EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

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

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

Alkyl (C16, C18, C22) trimethylammonium chloride

For other uses than as a preservative

Adopted by the SCCP
by written procedure on date 17 March 2006

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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 (C16, C18, C22) trimethylammonium chloride, (COLIPA[1] P72) bears the symbol (+) and can therefore be used in cosmetics at higher concentrations, as long as they are not employed as preservatives. Alkyl (C16, C18, C22) trimethylammonium chloride is currently authorized as 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 SCCP.

The European Commission has received a submission from industry proposing that Alkyl (C16, C18, C22) trimethylammonium chloride can be used for non-preservative purposes as specified in the safety dossier:

cetrimonium chloride (C16), steartrimonium chloride (C18):

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

behentrimonium chloride (C22):

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

[1] COLIPA – European Cosmetics Toiletry and Perfumery Association

2. TERMS OF REFERENCE

1. *On the basis of provided data the SCCP is asked to assess the risk to consumers when Alkyl (C16, C18, C22) trimethylammonium chloride is used in cosmetic products for non-preservative purposes as specified above.*

2. *Does the SCCP recommend any further restrictions or conditions for its use in the cosmetic products?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

a) cetrimonium chloride
b) steartrimonium chloride
c) behentrimonium chloride (INCI name)

3.1.1.2. Chemical names

a) C16-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) C18-alkyltrimethylammonium chloride
Stearyltrimethylammonium chloride
Stearyl trimethyl ammonium chloride
N-octadecyltrimethylammonium chloride
1-octadecanaminium, N,N,N-trimethyl-, chloride
N,N,N-trimethyl-1-octadecanaminium chloride

c) C22-alkyltrimethylammonium chloride
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

Trade names:

a)
Arquad 16-29
Arquad 16-25LO
Dehyquart A-CA
Genamin CTAC
Incroquat CTC30
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

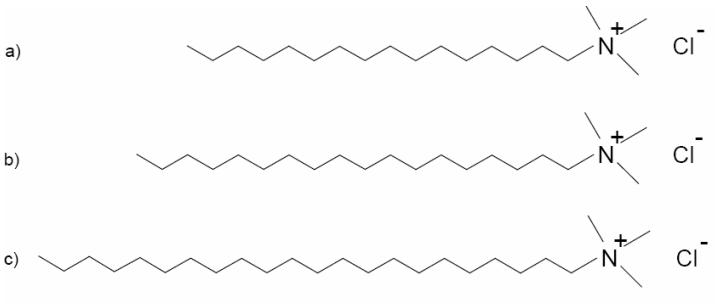
COLIPA n: P 72
a) C16
b) C18
c) C22

3.1.1.4. CAS / EINECS number

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

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

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

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

3.1.2. Physical form

a) liquid (24-30% aqueous solution)
b) liquid (49-51% aqueous solution), or solid (78-82% with residual water/isopropanol)
c) solid (77-84% with residual isopropanol)

3.1.3. Molecular weight

Molecular weight : a) 320.00
b) 348.05
c) 404.16

3.1.4. Purity, composition and substance codes

a) Cetrimonium chloride: no data

b) Steartrimonium chloride: no data

c) Behentrimonium chloride: no data

3.1.5. Impurities / accompanying contaminants

No data

3.1.6. Solubility

a) Cetrimonium chloride: no data

b) Steartrimonium chloride: no data

c) Behentrimonium chloride: no data

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: no data

3.1.8. Additional physical and chemical specifications

a) Cetrimonium chloride (aqueous solution, typical content 29%)

Melting point:	-5 °C
Boiling point:	no data
Flash point:	no data
Vapour pressure:	no data
Density:	970 kg/m³
Viscosity:	no data
pKa:	no data
Refractive index:	no data

b) Steartrimonium chloride (typical content 79%, with 21% isopropanol)

Melting point:	60-70 °C
Boiling point:	no data
Flash point:	no data
Vapour pressure:	no data
Density:	no data
Viscosity:	no data
pKa:	no data
Refractive index:	no data

c) Behentrimonium chloride (typical content 80%, with 20% isopropanol)

Melting point:	no data
Boiling point:	no data
Flash point:	no data
Vapour pressure:	no data
Density:	900 kg/m³
Viscosity:	no data
pKa:	no data
Refractive index:	no data

General comments on analytical and physico-chemical characterisation

The following properties do not or poorly comply with the basic requirements for a proper characterisation:

* No data is submitted on the impurities, log P_{ow}, pKa; limited data is submitted on purity.

3.2. Function and uses

Cetrimonium chloride, steartrimonium chloride, and behentrimonium chloride are currently authorized as preservatives up to a maximum concentration of 0.1% and listed on Annex VI of the Cosmetics Directive 76/768/EEC, Part 1, No. 44, under the entry "Alkyl (C12-C22) trimethyl ammonium, bromide and chloride (+)".

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

a) Cetrimonium chloride

Guideline:	OECD 401
Species/strain:	Wistar rat
Group size:	5 rats/sex/dose
Test substance:	Genamin CTAC (29% cetrimonium chloride)
Batch:	E06112547
Dose:	630 (only females), 1000, 1600, 2500, 3150 (only males), 4000 (only males) mg/kg bw
GLP:	in compliance

Results

Male rats	LD50:	2970 mg/kg bw
Female rats	LD50:	1550 mg/kg bw
Male and female rats	LD50:	2410 mg/kg bw

Conclusion
Based on the LD_{50} value of 1550 mg/kg bw in females, Genamin CTAC is considered to be moderately toxic under the experimental conditions.

Ref.: 6

Guideline:	Directive 92/069/EEC, part B, methode B.1b
Species/strain:	Sprague-Dawley rat
Group size:	5 rats/sex/dose
Test substance:	Quartamin 60W25 (25% cetrimonium chloride)
Batch:	3-4
Dose:	500 and 2000 mg/kg bw
GLP:	in compliance

Quartamin 60W25, dissolved in water, was administered once by oral gavage at doses of 500 and 2000 mg/kg bw to groups of 5 male and 5 female rats. The animals were observed for 14 days.

Lethality:

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

Conclusion
The LD_{50} was set at about 2000 mg/kg bw under the experimental conditions.

Ref.: 7

b) Steartrimonium chloride

Guideline: OECD 401
Species/strain: Sprague-Dawley rat
Group size: 5 rats/sex/dose
Test substance: Quartamin 86W (28% actives; steartrimonium chloride:cetrimonium chloride 80:20)
Batch: 1841
Dose: 2000 mg/kg bw
GLP: in compliance

Quartamin 86W, dissolved in water, was applied once by oral gavage at doses of 2000 mg/kg bw to groups of 5 male and 5 female rats. Animals were observed for 14 days.

