (faint erythema) **1** definite erythema, no eruption or broken skin **2** strong erythema, may have a few papules or deep fissures, moderate to severe erythema in the cracks **3** severe erythema, may have generalised papules and/or vesicles **4** bullae or eschar formations or severe erythema with oedema extending clearly beyond the patch area
GCP:	No statement

The test and reference substances were applied to the ventral side of the forearm using Finn chambers. Reference substances included deionised water as negative control and 4.0% sodium lauryl sulfate as positive control. The test site was covered by an adhesive occlusive plaster for 24 hours. Skin reactions were evaluated 15 minutes and 24 and 48 hours after removal of test patches.

Results
The mean irritation scores were the following:

- negative control	0.04 (15 min),	0.00 (24 hours),	0.00 (48 hours)
- test article	0.22 (15 min),	0.11 (24 hours),	0.06 (48 hours)
- positive control	0.79 (15 min),	1.00 (24 hours),	0.86 (48 hours)

Conclusion
The study authors conclude that 3.52% *cetrimonium chloride* in a cosmetic formulation caused slight to moderate skin irritation when applied under occlusion for 24 hours.

Ref.: 44

B. *Cetrimonium chloride* - single patch test 2

Date of study:	April 1999
Method:	Epicutaneous patch test
Subjects:	Healthy male and female volunteers, age 19-66 years (mean 43.8 years) of which 19 were categorised as having sensitive skin
Group size:	6 male and 43 female subjects
Test substance:	Rinse-off formulation (Lotion stain remover Ref.453061): 64.5% water 10% propylene glycol 10% ethanol 5% benzyl alcohol 5% glycerine 3% cocobetain 2.5% *cetrimonium chloride*
Dosages:	160 µl per chamber
Exposure time:	1 hour
Scoring system:	**0** no reaction **0.5** doubtful reaction (faint erythema) **1** very slight reaction (pale pink, homogenous, regular erythema) **2** slight reaction (pink, homogenous, regular, non-vesicular erythema) **3** moderate reaction (red, homogenous, regular erythema) **4** severe reaction (bright red, pronounced, homogenous, regular erythema)

GCP: In compliance

160 µl of test substance was applied to the back of the volunteers. The test site was covered semi-occlusively by adhesive plaster for 1 hour. Skin reactions were evaluated 15 minutes and 24 and 48 hours after removal of test patches.

Results
The test article produced a single doubtful reaction (score 0.5) after patch removal, another very slight reaction (score 1) after 24 hours, and a very slight reaction (score 1) after 48 hours. In untreated control skin sites, 1 very slight reaction (score 0.5) was observed at 24 hours.

Conclusion
The study authors conclude that 2.5% *cetrimonium chloride* in a cosmetic formulation produced some skin irritation when applied under occlusion for 1 hour.

Ref.: 45

C. *Cetrimonium chloride* - single patch test 3

Date of study:	Not stated (report dated July 1996)
Method:	Epicutaneous patch test
Subjects:	Healthy male and female volunteers, age 17-68 years, of which 23 had atopy without visible skin lesions at the start of the study
Group size:	18 male and 32 female subjects
Test substance:	50% of rinse-off formulation (Proficare Krauteracid 8/5197/05/01/010): main components: 86.5% water 4.7% glyceryl stearate 3.6% stearyl alcohol 3.5% *cetrimonium chloride*
Dosages:	Not reported
Exposure time:	24 hours
Scoring system:	**-** negative, no reaction **-+** doubtful reaction **+** weak (non-vesicular) reaction **++** strong (oedematous or vesicular) reaction **+++** strong, infiltrated erythema and accompanying vesicles or superficial erosions **++++** bullae or extensive erosions IR irritant reaction
GCP:	No statement

The test substances was applied to the ventral side of the forearm using patches of filter paper, placed on an impermeable sheet and fixed to the skin with adhesive tape. The test site was covered occlusively for 24 hours. Skin reactions were evaluated 0, 24 and 48 hours after removal of test patches.

Results
No signs of irritation were detected in any subject at 0, 24 and 48 hours after application.

Conclusion
The study authors conclude that a 50% aqueous dilution of a cosmetic formulation containing 3.52% *cetrimonium chloride* caused no skin irritation when applied under occlusion for 24 hours.

