

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

CONCERNING

BENZETHONIUM CHLORIDE

Colipa n° P70

adopted by the SCCNFP during the 19th Plenary meeting
of 27 February 2002

1. Terms of Reference

1.1 Context of the question

The adaptation to technical progress of the 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

The SCCNFP is requested to answer the following questions :

- * Is Benzethonium chloride safe for use in cosmetic products under the proposed conditions of use?
- * 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 extend 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

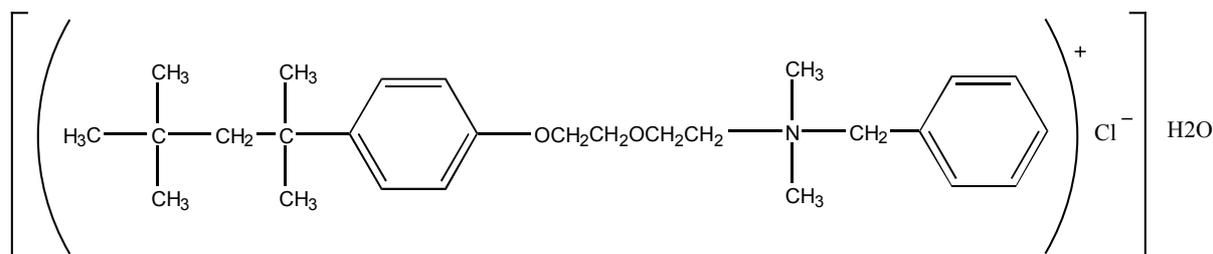
2.1.3. Trade names and abbreviations

Colipa No. : P 70

2.1.4. CAS number

121-54-0

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $\text{C}_{27}\text{H}_{42}\text{NO}_2\text{Cl}$
Mol weight : 448.15

2.1.7. Purity, composition and substance codes

No data

2.1.8. Physical properties

Subst. Code	:	
Appearance	:	colourless et white crystals
Melting point	:	164-166 °C
Boiling point	:	/
Density	:	27.5 lbs/ft ³
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 preservatives in rinse-off products only at a maximum authorised concentration of 0.1%.

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

TOXICOLOGICAL CHARACTERIZATION**2.3. Toxicity****2.3.1. Acute oral toxicity**

- Oral, Rat, LD₅₀ = 420 mg/kg
- Oral, rat, LD₅₀ = 295 mg/kg (95% conf. limits: 160 and 543 mg/kg) Ref. : 33
- I. P., Rat, LD₅₀ = 33 mg/kg
- I.V., Rat, LD₅₀ = 19 mg/kg

2.3.2. Repeated dose oral toxicity**Subacute study**

- In a 28-day feeding study, rats received diets with 0, 20, 100, 500 or 2500 ppm, providing intake levels of 0, 1.7, 8, 40 or 200 mg/kg bw/day. The changes in the top-dose group included growth retardation, caecum enlargement, signs of liver damage and decreased serum levels of inorganic phosphorus in males. The last finding was the only effect considered treatment-related in males fed 500 ppm. The diet with 100 ppm (8 mg/kg bw/day) was a clear NOAEL.

Ref. : 14

- A supplementary 28-day study in rats with the same feeding levels was conducted to verify and extend certain findings in the previous study. The results confirmed most of the changes seen at the top dose, including caecal enlargement. The latter finding was not accompanied by histopathological changes. Decreased levels of serum-P seen at the two higher levels in the previous study did not occur in the present study. Therefore, 500 ppm (or 40 mg/kg bw) was the NOAEL in the supplementary study.

Ref. : 15

2.3.3. Repeated dose dermal toxicity

Subacute study

In a dermal application of 2 ml 0.1 % solution to the skin of rabbits daily, 5 days/week for 4 weeks no systemic effects were observed (Summary Report).

