OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

BENZETHONIUM CHLORIDE

COLIPA n° P 70

adopted by the SCCNFP during the 26th plenary meeting
of 9 December 2003

1. Terms of Reference

1.1 Context of the question

The adaptation to technical progress of Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.

Request to adapt reference number 53 of Annex VI, part 1 – List of preservatives which cosmetic products may contain – to Council Directive 76/768/EEC.

1.2 Request to the SCCNFP

* Is Benzethonium Chloride safe for use in leave-on cosmetic products (except for products for oral use) at 0.1%?

* Does the SCCNFP propose any additional restrictions or conditions for its use in cosmetic products?

1.3 Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.

The extent to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

2. Toxicological Evaluation and Characterisation

2.1 General

2.1.1. Primary name

Benzethonium Chloride (INCI name)

2.1.2. Synonyms

4'-(1,1,3,3-tetramethylbutyl) phenoxy-ethoxyethylene-dimethyl-benzylammonium chloride
Hyamine 1622
Phemerol chloride

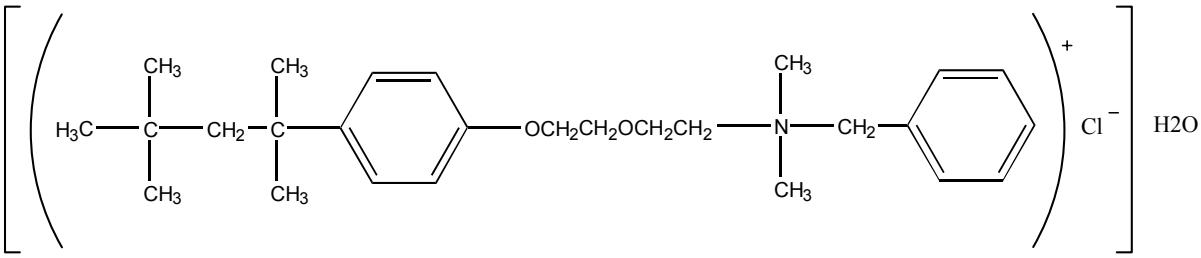
2.1.3. Trade names and abbreviations

COLIPA n° : P 70

2.1.4. CAS / EINECS number

CAS n° : 121-54-0
EINECS n° : 204-479-9

2.1.5 Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{27}H_{42}NO_2Cl$
Mol weight : 448.15

2.1.7. Purity, composition and substance codes

No data

2.1.8. Physical properties

Appearance	:	colourless et white crystals
Melting point	:	164-166 °C
Boiling point	:	/
Density	:	440.55 kg/m³
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{ow}	:	/

2.1.9. Solubility

Soluble in water, alcohol and other organic solvents.

2.2. Function and uses

Present authorised use : as a preservative in rinse-off products only at a maximum authorised concentration of 0.1%.

Requested use : as a preservative in rinse-off and leave-on products except oral care products at a maximum authorised concentration of 0.1%.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

See SCCNFP opinion on benzethonium chloride of 27 February 2002, doc. n° SCCNFP/0539/01

2.4. Irritation and corrosivity

See SCCNFP opinion on benzethonium chloride of 27 February 2002, doc. n° SCCNFP/0539/01

2.5. Skin sensitisation

See SCCNFP opinion on benzethonium chloride of 27 February 2002, doc. n° SCCNFP/0539/01

2.6. Teratogenicity

See SCCNFP opinion on benzethonium chloride of 27 February 2002, doc. n° SCCNFP/0539/01

2.7. Toxicokinetics (incl. Percutaneous absorption)

Study described in SCCNFP opinion on benzethonium chloride of 27 February 2002, doc. n° SCCNFP/0539/01

1% concentrations of Benzethonium Chloride were applied in water and ethanol:water (95:5) vehicles, to human and rat dermatomed skin membranes *in vitro* using a 'flow through diffusion cell system' (surface area exposed skin within the cells was 0.64 cm^2; formulation applied was 10 µl/cm^2 (= 100 µg Benzethonium Chloride/cm^2); receptor fluid was tissue culture medium with 4% BSA with 5% CO_2 in O_2). The solutions were left in place in donor chambers open to the atmosphere for 24 h. Receptor fluid was collected hourly for 0-6 h post dose and every other hour from 6-24 h post dose. Mean mass balance was ≥ 95% in all cases. The results are summarised in the scheme below.