Ref.: 46

D. *Cetrimonium chloride* - single patch test 4

Date of study:	Not stated (report dated Dec 1998)
Method:	Epicutaneous patch test
Subjects:	Healthy male and female volunteers, age 19-67 years, of which 7 had allergies and 8 had sensitive skin without visible skin lesions at the start of the study
Group size:	18 male and 32 female subjects
Test substance:	50% of rinse-off formulation (Kräuterazid 7/0609/05/05/02): main components: 84.8% water 4.7% glyceryl stearate 3.6% stearyl alcohol 3.2% *cetrimonium chloride* 1.9% isopropyl alcohol
Dosages:	2-5 mg test product per cm² skin covered by a standard test plaster
Exposure time:	48 hours
Scoring system:	**0** no irritation **(+)** slight or improbable erythema **+** distinct erythema (as well as urticarial) **++** strong erythema and/or papule formation **+++** abundant papules and/or vesicles **++++** blister formation or necrosis
GCP:	No statement

The test substance was applied to the skin of the back using standard test plasters. The test site was covered semi-occlusively for 48 hours. Skin reactions were evaluated 0 and 24 hours after removal of test patches.

Results
No signs of irritation were detected in any subject at 0 as well as at 24 hours after application.

Conclusion
The study authors conclude that a 50% aqueous dilution of a cosmetic formulation containing 3.2% *cetrimonium chloride* caused no skin irritation when applied under semi-occlusion for 48 hours.

Ref.: 47

E. *Cetrimonium chloride* - single patch test 5

Date of study:	Dec 1993
Method:	Epicutaneous patch test
Subjects:	Healthy male and female volunteers, age 20-56 years (mean 32.3 years) without skin diseases
Group size:	20 subjects
Test substance:	Dehyquart A-CA ex Henkel and Dehyquart A-CA ex Italien, dilute in water to generate a 1% *cetrimonium chloride* concentration (1% *cetrimonium chloride*) in water
Batch N°:	Dehyquart A-CA ex Henkel: 10393183 dated 07.12.1993 Dehyquart A-CA ex Italien: 151/1093 dated 07.12.1993
Dosages:	70 µl per chamber of Large-Finn-Chambers (12 mm diameter)
Exposure time:	24 hours
Scoring system:	**0** no reaction **0.5** very slight reaction **1** slight reaction

	2 moderate reaction
	3 strong reaction
	4 very strong reaction
	-1 no answer
GCP:	In compliance

The test and reference substances were applied to the back skin of 20 healthy subjects using large Finn chambers on Scanpor™ plaster. Reference substances included deionised water, physiological saline and cosmetic grade alcohol as negative controls and 0.5% sodium lauryl sulfate and 1.0% sodium laureth sulfate (Texapon N 25) as positive controls. The test site was covered occlusively by adhesive plaster for 24 hours. Skin reactions were evaluated 6, 24, 48 and 72 hours after removal of test substances.

Results
The two Dehyquart A-CA samples caused no erythema, oedema, eschar or fissures in any subjects. Sodium lauryl sulfate and sodium laureth sulfate caused slight to moderate erythema, slight oedema (only sodium lauryl sulfate) and slight (sodium laureth sulfate) to moderate (sodium lauryl sulfate) eschar formation. The negative control substances and empty Finn chambers caused no skin reaction.

Conclusion
The study authors conclude that neither Dehyquart A-CA Henkel nor Dehyquart A-CA Italien at 1% active substance (*cetrimonium chloride*) in water, applied for 24 hours under occlusion, was irritating to skin under the test conditions used.

Ref.: 48

F. *Cetrimonium chloride* - single patch test 6

Date of study:	June 1997
Method:	Epicutaneous patch test
Subjects:	Healthy male and female volunteers, age 18-66 years, of which 8 had eczema and 11 had sensitive skin without visible skin lesions at the start of the study
Group size:	50 male and female subjects
Test substance:	50% in water of rinse-off formulation (Wellazid 8/6161/05/07/465): main components: 89.1% water 4.0% glyceryl stearate 3.1% stearyl alcohol 2.0% *cetrimonium chloride*
Dosages:	50 µl per chamber of square Finn-Chambers (10 mm side length)
Exposure time:	24 hours
Scoring system:	**0** no erythema, no fissures, no scaling **1** slight erythema, minimal fissures, minimal scaling **2** clear erythema, clearly visible fissures, clearly visible scaling **3** severe erythema, distinct fissures, moderate scaling **4** very severe erythema, ulceration, distinct scaling
GCP:	In compliance

The test substances as well as 1% SDS in water as positive control and water alone as negative control were applied to the skin (site not stated) using square Finn chambers and adhesive tape. The test site was covered semi-occlusively for 24 hours. Skin reactions were evaluated 0, 24 and 48 hours after removal of test patches.

Results

No signs of irritation were detected in any subject at 0 as well as at 24 hours after application. The negative control (water) caused no skin reaction at any time points. The positive control (1% SDS) produced the following grades with regard to erythema/fissures/scales: 0.52, 0, and 0, respectively, after 0 hours, 0.96, 0.04, and 0.84, respectively, after 24 hours and 0.72, 0.04, and 0.38, respectively, after 48 hours.