Conclusion
The LD_{50} was considered to be > 2000 mg/kg bw under the experimental conditions.

Ref.: 8

Guideline: OECD 401
Species/strain: Wistar rat
Group size: 5 rats/sex/dose
Test substance: Genamin STAC (79.2% steartrimonium chloride in isopropanol)
Batch: 1061969521
Dose: 630 (only females), 800, 1250 (only females), 2000 mg/kg bw
GLP: in compliance

Genamin STAC, dissolved in water, was applied once by oral gavage at doses of 630, 800, 1250, and 2000 mg/kg bw to groups of 5 male and 5 female rats. Animals were observed for 14 days.

Lethality:

Dose (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

Except for one male and one female that were found dead on days 4 and 7, respectively, all deaths occurred within the first day. Female rats appear to be more susceptible than male rats to effects of acute oral doses of Genamin STAC.

Conclusion
The LD_{50} was set at about 700 mg/kg bw under the experimental conditions.

Ref.: 9

3.3.1.2. Acute dermal toxicity

No data

3.3.1.3. Acute inhalation toxicity

No data

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

a) Cetrimonium chloride

Guideline: OECD 404
Species/strain: New Zealand albino rabbit
Group size: 3
Test substance: Genamin CTAC (29% cetrimonium chloride)
Batch: E06112547
Dose: 0.5 ml
GLP: in compliance

A patch with 0.5 ml Genamin CTAC was placed on the shaved skin of three 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 30-60 minutes, 24 hours, 48 hours, 72 hours, 7 days, 14 days and 21 days after patch removal according to the scoring system of the test guideline.
No mortality or systemic clinical effects were observed. Slight erythema and oedema were observed at 30 minutes after patch removal. At 24, 48 and 72 hours, 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
Genamin CTAC is irritating to skin when tested at an active concentration of 29 %.

Ref.: 10

Guideline:	OECD 404
Species/strain:	New Zealand albino rabbit
Group size:	3 males
Test substance:	Quartamin 60W25 (25% cetrimonium chloride)
Batch:	3-4
Dose:	0.5 ml
GLP:	in compliance

A patch with 0.5 ml Quartamin 60W25 was placed on the shaved skin of three male 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 30-60 minutes, 24 hours, 48 hours, 72 hours and additionally 7 and 14 days after patch removal according to the scoring system of the test guideline.
No mortality or systemic 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 rabbits, respectively. The mean score values of the 24, 48 and 72-hour readings were 3.0 for erythema and 1.9 for oedema.

Conclusion
Quartamin 60W25 is irritating to skin when tested at an active concentration of 25 %.

Ref.: 11

b) Steartrimonium chloride

Guideline:	OECD 404
Species/strain:	New Zealand albino rabbit
Group size:	3
Test substance:	Quartamin 86W (28% actives; steartrimonium chloride:cetrimonium chloride 80:20)
Batch:	1841
Dose:	0.5 ml of 20% v/v and 2% v/v in distilled water
GLP:	in compliance

A patch with 0.5 ml of 20 % and 2 % in distilled water Quartamin 86W was placed on the shaved skin of three rabbits and covered with semi-occlusive dressing for 4 hours.

A patch with 0.5 ml test material was placed on the shaved skin of three 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 cotton swab soaked in distilled water. Skin reactions were evaluated 1, 24, 48, and 72 hours and additionally 7 and 14 days after patch removal according to the scoring system of the test guideline.
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 systemic 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
Under the experimental conditions, a 20 % solution of Quartamin 86W in water was irritating to the skin, while a 2 % solution was non-irritating.

Ref.: 12

Guideline:	/
Species/strain:	New Zealand albino rabbit
Group size:	1 female exposed for 4 hours, 3 females exposed for 3 minutes
Test substance:	Genamin STAC (79.2% steartrimonium chloride)
Batch:	1061969521
Dose:	0.5 g
GLP:	in compliance

A patch with 0.5 ml Genamin STAC was placed on the shaved skin of four rabbits and covered with semi-occlusive dressing. After the respective application times, 3 minutes using three rabbits and 4 hours using one rabbit, patches were removed and the area was wiped with a cellulose tissue. Skin reactions were evaluated 30-60 minutes, 24 hours, 48 hours, 72 hours after patch removal, and additionally after 7, 14 and 22 days for the 4-hour treatment according to the scoring system of the test guideline. 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
Genamin STAC caused irritation at an active concentration of 79.2% when applied for 4 hours on 1 animal. It was non-irritating when applied for only 3 minutes.

Comment
An exposure time of 3 minutes is irrelevant for skin irritation testing.

Ref.: 13

Behentrimonium chloride

Guideline:	/
Species/strain:	New Zealand albino rabbit
Group size:	3 females
Test substance:	Behentrimonium chloride (80% behentrimonium chloride)
Batch:	0040242
Dose:	0.5 ml of 10% solution in vehicle (0.5% methylcellulose in purified water)
GLP:	in compliance

A gauze pad with 0.5 ml Behentrimonium chloride was placed on the shaved skin of three female 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 additionally 5 days after patch removal according to the scoring system of the test guideline. 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
A 10% aqueous solution of the test substance (corresponding to 8% active behentrimonium chloride) produced minimal irritation when applied for 3 minutes under the experimental conditions.

Comment
An exposure time of 3 minutes is irrelevant for skin irritation testing.

Ref.: 14

Guideline:	/
Species/strain:	New Zealand albino rabbit
Group size:	3 males
Test substance:	Behentrimonium chloride (80% behentrimonium chloride)
Batch:	20010400390104
Dose:	0.5 ml of 6.25% solution in vehicle (0.5% methylcellulose in purified water)
GLP:	in compliance

A gauze pad with 0.5 ml Behentrimonium chloride 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 according to the scoring system of the test guideline. 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
A 6.25% aqueous solution of the test substance (corresponding to 5% active behentrimonium chloride) was non-irritating to skin when applied for 3 minutes on the experimental condition.

Comment
An exposure time of 3 minutes is irrelevant for skin irritation testing.