In a 16-day study, F 344/N rats (5 males + 5 females/group) received topical applications of a fixed 250 ml volume of ethanol solutions corresponding to 0, 6.3, 12.5, 25, 50 or 100 mg benzethonium chloride/kg bw/day. Animals were treated 5 days per week for a total of 12 doses. No mortality was observed. The body weight development was reduced at 50 or 100 mg/kg bw, probably related to the stress produced by the skin lesions. There were no other signs of predictive systemic toxicity; absolute and relative weights of thymus were also reduced at these dose levels. Skin alterations were found in most animals; thickening or hardening in rats at 25, 50 and 100 mg/kg bw; histopathological skin lesions - ranging from epithelial hyperplasia with minimal inflammation, which were found at all doses, and the intensity of which were dose related - were observed at all dose levels. The no systemic effect level was then at least 25 mg/kg bw/day.

Ref. : 17

In another 16 day study B6C3F1 mice (5 males + 5 females/group) received the same benzethonium chloride dose levels in a fixed 100 µl volume of ethanol solution, 5 days per week for a total of 12 doses. One male died at 100 mg/kg bw; a relation with the treatment cannot be excluded. The body weight development was higher than in the controls. The absolute and relative weights of thymus were decreased in 100 mg/kg bw females. There were no other signs predicting systemic toxicity.

The skin lesions were more or less similar to those observed in rats. The no systemic effect level was then at least 50 mg/kg bw/day.

Ref. : 17

2.3.4. Sub-chronic dermal toxicity

In a 13 week study, F344/N rats (10 males + 10 females/group) received topically in an ethanol vehicle (volume not exceeding 300 µl), 0, 1.56, 3.13, 6.25, 12.5, 25.0 mg benzethonium chloride/kg bw/day). Animals were treated 5 days per week. Clinical findings were recorded weekly, body weights at the beginning of treatment, weekly thereafter and at the end of the study. Necropsy was performed on all animals and brain, heart, right kidney, liver, lungs, right testis and thymus weighed.

Ref. : 29

A complete histopathological examination of tissues was performed in control and high dose groups. The skin at the site of application and untreated skin areas were examined histopathologically in all animals. No mortality was observed. At the higher dose, the final mean body weight and body weight gain of males was significantly lower than those of the controls. At the same dose level, a decrease in the weight of the thymus was noted, postulated to be due to the stress produced by the skin lesions. An increase of the myeloid cells in the bone marrow related to skin inflammation was found at 25 mg/kg bw. No other direct or potentially systemic alterations were found.

Clinical skin reactions were observed at the site of applications in animals receiving 3.13 mg/kg bw or more. Histopathological skin lesions, ranging from epithelial hyperplasia and inflammation to necrotizing ulceration involving the underlying and subcutaneous tissues. These changes were observed in all dosed animals, and their severity was dose related. The no systemic effect level was then at least 12.5 mg/kg bw/day.

Ref. : 17

In another 13 week study B6C3F1, mice (10 males and 10 females/group) were topically treated with the same dose levels in an ethanol vehicle not exceeding 100µl, 5 days per week. Clinical findings were recorded weekly, body weights at the beginning of treatment, weekly thereafter and at the end of the study. Necropsy was performed on all animals and brain, heart, right kidney, liver, lungs, right testis and thymus weighed. A complete histopathological examination of tissues was performed in control and high dose groups. The skin at the site of application and untreated skin areas were examined histopathologically in all animals. No mortality was observed. All mice survived to the end of the study. The final mean body weights of all dosed groups of males and females were similar to those of the controls, although the mean weight gain of 25 mg/kg bw/day males was reduced. Marginal increases of the relative weights of liver and kidney noted in 12.5 and 25 mg/kg bw/day males were due to the lower body weights and not considered of toxicological significance. No other direct or potentially systemic alterations were found.

Clinical findings included crusting, scales, thickening and reddening of the skin at the site of application in animals receiving 6.25 (males only), 12.5 or 25 mg/kg bw/day, Histopathological lesions from 6.25 mg/kg bw/day upwards included minimal epithelial hyperplasia, chronic inflammation and focal necrosis of the epithelium involving the underlying dermis and subcutaneous tissues. At 1.56 or 3.13 mg/kg bw/day minimal epithelial hyperplasia with or without chronic inflammation were present.