Conclusion
The study authors conclude that a 50% aqueous dilution of a cosmetic formulation containing 2.0% *cetrimonium chloride* caused no skin irritation when applied under semi-occlusion for 24 hours.

Ref.: 49

G. *Cetrimonium chloride* - single patch test 7

Date of study:	Aug-Sep 1999
Method:	Epicutaneous patch test
Subjects:	Healthy male and female volunteers, age 20-67 years, of which 36 had dry or seborrhoic skin without visible skin lesions at the start of the study
Group size:	24 male and 27 female subjects
Test substance:	25% of rinse-off formulation (Koleston 99 0-Masse, 83005095 B): main components: 60.3% water 10.0% stearyl alcohol 10.0% petrolatum 8.4% glyceryl stearate 4.1% ammonia 3.0% *cetrimonium chloride* 1.8% isopropyl alcohol
Dosages:	100 µl per chamber (round, 10 mm diameter)
Exposure time:	24 hours
Scoring system:	**0** no reaction **0.5** redness, faint with poorly-defined margins **1.0-1.9** weak, spotty erythema **2.0-2.9** moderate erythema **3.0-3.9** medium to strong erythema **4.0-4.9** strong large-surface erythema with oedema
GCP:	In compliance

The test substances as well as 2% sodium laureth sulfate and 0.5% sodium lauryl sulfate as positive controls and water alone as negative control were applied to the back of human volunteers using Finn chambers and adhesive tape. The test site was covered occlusively for 24 hours. Skin reactions were evaluated 15 minutes and 24 and 48 hours after removal of test patches.

Results
The negative control (water) produced no skin reaction at any time points.
At a concentration of 2%, the positive control substance sodium laureth sulfate caused 8 grade 0.5 and 3 grade 1 reactions at the 15 minutes reading, whereas no reactions were found at the 24- and 48-hour readings.
At a concentration of 0.5%, the positive control substance sodium lauryl sulfate caused 9 grade 0.5, 16 grade 1, 6 grade 1.5, and 11 grade 2 reactions at the 15 minutes reading, 13 grade 0.5, 9 grade 1, and 1 grade 1.5 reactions at the 24-hour reading, and finally 8 grade 0.5 reactions at the 48-hour reading.
The test substance caused 2 grade 0.5 and 1 grade 1 reactions at the 15 minutes reading, 3 grade 0.5 and 1 grade 1 reactions at the 24-hour reading, and finally 1 grade 0.5 and 1 grade 1 reactions at the 48-hour reading.

Conclusion
The study authors conclude that a 25% aqueous dilution of a cosmetic rinse-off formulation containing 3.0% *cetrimonium chloride* caused some skin irritation when applied under occlusion for 24 hours.

Ref.: 50, 51

H. *Cetrimonium chloride* - single patch test 8

Date of study:	Mar-Apr 2001
Method:	Epicutaneous patch test
Subjects:	Healthy female volunteers
Group size:	11 female subjects
Test substances:	Diluted hair conditioners: 10% of FE0299.01, FE0300.01, FE0301.01, FE0302.01, FE0303.01 or FE0304.01, 5% of FE0305.02 or FE0306.01FE main components of FE0304.01: 13.3% *cetrimonium chloride* (30% active ingredient) 3% surfactant 1% thickener 1% softener
Dosages:	200 µl per Webril patch (2 cm diameter), fixed with Micropore tape
Exposure time:	24 hours
Scoring system:	**0** no apparent cutaneous involvement **0.5** faint, barely percetible erythema or slight dryness (glazed appearance) **1** faint but definite erythema, no eruptions or broken skin or no erythema but definite dryness; may have epidermal fissuring **1.5** well-defined erythema or faint erythema with definite dryness; may have epidermal fissuring **2** moderate erythema; may have a few papules or deep fissures, moderate-to-severe erythema in the cracks **2.5** moderate erythema with barely perceptible oedema or severe erythema not involving a significant portion of the patch (halo effect around the edges); may have a few papules or moderate-to-severe erythema **3** severe erythema (beet redness), may have generalized papules or moderate-to-severe erythema with slight oedema (edges well defined by raising) **3.5** moderate-to-severe erythema with moderate oedema (confined to patch area) or moderate-to-severe erythema with isolated eschar formation or vesicles **4** generalized vesicles or eschar formation or moderate-to-severe erythema and/or oedema extending beyond the area of the patch
GCP:	In compliance

The test substances were applied to the skin (upper arm) of the volunteers using Webril patches and adhesive Micropore tape. The test site was covered for 24 hours with a semi-occlusive dressing. Skin reactions were evaluated 24 and 48 hours after removal of test patches.