Ref.: 15

3.3.2.2. Mucous membrane irritation

a) Cetrimonium chloride

Guideline:	OECD 405
Species/strain:	New Zealand albino rabbit
Group size:	3 (sex not stated in report)
Test substance:	Genamin CTAC (29% cetrimonium chloride)
Batch:	E06112547
Dose:	0.1 ml
GLP:	in compliance

An examination of the eyes using fluorescein solution and UV light was performed 24 hours before start of the experiment to ensure that only rabbits without any cornea damage were used in the test. A volume of 0.1 ml of the Genamin CTAC 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 according to the scoring system of the test guideline. 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. 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
Genamin CTAC, at an active test concentration of 29 % causes irreversible ocular damage.

Ref.: 16

Guideline:	OECD 405
Species/strain:	New Zealand albino rabbit
Group size:	3 males
Test substance:	Quartamin 60W25 (25% cetrimonium chloride)
Batch:	3-4
Dose:	0.1 ml
GLP:	in compliance

A volume of 0.1 ml of the Quartamin 60W25 was placed into the conjunctival sac of the right eye of each animal. 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. 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
Quartamin 60W25, at an active test concentration of 25%, causes irreversible ocular damage.

Ref.: 17

Guideline:	/
Species/strain:	New Zealand albino rabbit
Group size:	3 males, 3 females
Test substance:	Shampoo formulation containing 8% cetrimonium chloride (25% active)
Batch:	/
Dose:	0.01 ml
GLP:	in compliance

A volume of 0.01 ml of the test substance was applied to the central cornea of the right eye of each animal. The untreated left eye served as control. The effects on ocular tissues (cornea, conjunctiva and iris) are graded using a scoring scale. The readings are made until responses are cleared. The individual tissue grades are usually weighted and combined into a Maximum Average Score (MAS; 0 to 110 scale), which gives an indication of the average level of response on the day of the highest average reading. The rate of reversal of rabbit eye responses is reported as median days to clear. Corneal opacity was observed in 3/6 animals. Iritis was found in 4/6 rabbits and conjunctival irritation was evident in 6/6 animals. The maximal average score (MAS) of the tested formulation was 20.8 (day 3) and the corresponding median days to clear were 15.

Conclusion
The study is irrelevant

Ref.: 18

b) Steartrimonium chloride

Guideline:	OECD 405
Species/strain:	New Zealand albino rabbit
Group size:	3 (sex not stated in report)
Test substance:	Quartamin 60W (28% actives; steartrimonium chloride:cetrimonium chloride 80:20)
Batch:	1841
Dose:	0.1 ml of 2% v/v in distilled water
GLP:	in compliance

A volume of 0.1 ml of the Quartamin 86W 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 article according to the scoring system of the test guideline. When ocular reactions were noted at 72 hours, additional examinations were performed at 7 and 14 days after the instillation. No corneal opacity or iritis was found at any reading 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 ceased. 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
Quartamin 60W, as a 2% solution in distilled water (0.56% active steartrimonium/cetrimonium chloride), produced transient conjunctival irritation.

Ref.: 19

c) Behentrimonium chloride

Guideline:	OECD 405
Species/strain:	New Zealand albino rabbit
Group size:	3 males
Test substance:	Behentrimonium chloride (80% behentrimonium chloride)
Batch:	0040242
Dose:	0.1 ml of 10% solution in vehicle (0.5% methylcellulose in purified water)
GLP:	in compliance

A volume of 0.1 ml of the Behentrimonium chloride 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 22 after the instillation. At 48 hours and thereafter, additional examinations using fluorescein solution were performed. Corneal opacity was observed in 1 of 3 rabbits with grade 2 at 24 and 48 hours (with 1/4 to ½ 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
A 10% test substance solution (corresponding to 8% active behentrimonium chloride) in 0.5% methylcellulose in purified water caused irreversible ocular damage.

Ref.: 20

Guideline:	OECD 405
Species/strain:	New Zealand albino rabbit
Group size:	3 males
Test substance:	Behentrimonium chloride (80% behentrimonium chloride)
Batch:	20010400390104
Dose:	0.1 ml of 6.25% solution in vehicle (0.5% methylcellulose in purified water)
GLP:	in compliance

A volume of 0.1 ml (6.25% solution in vehicle - 0.5 % methylcellulose in purified water) of the Behentrimonium chloride 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. 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, 7 and 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
A 6.25% test substance solution (corresponding to 5% active behentrimonium chloride) in 0.5% methylcellulose in purified water caused conjunctival irritation, after a single administration followed by a rinse.

Ref.: 21

Guideline:	OECD 405
Species/strain:	New Zealand albino rabbit
Group size:	3 males
Test substance:	Behentrimonium chloride (80% behentrimonium chloride)

Batch: 20010400390104
Dose: 0.1 ml of 3.75% solution in vehicle (0.5% methylcellulose in purified water)
GLP: in compliance

A volume of 0.1 ml of the Behentrimonium chloride 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. Ocular reactions were noted at 72 hours. At 24 hours, examinations using fluorescein solution were performed. Corneal opacity or iritis were not 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
A 3.75% test substance solution (corresponding to 3% behentrimonium chloride) in 0.5% methylcellulose in purified water caused transient conjunctival irritation.

Ref.: 22

3.3.3. Skin sensitisation

a) Cetrimonium chloride

Maximisation (Magnusson and Kligman) Test

Guideline: OECD 406
Species/strain: Dunkin-Hartley albino Guinea pigs
Group size: 10 males and 10 female in test group, 5 males and 5 female in control group
Test substance: Quartamin 60W25 (25% cetrimonium chloride)
Batch: 3-4
Dose: intradermal induction: 0.125% test substance in both vehicles, physiological saline and Freund's complete adjuvant (FCA)
dermal induction: 3% test substance in distilled water
challenge: 0.5% test substance in distilled water
GLP: in compliance

The test group consisted of 10 male and 10 female Guinea pigs and 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 suprascapular 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 result is unclear. The observed skin reactions to the test substance occurred at slightly higher incidence in the animals of the treatment group than of the control group. Reactivity to the vehicle was similar in animals of both groups.

Comment
The challenge concentration is lower than the requested use concentration for leave-on and rinse-off hair care products.

Ref.: 23

Buehler test

Guideline:	OECD 406
Species/strain:	Pirbright white Guinea pigs
Group size:	30 females in test group, 10 females in control group
Test substance:	Genamin CTAC (30% cetrimonium chloride)
Batch:	E06178641
Dose:	dermal induction: 4% test substance in distilled water challenge: 1% test substance in distilled water
GLP:	in compliance

The test group consisted of 30 female Guinea pigs, 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
Genamin CTAC (30% active) when diluted to 4% (induction) and 1% (challenge): sensitization was not shown under the experimental conditions described.