The no systemic effect level was there at least 12.5 mg/kg bw/day.

Ref. : 17

2.3.5. Chronic toxicity

In a one year study, groups of 3 dogs were fed 0, 5, 100 or 500 ppm (providing intake levels of 0, 0.4, 8 or 40 mg/kg bw/day) in the diet. No changes were observed in growth rate, haematology or in gross or microscopic pathology. The NOAEL can be considered greater than 40 mg/kg bw.

A two-year study has been conducted with groups of 5 rats/sex, fed diets containing 0, 50, 200, 1000, 2500 or 5000 ppm (providing 0, 4, 16, 80, 200 or 400 mg/kg bw/day). The top dose induced mortality. With 2500 and 5000 ppm testicular atrophy and caecal enlargement occurred.

With 1000 ppm there was only caecal enlargement. The NOAEL can be considered at least 80 mg/kg bw.

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Skin irritation in rabbits did not occur when 2 ml of a 0.1 % dilution were applied daily 5 days a week for 4 weeks.

Topical application of a 1% (w/v) aqueous solution of benzethonium chloride (100 µl/ 10 cm²) to the dorsal skin of three male and three female rats for 5 days, only produced slight irritation (very slight to well-defined erythema and occasional very slight oedema).

Ref. : 31

In humans, 0.1 ml of a 5 % aqueous solution applied under patches for 48 hours, was irritating.

A skin irritation study was conducted with five female subjects. Benzethonium chloride was administered as 5%, 2.5%, 1.25%, 0.63%, 0.31% and 0.15% solutions (w/v) in water (0.2 ml under occlusive dressing, four applications over nine days).

Three of the five subjects exhibited responses to the 5%, 2.5%, 1.25% and/or the 0.63% level.

No reactions were observed with the 0.31% concentration and this was considered an appropriate level for a subsequent repeated insult patch test (see point 2.5., Ref. 35 below).

Ref. : 34

2.4.2. Irritation (mucous membranes)

Very slight irritation to the eye of rabbits was produced at concentrations as low as 0.01 and 0.03%.

The test was performed on New Zealand White rabbits according to OECD Guideline n° 405 (3 rabbits). A 0.1 % aqueous solution of benzethonium chloride was minimally irritant to the rabbit eye without rinsing after instillation of the solution.

Ref. : 26

2.5. Sensitisation

A sensitisation test in humans with 0.12 % in formulations applied to the skin under closed patches was negative.

A maximisation test according to the Magnusson & Kligman method was performed according to OECD guideline n° 406 (20 tests and 10 controls DH Guinea Pig). benzethonium chloride concentrations were selected on the basis of the results of a screening test performed to detect the concentration giving a very slight erythema at 24 hours observation. Filter paper patches saturated with 0.2 or 0.5 % (w/w) aqueous solutions benzethonium chloride were applied under occlusion for a period of 24 hours. No skin reactions were noted at the challenge sites of the tested or control group animals at the 24 or 48 hours observation.

The (cumulative) irritation and/or sensitisation potential of benzethonium chloride (purity 100%; 0.3% aqueous solution) was examined in 152 human subjects. Aliquots (0.2 ml) of test and control solutions were applied under occlusive dressing to the same site three times a week for a total of 10 applications. Patches were removed after 24 hours exposure. Approximately 14 days following the 10th application, a challenge patch was applied to the original site and to a virgin site, and the sites were evaluated after 24 and 28 hours.

During the induction phase, six subjects showed mild to well-defined erythema and one subject exhibited mild to marked responses. With one exception (the subject that showed marked responses during induction) all observations were negative during the challenge phase. (The one responding subject was classified as a 'reactive individual' and his data were not considered in the final results). It was concluded that benzethonium chloride when administered at 0.3% did not elicit dermal irritation and/or sensitisation.