Species	Human		Rat	
formulation	water	ethanol/water	water	ethanol/water
Nominal dose P70	100 µg/cm^2	100 µg/cm^2	100 µg/cm^2	100 µg/cm^2
Mass balance	94.72%	97.60%	96.06%	95.36%
Total unabsorbed	90.59%	95.62%	80.34%	77.37%
Dose site epidermis*	3.47%	1.47%	11.20%	13.43%
Dose site dermis	0.6%	0.19%	4.51%	4.07%
Non-dose site skin	0.04%	0.03%	0.53%	0.66%
Receptor fluid/ rinse	0.03 %	0.29%	0.16%	0.33%
Total absorbed	4.14%	1.98%	16.4%	18.49%

* The authors assumed that the activity in dose site epidermis represented unremoved stratum corneum (only "minimally" 5 strippings were conducted and the amount found in stratum corneum was high, viz. 3-11%), and included this figure in the unabsorbed material. However, the reviewer conservatively considers this figure in the epidermis as absorbed.

The compound poorly penetrated human and rat skin in both test formulations. Total absorption was higher in rat versus human skin.
Under the conditions of this *in vitro* study (10 µl/cm^2 of a 1% formulation [= 100 µg Benzethonium Chloride/cm^2]), the total amount maximally absorbed by human skin during 24 hours was 4.14 µg Benzethonium Chloride/cm^2.

Remark
10 µl/cm^2 exceeds the volume (5 µl/cm^2) proposed by the SCCNFP and may result in a lower percentage of absorption. On the other hand, a 1% solution was tested instead of the in use concentration of 0.1% and hence the absolute amount absorbed is clearly an overestimate.

Ref. : 32

The i*n vitro* percutaneous absorption of [^{14}C]-Benzethonium Chloride through human skin 1.0% (w/v) solution in ethanol

A 1% concentration of Benzethonium Chloride was applied in ethanol:water (95:5) to human dermatomed skin membranes (430 - 500 µm thickness) *in vitro* using a 'flow through diffusion cell system' (skin temperature was 30.7 - 31.6°C; membrane integrity had been checked with tritiated water; surface area exposed skin within the cells was 0.64 cm^2; formulation applied was 10 µl/cm^2 (≈ 10 mg/cm²) leading to 100 µg Benzethonium Chloride/cm^2; receptor fluid was tissue culture medium with 4% BSA with 5% CO_2 in O_2). The solutions were left in place in donor chambers open to the atmosphere for 24 h. Receptor fluid was collected hourly for 0-6 h post dose and every other hour from 6-24 h post dose. Mean mass balance was ± 104%. The results are summarised in the scheme below.

	Without epidermis	Epidermis included*
Nominal dose P70	100 µg/cm^2	
Mass balance	103.88%	
Total unabsorbed	103.59%	102.01%
Dose site epidermis*	1.58%	1.58%
Dose site dermis	0.18%	0.18%
Non-dose site skin	0.07%	0.07%
Receptor fluid/ rinse	0.04%	0.04%
Total absorbed	**0.29%**	**1.87%**

* The authors assumed that the activity in dose site epidermis represented unremoved stratum corneum (only "minimally" 5 strippings were conducted and the amount found in stratum corneum was high, viz. 3-11%), and included this figure in the unabsorbed material. However, the reviewer conservatively considers this figure in the epidermis as absorbed.

Under the conditions of this *in vitro* study (10 µl/cm^2 of a 1% formulation [= 100 µg Benzethonium Chloride/cm^2]), the total amount maximally absorbed by human skin during 24 hours was 1.87% or 1.87 µg Benzethonium Chloride/cm^2.

Remark
10 µl/cm^2 exceeds the volume (5 µl/cm^2) proposed by the SCCNFP and may result in a lower percentage of absorption.

Ref. : 36

The i*n vitro* percutaneous absorption of [^{14}C]-Benzethonium Chloride through human skin 0.1% (w/v) in GMS-cream and ethanol

A 0.1% concentration of Benzethonium Chloride was applied in "GMS-cream" or ethanol:water (95:5) to human dermatomed skin membranes (390 - 500 µm thickness) *in vitro* using a 'flow through diffusion cell system' (skin temperature was 30.4 - 31.6°C; membrane integrity had been checked with tritiated water; surface area exposed skin within the cells was 0.64 cm^2; formulation applied was 2 mg/cm² leading to 2 µg Benzethonium Chloride/cm^2; receptor fluid was tissue culture medium with 4% BSA with 5% CO_2 in O_2). The solutions were left in place in donor chambers open to the atmosphere for 24 h. Receptor fluid was collected hourly for 0-6 h

post dose and every other hour from 6-24 h post dose. The results are summarised in the scheme below.