Comment
The challenge concentration is lower than the requested use concentration for rinse-off hair care products.

Ref.: 24

b) Steartrimonium chloride

Maximisation (Magnusson and Kligman) Test

Guideline:	OECD 406
Species/strain:	Dunkin-Hartley albino Guinea pigs
Group size:	10 males test group, 5 males in control group
Test substance:	Quartamin 86W (28% actives; steartrimonium chloride: cetrimonium chloride 80:20)
Batch:	1841
Dose:	intradermal induction: 0.1% v/v test substance in both vehicles, distilled water and Freund's complete adjuvant (FCA) dermal induction: 5% v/v test substance in distilled water challenge: 10% v/v and 5% v/v test substance in distilled water
GLP:	in compliance

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 and 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
Quartamin 86W produced no skin sensitisation under conditions of the test. However, the topical induction concentration (5%) was probably too low, as indicated by the challenge concentrations used (5% and 10%).

Ref.: 25

Buehler test

Guideline	:	OECD 406
Species/strain	:	Pirbright white Guinea pigs
Group size	:	20 females in test group, 10 females in control group
Test substance	:	Genamin STAC (79.8% steartrimonium chloride)
Batch no	:	E061859561
Dose :		dermal induction: 4% test substance in ethanol: water 80:20 challenge: 1% test substance in isopropanol
GLP	:	in compliance

The test group consisted of 20 female Guinea pigs, 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. 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, well-defined to severe erythema and very slight to well-defined oedema the treated skin area. After challenge, skin reactions were observed neither in the treated group nor the control group.

Conclusion
Genamin STAC (79.8% active) when diluted to 4% (induction) and 1% (challenge): sensitization was not shown under the experimental conditions described.

Comment
The challenge concentration is lower than the requested use concentration for rinse-off hair care products.

Ref.: 26

c) Behentrimonium chloride

Buehler test

Guideline:	OECD 406
Species/strain:	Pirbright white Guinea pigs
Group size:	20 females in test group, 10 females in control group
Test substance:	Genamin KDMP (77-83% behentrimonium chloride)
Batch:	E06186598
Dose :	dermal induction: 20% test substance in ethanol : water 80 : 20 challenge: 0.8% test substance in isopropanol
GLP:	in compliance

The test group consisted of 20 female Guinea pigs, the control group of 10 female Guinea pigs. On day 1, a 20 % solution of the test substance in 80 % 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. 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 treatedgroup 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
Genamin KDMP (about 80% active) when diluted to 20% (induction) and 0.8% (challenge): sensitization was not shown under the experimental conditions described.

Comment
The challenge concentration is lower than the requested use concentration.

Ref.: 27

Buehler test

Guideline:	OECD 406
Species/strain:	Hartley Guinea pigs
Group size:	10 males and 10 females in test group, 5 males and 5 females in control group
Test substance:	Behentrimonium chloride (80% behentrimonium chloride)
Batch:	0040242
Dose:	dermal induction: 10% test substance in corn oil challenge: 0.5 % test substance in corn oil
GLP:	in compliance

The test group consisted of 10 male and 10 female Guinea pigs, the control group of 5 male and 5 females. On day 1, a 10 % solution of the test substance in corn oil was prepared and applied on a 8 cm² 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
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 welldefined 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. A grade 1 erythema was found in 1/19 and a grade 2 erythema in 2/19 animals at 72 hours.

Conclusion
Behentrimonium chloride (80 % active), when diluted to 10 % (induction) and 0.5 % (challenge): skin reactions were found in 26 % of the animals.

Comment
The challenge concentration is lower than the requested use concentration.

Ref.: 28

3.3.4.	Dermal / percutaneous absorption

In vitro

Guideline:	Draft OECD 428 (30)
Tissue:	Dermatomed full thickness skin (1000 µm) of back and flank of castrated, male pigs, stored at -20°C
Method:	Diffusion cell (static)
Test substance:	Dehyquart A-CA (25% cetrimonium chloride)
Batch:	/
Dose:	3.5% active test substance in emulsion formulation were applied to the test skin at 25 mg/cm2 for 30 minutes
Replicate cells:	6 cells
GLP:	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 containing 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. 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.

Amount of cetrimonium chloride in:	***Expressed as µg per skin sample***	***Expressed as µg/cm²***	***Expressed as % of dose***
Receptor fluid	**b.d. (*)**	**b.d.**	**b.d.**
Stratum corneum	5.0 – 57.0	1.3 – 14.3	0.7 ± 0.6
Dermis	3.0 – 29.0	0.7 – 7.3	0.3 ± 0.3
Total skin	**8.0 – 86.0**	**2.0 – 21.5**	**1.0 ± 0.9**
Rinsing solution	2807.0 – 3223.0	701.8 – 805.8	90.2 ± 4.5
Spatula/swabs/pipette	258.0 – 862.0	64.5 – 215.5	17.1 ± 6.2

Rinsing solution and spatula/swabs/pipette	**3320.0 – 3756.0**	**830.0 – 939.0**	**107.4 ± 6.1**
Total recovery	**3368.0 – 3842.0**	**842.0 – 960.5**	**108.4 ± 6.3**

(*) b.d.: below detection limit

Conclusion
The total duration of the study (72 hours) is longer than the standard 24-hour exposure time generally used in percutaneous absorption studies. Although the skin was not experimentally checked at the end of the 72-hour period, the absence of the test article in the receptor fluid up to 72 hours indicates that the skin remained intact during the duration of the study. The applied dose of 25 mg/cm² of formulation is higher than the usually recommended test amount of 2-5 mg/cm². Although this test condition represents a high skin area dose, it is considered to have no consequences on the interpretation of the results.

Comment
After dermal absorption: the test substance may not be systemically available as no cetrimonium chloride was detected in the receptor fluid.