Ref. : 35

2.6. Teratogenicity

Fertility and reproductive performance were examined in rats treated orally with 1.1, 3.6 and 35.6 mg/kg bw/day prior to and during mating and during the gestation and lactation period. The high-dose produced growth depression, increased irritability, respiratory signs in the parents and decreased viability and body weight of pups at birth. Fertility and general reproductive performance were not affected. The NOAEL has to be considered higher than 35.6 mg/kg bw/day for the fertility and reproductive performance.

Ref. : 5

An oral teratogenicity study in New Zealand white rabbits (15/group) with 1, 3 and 10 mg/kg bw/day on gestational days 7 to 19 revealed signs of maternal toxicity with 3 and 10 mg, increased mortality of mothers and pups with 10 mg, and an increased incidence of supernumerary ribs with 3 and 10 mg. Supernumerary ribs are known to occur secondary to maternal toxicity (Khera 1985). No teratogenic effects have been observed; the NOAEL for maternal toxicity and embryotoxicity was 1 mg/kg bw/day.

Ref. : 3

In a second teratogenicity study in New Zealand white rabbits (15 to 27/group) with oral dosing of 1.1, 3.6 and 35.6 mg/kg bw/day, on gestational days 7 to 19, the high dose induced maternal and foetal mortality. A dose-related increase in foetal resorptions occurred in all treatment groups although the change was statistically significant only in the high-dose group. No substance related malformations were found at any dose level. The NOAEL for maternal toxicity and embryo-toxicity in this study was 3.6 mg/kg bw/day.

Ref. : 8

- In a teratogenicity study in Long Evans (20 per Group) rats with oral dosing of 1.1, 3.6 or 35.6 mg/kg bw/day on gestational days 6 to 15 the high-dose group showed decreased maternal body weight and an increased number of smaller pups. An increased incidence of skeletal variants (ossification effects) occurred in all treated groups.

Skeletal malformation was increased in the high-dose group. Slight hydrocephalus was seen in one pup of the mid-dose group and in 5 pups (in 2 litters) of the high-dose group; workers assume that the delays of ossification, according to their low incidence (almost in one litter) "are

secondary to the maternal toxicity and do not represent a primary action of the substance on the embryo". Nevertheless, the mid dose was not clearly without effect on maternal toxicity.

Ref. : 6

The study has been renewed by the same workers in Long Evans rats (18 to 20/group) with oral dosing of 0, 0.06, 1.1, 3.6 or 35.6 mg/kg bw/day, on gestational days 6 to 15; this second teratogenicity study showed lower maternal body weights, increased variation of skeletal ossification and increased incidence of skeletal malformations (wavy ribs) in the top-dose group only. The last finding was considered to be within the limits for historical controls. Under the conditions of this study no teratogenic potential was found. The NOAEL for maternal toxicity and embryotoxicity was 3,6 mg/kg bw/day.

Ref. : 7

Peri-and postnatal effects were examined in rats dosed orally with 1.1, 3.6 and 35.6 mg/kg bw/day from day 15 of gestation through day 20 of lactation. A slight decrease in foetal viability occurred in all dosed groups and in postnatal survival in the mid- and top-dose group. Those findings may be related to the maternal toxicity.

Ref. : 9

An additional oral teratogenicity study has been carried out (1995). Sprague Dawley CD pregnant rats (24/group) were treated by oral gavage on gestational days 6 to 15. Benzethonium chloride doses were 0, 10, 30, 100 and 170 mg/kg bw/day in water vehicle, 10 ml/kg volume). All animals were autopsied on day 20 of gestation. Maternal examination included mortality clinical signs, body weight, food consumption and gross pathology; at cesarian section, corpora lutea, implantation sites, resorptions, foetal viability and foetal body weight were recorded. Gross external visceral and skeletal examinations were done on foetuses.

The high dose induced maternal mortality, reduced body weight development, body weight loss, reduction of the food consumption, together with other clinical signs, mainly alopecia, hypersalivation, fur staining, hypothermia, ptosis and abnormal faeces. Necropsy in rats that died during the study presented gastrointestinal lesions e.g. black spots on the mucosal surface of the stomach and gaseous distension of intestine or caecum, possibly corresponding to post-mortem alterations.