	[^{14}C]-BZC in "GMS-cream"		**[^{14}C]-BZC in ethanol:water (95:5, v/v)**	
Nominal dose P70	Without epidermis	Epidermis included*	Without epidermis	Epidermis included*
	2 µg/cm^2		2 µg/cm²	
Mass balance	109.95%		93.33%	
Total unabsorbed	108.22%	105.24%	93.05%	91.78%
Dose site epidermis*	2.98%	2.98%	1.27%	1.27%
Dose site dermis	1.08%	1.08%	0.16%	0.16%
Non-dose site skin	0.46%	0.46%	0.07%	0.07%
Receptor fluid/ rinse	0.20%	0.20%	0.05%	0.05%
Total absorbed	**1.73%**	**4.72%**	**0.28%**	**1.55%**

* The authors assumed that the activity in dose site epidermis represented unremoved stratum corneum (only "minimally" 5 strippings were conducted and the amount found in stratum corneum was high, viz. 3-11%), and included this figure in the unabsorbed material. However, the reviewer conservatively considers this figure in the epidermis as absorbed.

Under the conditions of this *in vitro* study, the total amount maximally absorbed by human skin during 24 hours was :

- 4.72% or 0.094 µg Benzethonium Chloride/cm^2 (2 mg/cm^2 of a 0.1% formulation in "GMS-cream [= 2 µg Benzethonium Chloride/cm^2])
- 1.55% or 0.031 µg Benzethonium Chloride/cm^2 (2 mg/cm^2 of a 0.1% formulation in ethanol:water (95:5, v/v) [= 2 µg Benzethonium Chloride/cm^2])

Ref. : 37

2.8. Mutagenicity

See SCCNFP opinion on benzethonium chloride of 27 February 2002, doc. n° SCCNFP/0539/01

2.9. Carcinogenicity

See SCCNFP opinion on benzethonium chloride of 27 February 2002, doc. n° SCCNFP/0539/01

2.10. Special investigations

See SCCNFP opinion on benzethonium chloride of 27 February 2002, doc. n° SCCNFP/0539/01

2.11. Safety evaluation

Determination of the NOAEL [SCCNFP/0539/01]

Benzethonium Chloride has moderate acute toxicity by the oral route and high toxicity following parenteral exposure.

It produced very slight irritation at the maximum "in use" concentration (0.1 %) and significant irritation when applied at a concentration of 5 %. It was not sensitiser to guinea pig or human skin.

The acceptable NOAEL in a 28 day repeated oral studies in rats was 40 mg/kg bw/day. The NOAEL in the same animal species was at least 80 mg/kg bw/day in a 2 years study. The NOAEL was 40 mg/kg bw/day in a one year toxicity study on dogs.
Benzethonium Chloride was administered dermally to rats and mice in subacute (16 days), subchronic (13 weeks) and carcinogenicity studies (2 years). In all studies, the maximum applied dose was limited by local skin effects of various degrees of severity. In spite of those local reactions, some adverse effects, for which a relation to the treatments cannot be excluded, were noted.
The non systemic observable effects were then respectively 50 mg/kg bw/day for the 16 day study on mice, 25 mg/kg bw/day for the 16 day study on rats, 12.5 mg/kg bw/day for the 13 week studies on mice and rats.

The initial data from teratogenicity studies on Long Evans rats allow 3.6 mg/kg bw/day to be considered as a no effect level for maternal toxicity and embryotoxicity. In a study reviewed on Sprague Dawley CD Rats, the no effect level for maternal toxicity and embryotoxicity was 100 mg/kg bw/day.
In rabbits, according to a first study the non effect level by oral route was 1 mg/kg bw/day. However, as the results were considered as questionable, the teratogenicity study has been renewed in the same conditions. The no effect level was then of 3.6 mg/kg bw/day.
There was no evidence of Benzethonium Chloride effect on fertility and reproductive performance, nor in peri or postnatal studies in rats at the highest level used (35,6 mg/kg bw/day).