Ref.: 31

3.3.5. Repeated dose toxicity

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

a) Cetrimonium chloride

Subacute oral toxicity in rats

Guideline: /
Species/strain: Sprague-Dawley CD rat / Charles River Wiga, Germany
Group size: 10 per sex/dose, additional recovery groups of 5 per sex of the control and high dose groups
Test substance: Cetyltrimethylammonium chloride (24-26% cetrimonium chloride)
Batch: 548050
Dose: 0, 30, 100 and 300 mg cetrimonium chloride/kg bw/day in distilled water, 5 days/week by gavage (total of 23/24 applications)
Exposure: 28 days
GLP: in compliance

Male and female rats of the Sprague-Dawley CD strain, weighed 71-108 g and 73-104 g, respectively, at the start of the study. Groups of 10 males and 10 females received 30, 100 and 300 mg/kg bw/day of cetrimonium chloride by oral gavage on 5 days per week (total of 23/24 applications). Control animals received 10 ml/kg bw/day of distilled water. Control and high dose groups were supplemented with additional 5 male and 5 female rats in order to study the reversibility of treatment-related effects after a subsequent 27-day treatment-free period. The effects of the test compound were assessed using twice daily clinical observations and mortality checks as well as weekly recordings of body weights and food consumption. At the end of the exposure period, ophthalmoscopic examinations, blood biochemical and haematological

investigations were performed. At terminal sacrifice, all animals were subjected to gross necropsy and organ weights were determined. A large number of organs and tissues from all animals in all study groups were preserved and the majority of these specimens were examined histopathologically. All animals survived. There were no effects on food consumption and body weight development. The mean water intake of male animals of the high dose group was higher than that of controls. Ophthalmological and haematological results revealed no treatment-related changes in any group. Clinical chemistry parameters were unaffected by treatment, with the exception of a minor increase in serum alanine aminotransferease (ALT) activity in males and females of the high dose group, which remained within the range of the historical controls. The only effect on organ weights was a slight increase in absolute and relative adrenal weights in males and a slight decrease in absolute and relative spleen weight in males. Macroscopic examination of animals at 300 mg/kg revealed thickening of the forestomach mucosa, associated with oedema and sporadic ulceration in male and female rats. The microscopic correlates included inflammatory oedema of the forestomach mucosa, sporadic ulceration and acanthosis up to papillomatous hyperplasia in both sexes of the high dose group. No histopathological alterations were found in adrenals and spleen or any other organs. No macroscopic or microscopic alterations were found in the mid and low dose groups. All treatment-related changes were shown to be reversible following the recovery period.

Conclusion
The forestomach and stomach changes are considered to be a result of local irritation and 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 minimal systemic toxicity. As such, the dose of 100 mg/kg bw/day was the no-observed-adverse-effect-level (NOAEL) in this study.

Ref.: 33

Subacute dermal toxicity in rabbits

Guideline:	/
Species/strain:	New Zealand White rabbits
Group size:	5 males and 5 females per dose
Test substance:	Ammonium, hexadecyltrimethyl-, chloride 54.5% in aqueous isopropanol
Batch:	not stated
Dose:	0 and 0.5 % w/v: 2 ml/kg (10 mg cetrimonium chloride/kg bw/day) in distilled water for 6.5-7 hours/day, 5 days/week by dermal application
Exposure:	4 weeks
GLP:	/

Five 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/day (0, 0.5% aqueous solutions, respectively). 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 coriaceousness (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.

Conclusion
The skin changes at the application site are a result of local irritation and do not present evidence of systemic toxicity. No evidence of systemic toxicity was observed at the dose level of 10 mg/kg/day in this study.

Comment
It was not possible to evaluate the study as only a summary was provided.

Ref.: 34

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

No data

3.3.5.3. Chronic (> 12 months) toxicity

No data was provided for cetyltrimethylammonium chloride, steartrimonium chloride or behentrimonium chloride.
Cetyltrimethylammonium bromide in drinking water for one year was shown to reduce body weight and the growth rate in rats at the highest dose (equivalent to 45 mg/kg bw). At the end of study period, these effects were shown to be more pronounced in males.
A full evaluation was not possible as this was reported in a peer-reviewed scientific paper.

Ref.: 35

3.3.6. Mutagenicity / Genotoxicity

Bacterial gene mutation assay

a) Cetrimonium chloride

No data

b) Steartrimonium chloride

Guideline:	OECD 471
Species/strain:	Salmonella typhimurium, TA98, TA100, TA1535, TA1537, TA1538
Replicates:	Triplicate plates
Test substance:	Quartamin 86W (28% actives; steartrimonium chloride:cetrimonium chloride 80:20)
Batch no:	1841
Concentration:	Experiment 1: 0.15, 0.5, 1.5, 5, 15 and 50 µg active substance/plate without metabolic activation (rat S9 mix) and 1.5, 5, 15, 50, 150 and 500 µg active substance/plate with S9 mix. Experiment 2: 0.5, 1.5, 5, 15 and 50µg active substance/plate without metabolic activation (rat S9 mix) and 0.5, 1.5, 5, 15, 50 and 150 µg active substance/plate with S9 mix.
GLP:	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-aminofluorene was used. N-ethyl-N'-nitro-N-nitrosoguanidine (for TA100, TA1535), 9-aminoacridine (for TA1537) and 4-nitroquinoline-1-oxid (for TA98) and 4-nitro ophenylenediamine (TA1538) were used as positive standards without metabolic activation. Two independent experiments were performed. Toxicity testing was done in a pretest using strain TA 100 and test substance concentrations of 50, 150, 500, 1500 and 5000 µg/plate with and without S9 mix. Due to toxicity at all dose levels, the pretest was repeated with concentrations of 0.5. In the pretest, 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
Quartamin 86W 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. It was toxic at higher test concentrations.

Ref.: 36

Guideline:	OECD 471
Species/strain:	Salmonella typhimurium, TA98, TA100, TA1535, TA1537
Replicates:	Triplicate plates
Test substance:	Genamin STAC (79.8% steartrimonium chloride)
Batch:	E061859561
Concentration:	Experiment 1: 4, 20, 100, 500, 2500 and 5000 µg active substance/plate without metabolic activation (rat S9 mix)

	Experiment 2: 0.8, 4, 20, 100, 500, and 2500 µg active substance/plate without metabolic activation (rat S9 mix).
GLP:	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 in the first experiment; it was lowered due to toxicity to 0.8 to 2500 µg/plate in the second experiment. Ethanol alone served as negative control. As a positive standard requiring metabolic activation 2-aminofluorene was used. Sodium azide (for TA 100, TA 1535), 9-aminoacridine (for TA 1537) and 2-nitrofluorene (for TA 98) were used as positive standards without metabolic activation. Two independent experiments were performed. Toxicity testing was done in combination with the second experiment. 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
Genamin STAC 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.