Results and conclusion
10% aqueous dilutions of a hair conditioner containing 4% active *cetrimonium chloride* caused no skin irritation when applied under occlusion for 24 hours.

Ref.: 52

I. *Cetrimonium chloride* - single patch test 9

Date of study:	Nov 1998
Method:	Epicutaneous patch test
Subjects:	Healthy female volunteers
Group size:	13 female subjects, 1 male subject
Test substances:	Diluted hair styling gels: 25, 50, 75 and 100% of 10000362 and 10000363; main components of 10000362: 2.1% alcohols 0.5% *cetrimonium chloride* 0.2% thickener 0.2% softener
Dosages:	200 µl non-woven cotton patch (approx. 4 cm²), fixed with tape
Exposure time:	24 hours
Scoring system:	**0** no apparent cutaneous involvement **0.5** faint, barely percetible erythema or slight dryness (glazed appearance) **1** faint but definite erythema, no eruptions or broken skin or no erythema but definite dryness; may have epidermal fissuring **1.5** well-defined erythema or faint erythema with definite dryness; may have epidermal fissuring **2** moderate erythema; may have a few papules or deep fissures, moderate-to-severe erythema in the cracks **2.5** moderate erythema with barely perceptible oedema or severe erythema not involving a significant portion of the patch (halo effect around the edges); may have a few papules or moderate-to-severe erythema **3** severe erythema (beet redness), may have generalized papules or moderate-to-severe erythema with slight oedema (edges well defined by raising) **3.5** moderate-to-severe erythema with moderate oedema (confined to patch area) or moderate-to-severe erythema with isolated eschar formation or vesicles **4** generalized vesicles or eschar formation or moderate-to-severe erythema and/or oedema extending beyond the area of the patch
GCP:	In compliance

The test substances (100, 75, 50, or 25% dilutions in water) were applied to the upper arm skin of the volunteers using non-woven cotton patches and adhesive tape. The test site was covered occlusively for 24 hours. Skin reactions were evaluated 24 and 48 hours after removal of test patches.

Results
25-100% aqueous dilutions of two styling gel formulations containing up to 0.5% *cetrimonium chloride* caused no skin irritation when applied under occlusion for 24 hours. The group average skin grades for all test articles fell within the lowest patch test grading scale category, with no apparent cutaneous involvement.

Ref.: 53

J. *Behentrimonium chloride* - single patch test 1

Date of study:	Sep 1998
Method:	Epicutaneous patch test

Subjects:	Healthy male and female volunteers, age 18-65 years without skin diseases
Group size:	5 male and 46 female subjects
Test substances:	Hair care product Fle 433833: aqueous solution containing 10.5% amodimethicone and *cetrimonium chloride* and trideceth 5.0% *behentrimonium chloride* 3.5% myristyl alcohol 3.0% glycerin 2.0% cetearyl alcohol 1.5% sodium PCA 1.5% cetyl esters 1.0% *cetrimonium chloride*
Dosages:	45 µl under Finn Chamber n°52268
Exposure time:	24 hours
Scoring system:	**0** no erythema, edema or vesicle formation **1** light erythema, edema or vesicle formation **2** moderate erythema, edema or vesicle formation **3** severe erythema, edema or vesicle formation
GCP:	No undersigned statement available

45 µl of the test substance was applied to the skin of the back of 51 volunteers using large Finn chambers. Deionised water was used as negative control substance. The test site was covered occlusively by adhesive plaster for 24 hours. Skin reactions were evaluated 15 minutes and 24 and 48 hours after removal of test patches.

Results
The test article caused slight erythema in 3 subjects at the 15 minutes reading, slight erythema in two subjects and dryness in a third at 24 hours, and slight erythema in one subject and dryness in a second at 48 hours. Water alone caused slight erythema in 2 subjects at the 15 minutes and 24 hour readings.

Conclusion
The study authors conclude that 5.0% *behentrimonium chloride* in a cosmetic formulation produced some skin irritation when applied under occlusion for 24 hours.

Ref.: 55

K. *Behentrimonium chloride* - additional studies

Finally, the submission contains two summaries of single patch studies with *behentrimonium*-containing formulations.
The first study was performed in 1997 on 44 female and 6 male human volunteers with a hair conditioner containing 5% *behentrimonium chloride* and an unknown concentration of *cetrimonium chloride*. 50 µl of test substance was applied to the skin of the back using large Finn chambers. An empty chamber served as negative control. The contact time was 48 hours and skin reactions were evaluated 30 minutes after removal of test patches.
The study authors conclude that the formulation was well-tolerated by human skin.

