



Scientific Committee on Consumer Products

SCCP

OPINION ON

ALKYL (C16, C18, C22) TRIMETHYLMAMMONIUM CHLORIDE

For other uses than as a preservative

COLIPA n° P72



The SCCP adopted this opinion at its 11th plenary on 21 March 2007

About the Scientific Committees

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

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SCCP

Questions concerning the safety of consumer products (non-food products intended for the consumer).

In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

Scientific Committee members

Claire Chambers, Gisela Degen, Ruta Dubakiene, Ramon Grimalt, Bozena Jazwiec-Kanyion, Vassilios Kapoulas, Jean Krutmann, Carola Lidén, Jean-Paul Marty, Thomas Platzek, Suresh Chandra Rastogi, Jean Revuz, Vera Rogiers, Tore Sanner, Günter Speit, Jacqueline Van Engelen, Ian White

Contact:

European Commission
Health & Consumer Protection DG
Directorate C: Public Health and Risk Assessment
Unit C7 - Risk Assessment
Office: B232 B-1049 Brussels
Sanco-Sc6-Secretariat@ec.europa.eu

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Dr. B. Jazwiec-Kanyion
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Prof. C. Lidén
Prof. J.-P. Marty
Prof. T. Platzeck
Dr. S.C. Rastogi
Prof. J. Revuz
Prof. V. Rogiers (rapporteur)
Prof. T. Sanner
Dr. J. van Engelen
Dr. I.R. White (Chairman)

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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, (COLIPA¹ 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% |

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 names and/or INCI names

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

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
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) C₂₂-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

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Incroquat Behenyl TMC/P
 Quartamin AB
 Varisoft BT 85

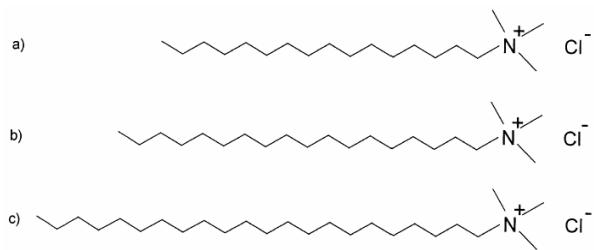
COLIPA P 72 [covers a), b) and c]

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

Molecular formula: a) C₁₉H₄₂ClN
 b) C₂₁H₄₆ClN
 c) C₂₅H₅₄ClN

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 chlorideGenamin 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% <i>behentrimonium chloride</i> (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 considered unacceptable:

- 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 mentions batch n°1081 for Quartamin 86W, a batch that has not been mentioned 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 Ames test (see 3.3.6.1.B), was not mentioned 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 (C₁₂-C₂₂) 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.

Industry proposes the following adaptation is made to Annex III of the Cosmetics Directive:

Annex III: entry of *cetrimonium chloride* and *steartrimonium chloride* in order to restrict their other uses to rinse-off hair care products at 2.5%, leave-on hair care products at 1%, and leave-on facial cream products at 0.5%.

Annex III: entry of *behentrimonium chloride* in order to restrict its other uses to rinse-off hair care products at 5%, leave-on hair care products at 3%, and leave-on facial cream products at 3%.

According to the submission, the proposed adaptations reflect the other uses of alkyl (C₁₆, C₁₈, C₂₂) trimethylammonium chlorides in currently marketed cosmetic products.

3.3 TOXICOLOGICAL EVALUATION

3.3.1 Acute toxicity

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 LD₅₀ 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% <i>cetrimonium chloride</i> 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 LD₅₀ 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 LD₅₀-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% <i>cetrimonium chloride</i> 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 LD₅₀ 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 <i>steartrimonium chloride</i>) |
| 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% <i>steartrimonium chloride</i> , 19.1% isopropanol and 0.9% water) |

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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 b.w.) | 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 LD₅₀ value of 702.5 mg/kg bw for female rats using the probit method, whereas the LD₅₀ 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 LD₅₀-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**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% <i>cetrimonium chloride</i> in water) |
| Batch: | E06112547 (04.10.1983) |
| Purity: | Not stated |
| Dose: | 0.5 ml of test substance on 6 cm ² |
| 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% <i>cetrimonium chloride</i> in water) |

Opinion on alkyl (C16, C18, C22) trimethylammonium chloride, non-preservative uses

| | |
|---------------------|---|
| Batch: | 3-4 |
| Purity: | Not stated |
| Dose: | 0.5 ml of test substance on 6 cm ² |
| Observation period: | 14 days |
| GLP/QAU: | In compliance |

A patch with 0.5 ml test material was placed on a \pm 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 <i>steartrimonium chloride</i>) 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 cm ² |
| Observation period: | 14 days |
| GLP/QAU: | In compliance |

A patch with 0.5 ml test material was placed on a \pm 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.