Ref.: 37

c) Behentrimonium chloride

Guideline:	OECD 471
Species/strain:	*Salmonella typhimurium*, TA98, TA100, TA1535, TA1537
Replicates:	Triplicate plates
Test substance:	Genamin KDMP (77-83% behentrimonium chloride)
Batch:	E06186598
Concentration:	4, 20, 100, 500, 2500 and 5000 µg active substance/plate with and without metabolic activation (rat S9 mix)
GLP:	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-aminofluorene was used. Sodium azide (for TA100, TA1535), 9-aminoacridine (for TA1537) and 2-nitrofluorene (for TA98) were used as positive standards without metabolic activation. Two independent experiments were performed. Toxicity testing was done in combination with the second experiment. 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
Genamin KDMP had no mutagenic activity on any of the bacterial tester strains used either with or without S9 mix at up to 500 (with S9 mix) or 100 (without S9 mix) µg/plate.

Ref.: 38

***In vitro* chromosome aberration test**

a) Cetrimonium chloride

Guideline:	OECD 473, Directive 84/449/EEC
Species/strain:	V79 Chinese hamster cells
Replicates:	Duplicate plates
Test substance:	Cetyltrimethylammonium chloride (24-26% cetrimonium chloride)
Batch no:	3118322
Concentration:	0.1, 0.3, 0.6, 1.0, 3.0 and 6.0 µg/ml without metabolic activation
	0.1, 0.5, 1.0, 3.0, 6.0 and 10.0 µg/ml with rat S9 mix
GLP:	in compliance

Liver S9 fraction from Aroclor 1254 induced rats were used as the exogenous metabolic activation system. Logarithmically growing cells were incubated with the test substance in serum-free culture medium at concentrations of 0.1 to 6 µg active substance/ml without S9 mix and at 0.1 to 10 µg active substance/ml with S9 mix for 4 hours. Cells were then 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. 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. It was concluded that cetrimonium chloride 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

***In vitro* cell transformation test**

a) Cetrimonium chloride

Guideline: /
Species/strain: Cryopreserved primary Syrian hamster embryo cells
Test substance: Cetyltrimethylammonium chloride (99.30% cetrimonium chloride)
Batch: not stated
Concentration: 0.1, 1.0 and 5.0 µg/ml
GLP: /

On Day 0, an ampoule of cryopreserved primary Syrian hamster embryo cells prepared as feeder-layer cells was rapidly thawed and plated in a 75-cm² flask containing 20 ml of culture medium. On day 3, an ampoule of cryopreserved primary cells prepared as target cells was also rapidly thawed and plated in a 75-cm² flask. On day 4, the feeder cells which were shifting from a stage of logarithmic growth to a stationary phase were irradiated with 5000 rad from a linear accelerator, trypsinized, and then plated at 6 x 10^4 cells/50-mm dish in 2 ml of complete medium. On day 5, the target cells which were approximately 80 - 90 % confluent were trypsinized and a suspension of 500 target cells in 2 ml of complete medium was then added to each of the dishes plated the day before with irradiated feeder layer cells. On day 6, an appropriate dose of the test chemical in a volume of 4 ml was added. Nine dishes were used for each dose level. 3-methylcholanthrene was used as positive control at 0.1, 0.5 and 1.0 µg/ml. On day 14, the cultures were fixed with absolute methanol for 10 minutes and stained with Giemsa solution for 45 minutes or more. The stained dishes were examined with a stereoscopic dissection microscope to count normal and transformed colonies. Randomly oriented three-dimensional growth with extensive crossing-over of the cells at the periphery of the colony was considered to be the endpoint of morphological transformation. The highest concentration caused cytotoxic effects as shown by the reduced number of surviving colonies (9 vs. 545 in negative control). No cell transformation was observed at any of the concentrations tested (588, 413 and 9 colonies analysed at 0.1, 1 and 5 µg/ml, respectively).
However, in the positive control (3-methylcholanthrene) only 1 transformed colony was found at the low dose (1/504) and none transformed colonies were found at the two higher doses (0/520, 0/490).

Conclusion
Due to the problem with the positive control, no conclusion can be drawn from the experiment.
Ref.: 40

3.3.7. Carcinogenicity

No data

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data

3.3.8.2. Teratogenicity

a) Cetrimonium chloride

Guideline:	/
Species/strain:	New Zealand White rabbits
Group size:	20 pregnant rabbits/group
Test substance:	Ammonium, hexadecyltrimethyl-, chloride (cetrimonium chloride)
Batch no:	/
Dose:	0.1, 0.5, 1.0 and 2.0% in deionised water at 2.0 ml/kg (equivalent to 10, 20, and 40 mg/kg bw/day), by dermal application
GLP:	in compliance

Twenty mated female rabbits per group were exposed topically to 2.0 ml/kg of the test substance at concentrations of 0, 0.5, 1.0, or 2.0 % for 2 hours daily from day 7 to day 18 of gestation. The control group was treated with water only. Prior to the initial treatment, the dorsal area of eachanimal was shaved and any skin lesions were documented. At the time of treatment, the animals were fitted with a collar to prevent oral ingestion of the test substance. After the 2-hour exposure period, the collars were removed and 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. Maternal effects: 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. Foetal effects: 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.

Conclusion
The 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, cetrimonium chloride was found to be non-foetotoxic and non-teratogenic. The NOEL for maternal systemic toxicity and embryofoetal toxicity was 40 mg cetrimonium chloride/kg bw/day.

Comment
It was not possible to evaluate the study as only a summary was provided.

Ref.: 41

b) Steartrimonium chloride

Guideline:	/
Species/strain:	CFY Sprague-Dawley rat
Group size:	20 mated female rats/group
Test substance:	Trimethylstearylammonium chloride (steartrimonium chloride)
Batch:	/
Dose:	0.1, 0.9, 1.5 and 2.5% in distilled water at 0.5 ml/rat (approximately 4.5, 7.5, and 12.5 mg/kg bw/day), by dermal application
GLP:	/

Concentrations of 0.9, 1.5 and 2.5% of the test substance in distilled water were utilised for this study. All animals were dosed with 0.5 ml of the proper test substance concentration from day 6 to 15 of gestation. 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. Twenty 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. Maternal effects: There were no systemic signs of toxicity, 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. Foetal effects: Litter values assessed by litter size, post-implantation loss, litter and mean foetal weights and the embryonic 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.

Conclusion
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.

Comment
It was not possible to evaluate the study as only a summary was provided.

Ref.: 42

3.3.9. Toxicokinetics

Cetrimonium bromide

Isomaa (43) reported 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.