The second report is dated 1986 and summarizes the results of a single patch study on 50 human volunteers with a 1% *behentrimonium chloride* solution. Contact time was 24 hours, but the applied dose is not mentioned. Assessment of skin irritation 48 and 72 hours after patch removal is reported to prove the test substance non-irritating to the skin of human volunteers.

Ref.: 56, 57

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

The submission of 2007 provided a detailed discussion of the results of the human skin irritation studies. The applicant emphasized that the human patch studies were performed under exaggerated exposure conditions (e.g. under (semi-)occlusion for 24 hours) and with the inclusion of subjects with sensitive or compromised skin. Summarising the results for CTAC, they concluded that up to 2.5% the compound produces no to mild skin irritation, even in the more vulnerable population groups.

In addition, the applicant presented a recalculation of the dose level of the substance as the amount applied per square cm, which they consider a more relevant dose metric for local effects (see table below).

CTAC concentration (%, w/w)	**CTAC skin area dose (µg/sq-cm)**	**Volunteer skin type**	**Application conditions**	**Exposure duration**	**Results**
1.6%	80	Healthy or sensitive skin or allergic volunteers	Semi-occlusion	48 hours	No skin irritation [47]
0.4%	255	Healthy skin	Semi-occlusion	24 hours	No skin irritation [52]
0.5%	500	Healthy skin	Occlusion	24 hours	No skin irritation [53]
1%	500	Healthy or sensitive or eczematous skin	Semi-occlusion	24 hours	No skin irritation [49]
1% (1)	619	Healthy	Occlusion	24 hours	No skin irritation [48]
0.75%	960	Healthy, dry or seborrhoic skin	Occlusion	24 hours	Minimal skin irritation [50]
2.5%	1000	Healthy or sensitive skin	Semi-occlusion	1 hour	Minimal to no skin irritation [45]
3.52%	1408	Healthy skin	Occlusion	24 hours	Slight to moderate skin irritation [44]
1.76%	-	Healthy or atopic	Occlusion	24 hours	No skin irritation [46]

(1) Following a comment issued in the opinion SCCP/0000/00, the actual concentration used in this study was checked and confirmed to be 1% active CTAC

- Not calculable

For the intended uses in cosmetic products, the following values were reported to be obtained:

- leave-on hair products (1% CTAC):
  700 cm² at 500 mg/application: 0.71 mg formulation /cm² i.e. 7 µg CTAC/cm²
- rinse-off hair products (2.5% CTAC):
  700 cm² at 80 mg/application: 0.11 mg formulation /cm² i.e. 2.9 µg CTAC/cm²
- leave-on face cream product (0.5% CTAC):
  565 cm² at 800 mg/application: 1.42 mg formulation /cm² i.e. 7 µg CTAC/cm²

As the human patch tests were all performed with CTAC amounts of 80 to > 1000 µg/cm², and effects were only observed at skin area doses of 960 µg CTAC/cm² and above, but not at skin area doses up to 619 µg CTAC/cm², the applicant considers that cosmetic products containing CTAC at the requested use conditions and concentrations are not expected to cause any skin irritation. This view is supported by the low reporting rate of undesirable effects as detailed in a report on post-marketing surveillance on P72-containing cosmetic products (see 3.3.12).

The same calculation and reasoning was presented for *behentrimonium chloride* at 3% in leave-on hair and face cream products and at 5% in rinse-off hair products, leading to maximum dose levels of 15 µg BTAC/cm².

In response to the previous assessment of SCCP that *no concentration- or contact time-dependency was noticed in the human patch tests*, the applicant claimed that, when the skin area dose is plotted versus the observed effects, a clear dose-relationship is observed.

3.3.11.2 Repeated insult patch test on human volunteers (taken from SCCP/1087/07)

In the Cosmetic Ingredient Review (CIR) on *cetrimonium chloride*, *cetrimonium bromide* and *steartrimonium chloride*, four repeated insult patch tests in human volunteers with *cetrimonium chloride* are described (performed 1983-1984). Concentrations ranged from 0.02 to 0.25% and in total more than 500 volunteers (about 100 per study) were occlusively patched with test formulation for 24 hours 3 times/week for 3 weeks and subsequently challenged 7 days later. On the basis of these studies, the CIR experts concluded that 0.25% (the highest concentration tested) of *cetrimonium chloride* showed to be a mild irritant, but no skin sensitiser.