There was no evidence of mutagenicity according to results obtained in the *in vitro* Ames tests, chromosome aberration test and sister chromatid exchange assay. There was as well no evidence of carcinogenicity according to the results obtained in a 103 week mice study (upper dose rate: 1.5 mg/kg bw/day).

Percutaneous absorption studies

In replacement of the original percutaneous absorption study (described under 4.4.1), industry provides 2 new study reports which are believed to correctly reflect the dermal absorption of Benzethonium Chloride through human dermatomed skin *in vitro*, when the substance is present in a concentration as high as 1% and in the maximum authorized concentration of 0.1%, respectively.

- In contrast to what the study report states, it is considered by the SCCNFP that the results of the new study, with 1.0% of compound applied in EtOH/water, confirm the results of the study described in the first submission, as far as the absorbed percentages are concerned. Namely 1.87% is found as total amount absorbed whereas this was 1.98% in the study of the first submission. It is true that the flow through rates differ, but these values are not used for the calculation of the Margin of Safety, since they do not take into account the amount of test compound retrieved in the epidermis.
- In the study in which 0.1% of the compound was applied, the maximum dermal absorption percentage of 4.72% is reported.

The recent studies constitute a clear improvement compared to the original study, with the exception of the following shortcomings :

- The raw data of both experiments are very limited (no tables of absolute amounts on the individual time points).
- The solubility of the test compound in the receptor fluid is not stated. Although the substance is likely to be soluble in the receptor fluid (viewing the fact that it is water soluble), it is not substantiated in the test report.
- The Log P_{ow} value has not been given.
- The exact quantitative composition of the "GMS-cream" is not stated.
- There appears to be a decay in radioactivity associated with the test compound. The performing laboratory had to further purify the received test material. The presence of unlabelled Benzethonium Chloride in the tested mixture has to be ruled out in order to avoid an underestimation of the percutaneous absorption value. In the second study, there even appears to be an additional problem with the content of [^{14}C]-Benzethonium Chloride at the start of the test (even after the additional purification step).

Despite the shortcomings mentioned above, the SCCNFP does not consider it necessary to perform additional percutaneous absorption studies with Benzethonium Chloride. Since only 5 tape strippings have been done for the measurement of the amount of test compound in the stratum corneum, it is very likely that the value measured for the epidermis, will constitute an overestimation of the actual amount.

Therefore, it is acceptable to perform the calculation of the Margin of Safety with the obtained value of 4.72% dermal absorption (dermis-value is included in this figure).

Calculation of the Margin of safety

According to data present in the first submission, the NOAEL in oral repeated dose studies in rats may be considered as being 3.6 mg/kg bw/day which corresponds to the lowest oral dose presenting no adverse effect from all acceptable given studies.

Based on the data from the recently conducted *in vitro* skin penetration studies, the following calculations can be made.

Percutaneous absorption	=	4.72%
Intended concentration in finished product	=	0.1%

Typical body weight		=	60 kg
Cumulative exposure to preservatives		=	17790 mg/day
Systemic exposure (SED) bw/day	17790 x 0.001 x 0.0472/60	=	0.014 mg/kg
NOAEL		=	3.6 mg/kg bw/day

Margin of Safety	**NOEL / SED**	**=**	**257**

2.12 Conclusions

For use in all types of leave-on cosmetics, the above Margin of Safety is considered acceptable.

2.13 References

32. Wilson D.M., White R.D., Gonder J., Preliminary pharmacokinetics study of dermally applied ^{14}C-Benzethonium Chloride in rats. Baxter Lab. Report, Study 10936, 4.10.02
36. Roper C.S. The *In vitro* Percutaneous Absorption of [^{14}C]-Benzethonium Chloride Through Human Skin as a 1.0% (w/v) solution in Ethanol. Inveresk Research, Tranent EH33 2NE Scotland. Project 203068, report number 21705, 15 October 2002.
37. Roper C.S. The *In vitro* Percutaneous Absorption of [^{14}C]-Benzethonium Chloride Through Human Skin at an incorporation rate of 0.1% (w/v) GMS Cream and Ethanol. Inveresk Research, Tranent EH33 2NE UK. Project 203089, report 21706, 15.10.02

3. OPINION OF THE SCCNFP

The SCCNFP is of the opinion that the data provided in the submitted dossier support the requested use of benzethonium chloride as a preservative in leave-on products, except products for oral use, up to a maximum concentration of 0.1%.

4. Other considerations

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5. Minority opinions

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