The treatment did not have any effect on the number of resorptions, litter size, foetal viability or foetal body weights at any dose level.

External visceral and skeletal examinations of the litters did not reveal variations or malformations attributable to the treatment. No significant differences in ossification were found among the five groups.

Under the conditions of this study, maternal toxicity was not evidenced up to 100 mg/kg bw/day, benzethonium chloride is not teratogenic nor embryotoxic in CD rats up to the maternal toxic and lethal dose of 170 mg/kg bw/day.

Ref. : 28

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Dermal absorption was examined by applying 10 ml of a 10 % aqueous solution of the ¹⁴C-labelled compound under occluded patches to the skin of two rabbits on 4 consecutive days. One rabbit had the skin abraded. Blood samples taken on each day showed an average concentration of 0.2 ppm, which corresponds to 0.003 % of the amount applied. No mention is made of

analyses in urine, faeces or carcasses and it is impossible to make any assessment of the total amount absorbed.

Ref. : 12

Maternal and foetal absorption of the ^{14}C -labelled compound was examined in pregnant rats treated orally with 1.1 and 3.6 mg/kg/day on days 6 through 15 of gestation. Average blood levels in the two groups were 1.5 and 0.97 ng/g respectively. In urine, the maximum levels were 52 and 149 ng/ml after a single oral dose. Virtually all radioactivity was found in the maternal faeces and carcass. Results of foetal analyses varied between not-detectable and 6.8 ng/g foetus.

Ref. : 13

The percutaneous absorption of a 0.5 % aqueous emulsion has also been investigated in human volunteers by measuring the rate of deposition in the stratum corneum and calculation of the permeability constant.

In a first study using 16 volunteers and a surface recovery method (at 0.5 hour then at hourly intervals from 1-6 hours) two penetration rates were noted. First a rapid transfer to the stratum corneum from the start to one hour, with $4.56 \mu\text{g}/\text{cm}^2$ in 30 minutes and $5.19 \text{g}/\text{cm}^2$ in 1 hour. The further penetration rate into the stratum corneum from 1 h to 6 h was calculated as $0.25 \mu\text{g cm}^{-2}\text{h}^{-1}$. After reaching of the steady flow a percutaneous penetration constant of $50 \mu\text{g cm}^{-2}\text{h}^{-1}$ was calculated.

In a second experiment using 6 volunteers the amount stored in the stratum corneum after 30 minutes application was determined in another way using an abrasion technique to remove surface layers. The result indicated a similar percutaneous penetration constant, namely about $50 \mu\text{g}/\text{cm}^2 \text{h}^{-1}$. According to the rationale of the recovery method, the first experiment recorded both the amount that entered the stratum corneum and stayed there over the entire duration of the experiment, and the amount that remained there the stratum corneum and was transferred to the viable tissue (circa $0.25 \mu\text{g cm}^{-2} \text{h}^{-1}$). The second experiment only confirmed the first figure. The value of this method for measuring skin absorption is controversial, but the data indicated appreciable absorption through the skin can occur with a 0.5 % formulation.

Data from *in vitro* studies using an aqueous emulsion of 0.5 % compound and excised abdominal skin did not, however, indicate any significant absorption. The concentration of benzethonium chloride in the receptor fluid remained below the detection limit ($0.1 \mu\text{g}/\text{ml}$) during the 72 hours exposure.

Ref. : 16

The additional data provided in Submission VII were essentially a detailed justification of the surface recovery technique which has been used to determine the benzethonium chloride skin absorption.

In a pilot toxicokinetic study, the levels and time course of appearance/disappearance of radioactivity in blood was examined over 48 hour in F344 rats (3/sex), after non-occluded dermal application of a 1% aqueous solution ($100 \mu\text{l}/10\text{cm}^2$) of ^{14}C -labelled benzethonium chloride. Quantifiable levels of radioactivity were not observed in blood samples, and it was concluded that a definitive toxicokinetic study was of no use.