Ref.: 43

3.3.10. Photo-induced toxicity

/

3.3.11. Human data

Skin irritation

a) Cetrimonium chloride

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 occlusively by an adhesive plaster for 24 hours. Skin reactions were evaluated 0.25, 24 and 48 hours after removal of test patches. The mean irritation scores for the positive control, negative control and test article were 0.79, 0.04 and 0.22, respectively, after 0.25 hours, 1.0, 0, and 0.11, respectively, after 24 hours, and 0.86, 0, and 0.06, respectively, after 48 hours. 3.52% cetrimonium chloride in a cosmetic formulation caused slight to moderate skin irritation when applied under occlusion for 24 hours.

Ref.: 44

The test substance was applied to the skin of the back. The test site was covered semiocclusively by adhesive plaster for 1 hour. Skin reactions were evaluated 0.25, 24 and 48 hours after removal of test patches. The test article produced a single doubtful reaction after patch removal, another very slight reaction after 24 hours, and a very slight reaction after 48 hours. In untreated control skin sites, 1very slight reaction was observed at 24 hours. 2.5% cetrimonium chloride in a cosmetic formulation produced minimal skin irritation when applied under occlusion for 1 hour.

Ref.: 45

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

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

The test and reference substances were applied to the back skin of 20 healthy subjects using large Finn chambers on ScanporTM 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. The two Dehyquart A 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) and slight to moderate (sodium lauryl sulfate) eschar formation. The negative control substances and empty Finn chambers caused no skin reaction. Dehyquart A at 1% active substance (cetrimonium chloride) in water, applied for 24 hours under occlusion, was not irritating to skin under the test conditions used.

Ref.: 48

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.
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. 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

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 skin of the back using Finn chambers and 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.
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 0-hour reading, no reactions were found at the 24- and 48-hour readings. At a concentration of 0.5 %, 9 grade 0.5, 16 grade 1, 6 grade 1.5, and 11 grade 2 reactions at the 0-hour reading, 13 grade 0.5, 9 grade 1, and 1 grade 1.5 reactions at the 24-hour reading, and 8 grade 0.5 reactions at the 48-hour reading were observed. A 25% aqueous dilution of a cosmetic formulation containing 3.01% cetrimonium chloride caused minimal skin irritation when applied under occlusion for 24 hours.

Ref.: 50, 51

The test substances (10% dilutions in water) were applied to the skin (upper arm) using Webrill patches and adhesive Micropore tape. The test site was covered for 24 hours (semiocclusive). Skin reactions were evaluated 24 and 48 hours after removal of test patches. A 10% aqueous dilution of a rinse-off hair conditioner formulation containing up to 4.0% cetrimonium chloride caused no skin irritation when applied under semi-occlusion for 24 hours.

Ref.: 52

The test substances (100, 75, 50, or 25% dilutions in water) were applied to the skin (upper arm) 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. A 25-100% aqueous dilution of a styling gel formulation 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

Repeat Insult Patch Test

114 subjects received occlusive patches containing 0.3 ml of 0.25% cetrimonium chloride on the upper arm for 24 hours on Mondays, Wednesdays and Fridays for 3 weeks. Seventeen days after the last application, a challenge patch of 0.25% cetrimonium chloride was applied to a previously untreated site. Mild irritation was observed in several subjects during induction, but no sensitisation was observed. One case of contact urticaria occurred, but the authors could not determine whether it was treatment-related because the subject refused to participate in a follow-up diagnostic patch test.

Comment
Human RIPT for testing allergenic potential is not considered ethical by the SCCP.

Ref.: 54

c) Behentrimonium chloride

Method:	Epicutaneous patch test
Subject:	Healthy male and female volunteers, age 18-65 years without skin diseases
Group size:	5 male and 46 female subjects
Test substance:	Formulation with 5.0% behentrimonium chloride
Batch:	OSFI 26.03.98 (formulation No. 433833)
Dosages:	45 µl per chamber of large Finn chambers (12 mm diameter)
Exposure:	24 hours

GCP: in compliance

The test substance was applied to the skin of the back 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 0.25, 24 and 48 hours after removal of test patches. The test article caused slight erythema in 3 subjects at the 0.25-hour 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 0.25- and 24-readings.

Conclusion
5.0% behentrimonium chloride in a cosmetic formulation produced minimal skin irritation when applied under occlusion for 24 hours.

Ref.: 55

The test substance was applied to the skin of the back using large Finn chambers. As negative control an empty chamber was used. The test site was covered occlusively by adhesive plaster for 48 hours. Skin reactions were evaluated 0.5 hours after removal of test patches. The test article caused a primary irritation index of 0.04. 5.0% behentrimonium chloride in a cosmetic formulation produced minimal skin irritation when applied under occlusion for 48 hours.

Ref.: 56

The test substance was applied to the skin of the back using usual test chambers. The test site was covered occlusively by adhesive plaster for 24 hours. Skin reactions were evaluated 0 and 24 hours after removal of test patches. No signs of irritation were detected in any subject at 0 as well as at 24 hours after application. 1.0% behentrimonium chloride in water produced no skin irritation when applied under occlusion for 24 hours.

Ref.: 57

Method:	Human repeated insult patch test
Subject:	Healthy male and female volunteers, age 18-70 years (mean 44) without skin diseases
Group size:	31 male and 81 female subjects, of which 104 completed the study
Test substance:	Rinse-off formulation with 3.4% behentrimonium chloride
Batch No.:	Not reported (formulation No. FE0394.01)
Dosages:	0.2 g on 2x2 cm Webril patches, 9 semi-occlusive patches during 3 weeks
Exposure time:	24 hours
GCP:	Statement on Good Clinical Practice included

The test substance was applied to the upper arm or the back using 2 x 2cm.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 72 hours (for Friday patches) for skin reactions. The resting period, after completion of the induction phase, was 10-15 days. 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. 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
3.4% behentrimonium chloride did not cause skin sensitization under the experimental conditions described.

Comment
The study is not considered ethical by the SCCP.

Ref.: 58

3.3.12. Special investigations

/

3.3.13. Safety evaluation (including calculation of the MoS)

Not applicable

3.3.14. Discussion

The designation alkyl (C16, C18, C22) trimethylammonium chlorides comprise the following substances: cetrimonium chloride, steartrimonium chloride, and behentrimonium chloride. Alkyl chain blends from plant oils are used for the production of alkyltrimethylammonium chlorides.

Acute toxicity
The available acute oral toxicity studies show comparable results when doses are expressed as active substance, as shown in the table.