Opinion on alkyl (C16, C18, C22) trimethylammonium chloride, non-preservative uses

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% <i>steartrimonium chloride</i> , 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 |

Opinion on alkyl (C16, C18, C22) trimethylammonium chloride, non-preservative uses

| | |
|---------------------|---|
| Species/strain: | New Zealand white albino rabbit |
| Group size: | 3 males exposed for 3 minutes |
| Test substance: | Formulation (77-83% <i>Behentrimonium chloride</i> , 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 were 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% <i>Behentrimonium chloride</i> , 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.

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.

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 <i>steartrimonium chloride</i>) 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 were 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% <i>Behentrimonium chloride</i> , 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 (with 1/4 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 were 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

H. 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

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% <i>cetrimonium chloride</i> 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 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 |

Opinion on alkyl (C16, C18, C22) trimethylammonium chloride, non-preservative uses

| | |
|---------------------|--|
| 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% <i>cetrimonium chloride</i> in water) |
| Batch: | E06178641 (26.07.1993) |
| Purity: | 28.7% (DIN ISO 2871) |
| Dosages: | Induction: topical application of 4% Genamin CTAC on 4 cm ² Challenge: topical application of 1% Genamin CTAC on 4 cm ² |
| Observation period: | 30 days |
| GLP/QAU: | not available |

The study authors report 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 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 <i>steartrimonium chloride</i>) |
| Batch: | 1841 (batch nr. not documented) |

Opinion on alkyl (C16, C18, C22) trimethylammonium chloride, non-preservative uses

| | |
|---------------------|--|
| 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 cm ² |
| | Challenge: topical application of 10 and 5% Quartamin 86W in distilled water on 8 cm ² |
| 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 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% <i>steartrimonium chloride</i> , 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 cm ² Challenge: topical application of 1% Genamin STAC in isopropanol on 4 cm ² |
| 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 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% <i>Behentrimonium chloride</i> , 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 cm ² Challenge: topical application of 0.8% Genamin KDMP in isopropanol on 4 cm ² |
| 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 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% <i>Behentrimonium chloride</i> , 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 cm ² Challenge: topical application of 0.5% Genamin KDMP in corn oil on 8 cm ² |
| 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

G. 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 in 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

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: <i>in vitro</i> 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 |
| Nº of samples: | 6 (not stated whether they originated from one or more animals) |
| Test substance: | Presumably a hair care formulation containing 3.5% <i>cetrimonium chloride</i> (see also remarks below) |
| Batch: | Not stated |
| Purity: | Not stated |
| Applied amount: | 25 mg/cm ² (100 mg/cm ²), 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 ($1.25\text{-}14.25 \mu\text{g/cm}^2$) and in residual skin ($0.75\text{-}7.25 \mu\text{g/cm}^2$, corresponding to 0.086-0.83%, with a mean of $0.27\pm0.28\%$). The following table shows the exact values expressed as $\mu\text{g/cm}^2$:

| Amount of cetrimonium chloride in: | Expressed as $\mu\text{g/cm}^2$ [mean \pmSD (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. Thus the amount found in the dermis ($7.25 \mu\text{g/cm}^2$ as upper level) will be used 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

D. 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.

Opinion on alkyl (C16, C18, C22) trimethylammonium chloride, non-preservative uses

- 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

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% <i>cetrimonium chloride</i> 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

Opinion on alkyl (C16, C18, C22) trimethylammonium chloride, non-preservative uses

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.

As such, 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 concludes that the toxic response only consists of skin irritation, and proposes 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 (\geq 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

B. Steartrimonium chloride - chronic toxicity

No data submitted

C. Behentrimonium chloride - chronic toxicity

No data submitted

3.3.6 Mutagenicity / genotoxicity

3.3.6.1 Mutagenicity/Genotoxicity *in vitro*

A. Cetrimonium chloride - Ames test

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 - Ames test 1

| | |
|-----------------|---|
| Date of study: | Mar-May 1996 |
| Guideline: | OECD TG 471, Annex V to Dir. 67/548/EEC, Method B.13/14: |
| Species/strain: | Salmonella typhimurium, TA98, TA100, TA1535, TA1537, TA 1538 |
| Replicates: | 3 |
| Test substance: | Quartamin 86W (unknown percentage of <i>steartrimonium chloride</i>) |
| 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 - Ames test 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 - Ames test 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: | <i>Salmonella typhimurium</i> , TA98, TA100, TA1535, TA1537 |
| Replicates: | 3 |
| Test substance: | Genamin STAC (79.8% <i>steartrimonium chloride</i> , 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 - Ames test

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-

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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**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.