Ref.: 54

Behentrimonium chloride - repeated insult patch test

Date of study:	Feb-Mar 2004
Method:	Human repeated insult patch study
Subjects:	Healthy male and female volunteers, age 18-70 years (mean 44 years) without skin diseases
Group size:	31 male and 81 female subjects, of which 104 completed the study
Test substances:	Hair conditioner FE0394.01: main components: 3.4% *behentrimonium chloride* 6.8% alcohols 4.2% softener
Dosages:	200 mg on 2 x 2 cm Webril patches
Exposure time:	24 hours
Scoring system:	**-** no reaction **?** minimal or doubtful response, slightly different from surrounding normal skin **+** definite erythema, no oedema **++** definite erythema, definite oedema **+++** definite erythema, definite oedema and vesiculation

GCP: In compliance

The test substance was applied to the upper arm or the back of the human volunteers using 2 x 2 cm Webril patches and covered semi-occlusively using hypoallergenic tape. The subjects removed the patches after 24 hours. Patches were applied on Mondays, Wednesdays and Fridays for three consecutive weeks. The skin sites were evaluated after 48 hours (for Monday and Wednesday patches) or after 72 hours (for Friday patches) for skin reactions. After completion of this induction phase, a resting period of 10-15 days was respected. For challenge, identical patches were applied to sites previously unexposed to the test material. The patches were removed by the subjects after 24 hours and sites graded after additional 24 and 48 hour periods.

Results
One subject showed a definite erythema and definite oedema after the first induction patch and test material application was discontinued in this subject. In three subjects, the test material produced minimal/doubtful responses at a few induction readings. In two subjects some readings revealed minimal/doubtful responses or definite erythema, no oedema. These very slight irritation reactions did not tend to increase in frequency or severity with the number of patches applied. No skin reactions were observed in response to the challenge patch.

Conclusion
The study authors conclude that 3.4% *behentrimonium chloride* in a cosmetic formulation was confirmed as not causing skin sensitisation when assessed in a repeated human insult patch test. Moreover the application of semi-occlusive patches for 24 hours did not produce skin irritation.

Ref.: 58

Comment
As mentioned in section 3-4.11 of the SCCP Notes of Guidance (SCCP/1005/06), predictive human sensitisation tests are considered unethical.

3.3.12 Special investigations (submission of July 2007)

Report on post-marketing surveillance data

The presented report is based on the COLIPA Guidelines on the management of undesirable event reports (Ref. B). It covers 18 months of post-marketing experiences with CTAC, BTAC and/or STAC-containing hair products of 5 cosmetic companies. Due to the issues raised by the SCCP in 2007, the focus of the study was placed on skin irritative effects.

In the report, 'skin irritation' is defined as *erythema and/or sensorial manifestations such as stinging, itching, burning sensation which in some cases may be accompanied by small papules and/or transient superficial scales or crusts, appear a few minutes to a few hours after product application. They are circumscribed to the area of the application and usually disappear quickly, within minutes, hours or a few days without leaving any sequelae.*

The available information on the participating companies and the type of products involved is given in the following table:

Companies (reporting geographical zones)	Rinse-off hair conditioners	Leave-on hair conditioners	Hair styling products
Company 1 (FR, DE, GB, IT)	CTAC (≤ 2.50%) and/or BTAC (≤ 4.94%)	CTAC (≤ 0.72%) and/or BTAC (≤ 2.37%)	CTAC (≤ 0.50%) and/or BTAC (≤ 2.96%)
Company 2 (FR, DE, GB, ES, IT)	CTAC (≤ 2.50%) and BTAC (≤ 4.94%)	CTAC (≤ 0.72%) and BTAC (≤ 2.37%)	CTAC (≤ 0.50%) and BTAC (≤ 2.96%)

Company 3 (DE)	CTAC (≤ 2.50%) and/or BTAC (≤ 4.94%) and/or STAC (≤ 1.90%)	CTAC (≤ 0.72%) and/or BTAC (≤ 2.37%) and/or STAC (≤ 0.30%)	CTAC (≤ 0.50%) and/or BTAC (≤ 2.96%) and/or STAC (≤ 0.40%)
Company 4 (FR, GB)	CTAC (≤ 2.50%) and/or BTAC (≤ 4.94%)	CTAC (≤ 0.72%) and/or BTAC (≤ 2.37%)	CTAC (≤ 0.50%) and/or BTAC (≤ 2.96%)
Company 5 (DE, GB, DK, GR, SE, NL)	CTAC (≤ 2.50%) and/or BTAC (≤ 4.94%)	CTAC (≤ 0.72%) and/or BTAC (≤ 2.37%)	CTAC (≤ 0.50%) and/or BTAC (≤ 2.96%)

Reporting was performed during 18 months for:
- undesirable effects compatible with skin irritation (causality assessment likely/very likely)
- serious[2] undesirable effects compatible with skin irritation (causality assessment likely/very likely)

and was presented as the number of (serious) undesirable effects per million units sold.