Substance	**Oral LD_{50} of test substance (mg/kg bw)**	**Active substance (%)**	**INCI**	**Calculated LD50 for active substance (mg/kg bw)**	**Ref.**
Genamin CTAC	1550	29	Cetrimonium chloride	450	6
Quartamin 60W25	≈2000	25	Cetrimonium chloride	500	7
Quartamin 86W	>2000	28	Steartrimonium chloride	>560	8
Genamin STAC	702.5	79	Steartrimonium chloride	555	9

No data on acute toxicity were submitted for behentrimonium chloride.

Repeated-dose toxicity
In the 28-day oral gavage study in rats using cetrimonium chloride, local effects in the gastrointestinal tract and slight effects on organ weights without histopathological correlates were observed at a dose of 300 mg/kg b.w./day. A dose of 100 mg/kg b.w./day has been considered as the NOEL. In the 1-year chronic study in rats receiving cetrimonium bromide in the drinking water, the highest dose of 45 mg/kg b.w./day was a NOAEL for systemic toxicity. However, this dose produced local effects in the gastrointestinal tract secondary to the irritant nature of the substance. The NOEL in this study was 10 mg/kg b.w./day. A dose of 10 mg/kg/day was also tested in a 28-day repeated dermal toxicity study in rabbits and produced no systemic toxicity. For the MOS calculation, the NOAEL for systemic effects of 100 mg/kg/day from the 28-day study or 45 mg/kg/day from the 1-year study may be used, since the effects on the gastrointestinal tract observed in the studies are considered to be related to the oral exposure route and irrelevant for the risk assessment of topical application.

Reproductive toxicity
It was not possible to evaluate the studies as only summaries were provided.

Skin irritation
The results of available skin irritation studies in rabbits are shown in the table.

Substance	Exposure time	Active substance (%)	INCI	Effect level	Ref.
Behentrimonium chloride	3 minutes	8	Behentrimonium chloride	very slightly irritating	14
Behentrimonium chloride	3 minutes	5	Behentrimonium chloride	not irritating	14
Genamin CTAC	4 hours	29	Cetrimonium chloride	irritating	10
Quartamin 60W25	4 hours	25	Cetrimonium chloride	irritating	11
Quartamin 86W	4 hours	5.6	Steartrimonium chloride	irritating	12
Quartamin 86W	4 hours	0.56	Steartrimonium chloride	irritating	12
Genamin STAC	3 minutes	79	Steartrimonium chloride	not irritating	12
Genamin STAC	4 hours	79.2	Steartrimonium chloride	corrosive	13

An exposure time of 3 minutes is irrelevant for skin irritation testing.

Eye irritation
The results of available eye irritation studies in rabbits are shown in the table.

Substance	Exposure time	Active substance (%)	INCI	Results	Ref.
Behentrimonium chloride	30 seconds	8.0	Behentrimonium chloride	serious damage to eyes	20
Behentrimonium chloride	30 seconds	5.0	Behentrimonium chloride	transient irritation	21
Behentrimonium chloride	30 seconds	3.0	Behentrimonium chloride	Slight irritation	22
Genamin CTAC	24 hours	29	Cetrimonium chloride	serious damage to eyes	16
Quartamin 60W25	24 hours	25	Cetrimonium chloride	serious damage to eyes	17

Substance	Exposure time	Active substance (%)	INCI	Results	Ref.
Rinse-off formulation	24 hours	2.0	Cetrimonium chloride	transient irritation (LVET)	18
Quartamin 86W	24 hours	0.56	Steartrimonium chloride	transient mild irritation	19

These substances are irritant to the eyes.

Skin sensitization
The available studies in Guinea pigs, comprising Maximisation and Buehler tests, indicate that alkyl (C22) trimethylammonium chloride (Behentrimonium chloride) is a skin sensitizer, and that alkyl (C16 and C18) trimethylammonium chlorides may not be skin sensitizers. The studies are summarised in the table:

Substance	INCI Name	Sensitisation test protocol	Concentration of active substance during induction / challenge	Results	Ref.
Genamin KDMP	Behentrimonium chloride	Buehler test	16 (topical) / 0.64	No sensitisation	27
Behentrimonium chloride	Behentrimonium chloride	Buehler test	8.0 (topical) / 0.4	26% sensitised	28
Quartamin 60W25	Cetrimonium chloride	Maximisation test	0.031 (intradermal), 0.75 (topical) / 0.125	Unclear *	23
Genamin CTAC	Cetrimonium chloride	Buehler test	1.2 (topical) / 0.3	No sensitisation	24
Quartamin 86W	Steartrimonium chloride	Maximisation test	0.025 (intradermal), 0.125 (topical) / 2.5	Unclear **	25
Genamin STAC	Steartrimonium chloride	Buehler test	3.2 (topical) / 0.8	No sensitisation	26

* = unclear result due to reactions to the substance in control group and to vehicle in both groups.
** = unclear result due to too low topical induction concentration.

The challenge concentration was lower than the requested use concentration for some applications.

Dermal / percutaneous absorption
Only the dermal absorption of cetrimonium chloride was tested. Although the test conditions represent a high skin area dose, no cetrimonium chloride was detected in the receptor fluid.

Mutagenicity / genotoxicity
The available mutagenicity/genotoxicity studies on alkyl (C16, C18, C22) trimethylammonium chlorides, including three bacterial gene mutation tests and one chromosomal aberration test in V79 cells, did not indicate mutagenic effects.
However, the substances have not been completely tested in accordane with the existing guideline.

4. CONCLUSION

The SCCP is of the opinion that the information submitted is inadequate to assess the safe use of the substance.

Before any further consideration, the following information is required:

- a percutaneous absorption study on steartrimonium chloride and behentrimonium chloride according to the SCCNFP Notes of Guidance;
- skin sensitisation studies according to the SCCNFP Notes of Guidance;
- data on the genotoxicity/mutagenicity following the relevant SCCNFP opinions and in accordance with the Notes of Guidance.

5. MINORITY OPINION

Not applicable

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Dr. C. Chambers
Prof. R. Dubakiene
Dr. R. Grimalt
Dr. B. Jazwiec-Kanyion (Rapporteur)
Prof. V. Kapoulas
Prof. J. Krutmann
Prof. C. Lidén
Prof. J.-P. Marty
Dr. S.C. Rastogi
Prof. J. Revuz
Prof. V. Rogiers
Prof. T. Sanner
Prof. G. Speit
Dr. I.R. White (Chairman)