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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, *steartimonium 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 *steartimonium 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*, *benzyldimethylstearylammmonium chloride* and *trimethylstearylammmonium 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

A. Cetrimonium chloride

A publication of 1975 reports a toxicokinetic study in rats using ¹⁴C-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 ¹⁴C-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

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

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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% <i>cetrimonium chloride</i> |
| Dosages: | 160 µl per chamber |
| Exposure time: | 1 hour |
| Scoring system: | <p>0 no reaction</p> <p>0.5 doubtful reaction (faint erythema)</p> <p>1 very slight reaction (pale pink, homogenous, regular erythema)</p> <p>2 slight reaction (pink, homogenous, regular, non-vesicular erythema)</p> <p>3 moderate reaction (red, homogenous, regular erythema)</p> <p>4 severe reaction (bright red, pronounced, homogenous, regular erythema)</p> |
| 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% <i>cetrimonium chloride</i> |
| 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: | Rinse-off formulation (Kräuterazid 7/0609/05/05/02): main components: 84.8% water 4.7% glyceryl stearate 3.6% stearyl alcohol 3.2% <i>cetrimonium chloride</i> 1.9% isopropyl alcohol |
| Dosages: | 2-5 mg test product per cm ² skin covered by a standard test plaster |

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| | |
|-----------------|--|
| Exposure time: | 48 hours |
| Scoring system: | <p>0 no irritation</p> <p>(+) slight or improbable erythema</p> <p>+ distinct erythema (as well as urticarial)</p> <p>++ strong erythema and/or papule formation</p> <p>+++ abundant papules and/or vesicles</p> <p>++++ blister formation or necrosis</p> |
| 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: | 1% of Dehyquart A-CA ex Henkel (1% <i>cetrimonium chloride</i>) in water 1% of Dehyquart A-CA ex Italien (1% <i>cetrimonium chloride</i>) 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: | <p>0 no reaction</p> <p>0.5 very slight reaction</p> <p>1 slight reaction</p> <p>2 moderate reaction</p> <p>3 strong reaction</p> <p>4 very strong reaction</p> <p>-1 no answer</p> |
| 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

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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

Comment

It is not clear whether a 1% aqueous solution of Dehyquart A-CA (containing 1% *cetrimonium chloride*) was applied on the skin or whether a formulation with a final concentration of 1% *cetrimonium chloride* has been applied on the skin. Dehyquart A-CA, previously mentioned in the submission, is reported to contain 24-26% *cetrimonium chloride*.

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% <i>cetrimonium chloride</i> | | | | | | | | | | |
| Dosages: | 50 µl per chamber of square Finn-Chambers (10 mm side length) | | | | | | | | | | |
| Exposure time: | 24 hours | | | | | | | | | | |
| Scoring system: | <table border="0"> <tr> <td>0</td><td>no erythema, no fissures, no scaling</td></tr> <tr> <td>1</td><td>slight erythema, minimal fissures, minimal scaling</td></tr> <tr> <td>2</td><td>clear erythema, clearly visible fissures, clearly visible scaling</td></tr> <tr> <td>3</td><td>severe erythema, distinct fissures, moderate scaling</td></tr> <tr> <td>4</td><td>very severe erythema, ulceration, distinct scaling</td></tr> </table> | 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 |
| 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: 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*
 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 perceptible 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

Under the conditions of the study, all test articles were found to be non-irritating.

Ref.: 52

Comment

Not all compositions of the tested formulations have been stated.

I. Cetrimonium chloride - single patch test 9

Date of study: Nov 1998

Opinion on alkyl (C16, C18, C22) trimethylammonium chloride, non-preservative uses

| | |
|------------------|---|
| 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% <i>cetrimonium chloride</i> 0.2% thickener 0.2% softener |
| Dosages: | 200 µl non-woven cotton patch (approx. 4 cm ²), fixed with tape |
| Exposure time: | 24 hours |
| Scoring system: | <p>0 no apparent cutaneous involvement</p> <p>0.5 faint, barely perceptible erythema <u>or</u> slight dryness (glazed appearance)</p> <p>1 faint but definite erythema, no eruptions or broken skin <u>or</u> no erythema but definite dryness; may have epidermal fissuring</p> <p>1.5 well-defined erythema <u>or</u> faint erythema with definite dryness; may have epidermal fissuring</p> <p>2 moderate erythema; may have a few papules <u>or</u> deep fissures, moderate-to-severe erythema in the cracks</p> <p>2.5 moderate erythema with barely perceptible oedema <u>or</u> severe erythema not involving a significant portion of the patch (halo effect around the edges); may have a few papules <u>or</u> moderate-to-severe erythema</p> <p>3 severe erythema (beet redness), may have generalized papules <u>or</u> moderate-to-severe erythema with slight oedema (edges well defined by raising)</p> <p>3.5 moderate-to-severe erythema with moderate oedema (confined to patch area) <u>or</u> moderate-to-severe erythema with isolated eschar formation or vesicles</p> <p>4 generalized vesicles or eschar formation <u>or</u> moderate-to-severe erythema and/or oedema extending beyond the area of the patch</p> |
| 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

Comment

Not all compositions of the tested formulations have been stated.