The results of the study show that overall, the reporting rates were low (< 0.2 events per million units sold). No serious undesirable effects were reported during the period covered by the report.

Comment
Although the study appears to generate relevant information, compiled by different companies, no details are given on the exact protocol followed or on the exact number of people involved (raw data are missing). The only indication towards a large-scale study is the expression of the results as the number of (serious) undesirable effects *per million units sold*.

3.3.13 Safety evaluation (including calculation of the MoS)

The submission contains the following table summarising the exposure to alkyl (C_{16}, C_{18}, C_{22}) trimethylammonium chlorides from:

a) use as a preservative at a maximum concentration of 0.1%, and

b) the proposed use of
   - *cetrimonium chloride* and *steartrimonium chloride* in rinse-off hair care products at up to 2.5%, leave-on hair care products at up to 1%, and leave-on facial cream products at up to 0.5%.
   - *behentrimonium chloride* in rinse-off hair care products at up to 5%, leave-on hair care products at up to 3%, and leave-on facial cream products at up to 3%.

It was suggested by the applicant to perform the calculation of the MoS with the proposed uses of *behentrimonium chloride* because this constitutes the worst case scenario due to its higher use concentrations compared to *cetrimonium chloride* and *steartrimonium chloride*.

Product category	Type of cosmetic product	Daily exposure to product (g/day)	Max. conc. of behentrimonium chloride (%)	Exposure to behentrimonium chloride (g/day)

[2] A 'serious' adverse event/effect is any event/effect that:
- is fatal
- is life-threatening
- is permanent/significantly disabling
- requires or prolongs hospitalization
- causes congenital anomaly
- requires intervention to prevent permanent impairment or damage

Product category	Type of cosmetic product	Daily exposure to product (g/day)	Max. conc. of behentrimonium chloride (%)	Exposure to behentrimonium chloride (g/day)
Leave-on	Facial cream	1.6	3	0.048
	General purpose cream	2.4	0.1	0.0024
	Body lotion	8.0	0.1	0.008
	Antiperspirants	0.5	0.1	0.0005
	Hair styling	1.0	3	0.03
	Eye make-up	0.02	0.1	0.00002
	Mascara	0.025	0.1	0.000025
	Lipstick	0.04	0.1	0.00004
	Eyeliner	0.005	0.1	0.000005
Rinse-off	Make-up remover	0.5	0.1	0.0005
	Hair conditioner	0.04	5	0.002
	Shampoo	0.08	5	0.004
	Shower gel	0.1	0.1	0.0001
	Mouthwash	3.0	0.1	0.003
	Toothpaste	0.48	0.1	0.00048
TOTAL				**0.09907**

CALCULATION OF THE MARGIN OF SAFETY

Maximum amount of ingredient applied daily		=	99.1 mg/day
Typical human body weight		=	60 kg
Maximum absorption through the skin (*in vivo* rat study *laurtrimonium bromide*)		=	3.15%
Systemic exposure dose (SED)	(99.1 x 3/100) / 60	=	0.052 mg/kg bw/day
No observed adverse effect level (12m-oral-rat)	NOAEL	=	10 mg/kg bw/day
Margin of Safety	**NOAEL / SED**	**=**	**192**

3.3.14 Discussion

Some shortcomings with regard to the data on identification, stability and physico-chemical properties of the three quaternary ammonium compounds under study, are mentioned as ***General comments on Section 3.1***. Nevertheless, they are not considered crucial for the final safety evaluation of P72.

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.

No adequate *in vitro* dermal absorption study is available, in particular no study to evaluate leave-on products. The presented assay for *cetrimonium chloride* was clearly performed under rinse-off conditions only and the test substance was not clearly identified. A detailed list of shortcomings is mentioned under 3.3.4.1.
The described *in vivo* study in the rat with *laurtrimonium bromide*, a shorter chain structure analogue of *cetrimonium chloride*, showed that under leave-on conditions, there was an

increase in excretion of radioactivity as a function of time 2 days after dermal application, whereas this was not the case under rinse-off conditions, when the skin was rinsed after 30 minutes of dermal exposure. The increase was attributed by the study authors to a slight skin damage caused by the test substance. The percutaneous absorption did not exceed 3.15% under leave-on conditions. The SCCS acknowledges that rat skin usually is more permeable than human skin and that the figure of 3.15% may represent an overestimation. However, it must not be ignored that none of the dermal absorption studies presented were performed according to the criteria set out by the SCCS. There is no study with any of the compounds under consideration. Therefore the use of the 3.15% value is a compromise as opposed to the use of 100% dermal absorption due to the absence of any assay with the actual quaternary ammonium compounds under study.

A toxicokinetic study indicated that ^{14}C-labeled 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. Viewing the date of the study (1975), the low number of experimental animals and the low (and single) dosage tested, the study was not considered sufficiently sound to be at the basis of a re-calculation of the MoS.

A 28-day oral repeated dose toxicity study revealed *cetrimonium chloride* to cause significant forestomach and stomach changes caused by its irritative effects. Slight weight changes in adrenals and spleen and an increase in alanine transaminase (ALT) levels were regarded as possible signs of systemic toxicity and occurred at the highest dosage level tested (300 mg/kg bw/day). 100 mg/kg bw/day was regarded as the NOEL value for Dehyquart A-CA, corresponding to 24-26 mg *cetrimonium chloride*/kg bw/day.

A 28-day dermal repeated dose toxicity study revealed that the only tested dosage of 10 mg *cetrimonium chloride*/kg bw/day caused no systemic toxicity, but it did cause a number of local effects on the skin at the only tested dose of 0.5%.
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, which can be considered to be the NOAEL of *cetrimonium bromide*.

The SCCS has chosen the chronic toxicity oral NOAEL of 10 mg/kg/day as the adequate value to be used in the calculation of the MoS for the following reasons:

- The dermal study with the rabbit appeared to be the most representative as far as the route of administration was concerned. Nevertheless it failed to identify a systemic NOAEL value, as 1) only one dosage level was tested and 2) the observed effects noted at that dosage level were solely of irritative nature.
- The chronic study is quite old, but its description is adequate. Moreover, the NOAEL is backed up by the NOEL of a more recent, GLP-compliant, 28-day study generating a NOEL of 24 mg/kg/day.
- The results for *cetrimonium bromide* are considered representative for *cetrimonium chloride*, as the SCCS agrees with the proposed read-across approach for the three compounds under study and their salts.

Genotoxicity
The presented mutagenicity studies (Bacterial reverse mutation assays for the three compounds under study and a chromosome aberration test and *in vitro* Syrian hamster cell transformation assay for *cetrimonium chloride* only) were all negative. Testing was limited to low concentrations due to the high cytotoxicity.

Developmental toxicity
Dermal developmental toxicity studies with *cetrimonium chloride* in the rabbit and the rat revealed dose-dependent irritative 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.

Local toxicity
As is the case for the majority of quaternary ammonium compounds, concentrated *cetrimonium chloride*, *steartrimonium chloride* and *behentrimonium chloride* are corrosive 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. When kept in contact with the skin for 4 hours, the test compounds were shown to be severely irritating down to concentrations of about 25%. After 3 minutes of skin contact, an 8% dilution of *behentrimonium chloride* caused some skin irritation and a 3% solution was shown to be non-irritant. The contact time of 3 minutes was considered relevant for mimicking rinse-off use conditions, but does not provide adequate data to predict the irritant potential in a leave-on setting. Dermal effects were noted in a 28-day dermal study with the rabbit at 10 mg/kg bw/day.

The submission 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. No concentration-or contact time-dependency was noticed. According to the applicant, concentration-dependent increase in skin irritation is present when the applied dose is expressed as amount per cm^2. Although this may be accurate, a comparison between the results of the presented patch tests is not evident, as the formulations used are diverse. Nevertheless 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 irritative effects under their intended use.

The three compounds appeared to cause irreversible ocular damage in the rabbit eye when applied at concentrations above 8%. Diluted to 5%, *behentrimonium chloride* caused persistent conjunctival irritation while a 3% dilution showed some irritating effects to the eye.

The submission 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. Mostly, the individual values of the positive controls, which would be useful to place some doubtful results into perspective, are lacking. However, viewing the fact that the test substances are known to be corrosive, the limited positive responses noted in the challenge phases, are considered to be the result of an irritative effect rather than to represent a sensitising potential. Therefore, there appears to be no reason to repeat the sensitisation studies or to consider that these quaternary ammonium compounds as sensitising to the skin.

Finally, two repeated insult patch tests on human volunteers show *cetrimonium chloride* to be non-sensitising at concentrations up to 3.4%. The irritative/non-irritative character, however, was not the same in both tests.

4. 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 (C_{16}), *steartrimonium chloride* (C_{18}):

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 (C_{22}):

Rinse-off hair care products up to	5.0%
Leave on hair care and facial cream products up to	3.0%

5. MINORITY OPINION

Not applicable

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