1. 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 |

Opinion on alkyl (C16, C18, C22) trimethylammonium chloride, non-preservative uses

| | |
|------------------|---|
| Group size: | 5 male and 46 female subjects |
| Test substances: | Hair care product Fle 433833: aqueous solution containing 10.5% amodimethicone and <i>cetrimonium chloride</i> and trideceth 5.0% <i>behentrimonium chloride</i> 3.5% myristyl alcohol 3.0% glycerin 2.0% cetearyl alcohol 1.5% sodium PCA 1.5% cetyl esters 1.0% <i>cetrimonium chloride</i> |
| 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

Comment

The test formulation also contains *cetrimonium chloride*, but its exact concentration is not mentioned.

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

3.3.11.2 Repeated insult patch test on human volunteers

In the Cosmetic Ingredient Review (CIR) on *cetrimonium chloride*, *cetrimonium bromide* and *stearyltrimonium 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% <i>behentrimonium chloride</i> 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 vesication |
| 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

No data submitted

3.3.13 Safety evaluation (including calculation of the MoS)

The submission contains the following table summarising the exposure to alkyl (C16, C18, C22) 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 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) |
|-------------------------|---------------------------------|--|--|--|
| 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 |
| Rinse-off | Eyeliner | 0.005 | 0.1 | 0.000005 |
| | 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 |
| TOTAL | | | | 0.09907 |

CALCULATION OF THE MARGIN OF SAFETY

| | | |
|--|---|---|
| Maximum amount of ingredient applied daily | = | 99.1 mg/day |
| Typical human body weight | = | 60kg |
| Maximum absorption through the skin (<i>in vivo</i> rat study <i>laurtrimonium bromide</i>) | = | 3.15% |
| Systemic exposure dose (SED) | = | $(99.1 \times 3/100) / 60 = 0.052 \text{ mg/kg bw/day}$ |
| No observed adverse effect level (12m-oral-rat) | = | NOAEL = 10 mg/kg bw/day |

| Margin of Safety | NOAEL / SED | = 192 |
|-------------------------|--------------------|--------------|
| | | |

3.3.14 Discussion

It is the opinion of the SCCP that the submission lacks a number of data with respect to the identification, stability and physico-chemical data of the three quaternary ammonium compounds under study. The lacking data as mentioned in the present opinion as **General comments on Section 3.1** are required for completion of the dossier.

With regard to the toxicological properties of P72, the table in the Annex to this opinion summarizes the test results obtained for each of the quaternary ammonium compounds under study. In view of their corrosive potential, systemic toxicity and local toxicity are discussed separately.

Systemic toxicity

Cetrimonium chloride, *steartrimonium chloride* and *behentrimonium chloride* display relatively low acute systemic toxicity. The oral LD₅₀ value of *cetrimonium chloride* was determined to be 400-600 mg/kg bw, while its dermal LD₅₀ 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 LD₅₀ 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 dermal absorption will very much depend on the state of the skin. When the final formulation is irritating because of the presence of quaternary ammonium derivatives, the absorption may become quite high. If skin absorption increases, the Margin of Safety will be inadequate, rendering quaternary ammonium compounds as being unsuitable for use in leave-on products.

Basically, the composition of the final topical product/formulation will be of crucial importance for the irritating properties of the product and which will influence dermal absorption.

Opinion on alkyl (C16, C18, C22) trimethylammonium chloride, non-preservative uses

A toxicokinetic study indicated that ¹⁴C-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.

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%. 10 mg/kg bw/day was thus determined to be the NOAEL (28-dermal-rabbit) for *cetrimonium chloride* for systemic effects.

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*.

Genotoxicity

The presented mutagenicity studies (Ames tests 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

Finally, dermal developmental toxicity studies with *cetrimonium chloride* in the rabbit and the rat revealed dose-dependent irritative effects, though 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 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.

The submission contains 8 human single patch tests with *cetrimonium chloride*-containing shampoos and hair care formulations. Concentrations ranging from 0.5 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. 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 under 3.3.13 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 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.

5. MINORITY OPINION

Not applicable

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