An average of 0.37 and 7.3% of the administered radioactivity was recovered in the composites of urine and faeces, respectively. The relatively high amount recovered in rat urine/faeces was consistent with the results obtained in an *in vitro* study with rat skin (see Ref. 30 below).

Ref. : 32

1% concentrations of ^{14}C -labelled 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 $10\text{ }\mu\text{l}/\text{cm}^2$ (= $100\text{ }\mu\text{g}$ 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 $\geq 95\%$ in all cases. The results are summarised in the scheme below.

Species	Human		Rat	
	water	ethanol/water	water	ethanol/water
Nominal dose P70	$100\text{ }\mu\text{g}/\text{cm}^2$	$100\text{ }\mu\text{g}/\text{cm}^2$	$100\text{ }\mu\text{g}/\text{cm}^2$	$100\text{ }\mu\text{g}/\text{cm}^2$
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 ($10\text{ }\mu\text{l}/\text{cm}^2$ of a 1% formulation [= $100\text{ }\mu\text{g}$ Benzethonium Chloride/ cm^2]), the total amount maximally absorbed by human skin during 24 hours was $4.14\text{ }\mu\text{g}$ benzethonium chloride/ cm^2 .

Remark :

$10\text{ }\mu\text{l}/\text{cm}^2$ exceeds the volume ($5\text{ }\mu\text{l}/\text{cm}^2$) 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

2.8. Mutagenicity

An Ames test with *S. typhimurium* strains TA98, TA100, TA 1535, TA 1537 was negative in the absence (0.01 to $1.0\text{ }\mu\text{g}$ benzethonium chloride/plate) or presence (1.0 to $100.0\text{ }\mu\text{g}$ benzethonium

chloride/plate) of metabolic activation. Activation was brought about by the addition of S9 mix from male rat liver induced by „Aroclor 1254“.

Ref. : 29

A chromosome aberration test in Chinese hamster ovary cells negative in the absence (0.96 to 9.6 µg benzethonium chloride/ml) or the presence (3.0 to 30 µ benzethonium chloride/ml) of metabolic activation. Activation was brought about by the addition of S9 mix from male SD rat liver induced with „Aroclor 1254“: no statistical significant or dose related increase in chromosomal aberrations, no cell cycle delay were noted.

Ref. : 29

A sister chromatid exchange assay without activation (0.96 to 9.6 µ benzethonium chloride /ml) and with activation (3.0 to 30 µg benzethonium chloride/ml) was negative. Activation was brought about by the addition of S9 mix from male SD rat liver cells induced with „Aroclor 1254“. No cell cycle delay was noted.

Ref. : 29

2.9. Carcinogenicity

Groups of 60 males and 60 females B6C3F1 mice aged of 5 to 6 weeks were topically treated with 0, 0.15, 0.5 or 1.5 mg benzethonium chloride/kg b.w./day in ethanol vehicle (volume 50-131µl) for males and females 5 days per week for 103 weeks. An interim examination was performed after 15 months. All animals were observed twice daily for moribundity and mortality. Clinical signs were recorded monthly and body weights were recorded weekly through week 10, once during week 12 and monthly thereafter. Necropsy was performed on all animals. At the 15-month interim sacrifice, the left kidney, right kidney and liver were weighted. A complete histopathological examination of tissues was performed in control and high dose animals. The skin at the site of application and untreated skin areas were examined histopathologically in all animals. Survival of dosed mice was similar to that of the controls throughout the study. Mean body weights of all dosed groups were similar to those of the corresponding controls.

Reddening of the skin was observed at the site of application in all dosed male groups and in 0.15 mg/kg b.w./day females. Crusts were observed in 0.5 mg/kg bw/day females. There were no other clinical findings considered to be treatment related.

There were no increased incidences of neoplasms, in particular, of those associated with the skin, that were attributed to the treatment with benzethonium chloride. Treatment related non-neoplastic lesions at the site of application were epithelial hyperplasia of minimal to mild severity. Epithelial hyperplasia was commonly observed in 1.5 mg/kg bw/day males and females at the 15-months interim evaluation. At the end of the study, a dose related increase in the incidence of epithelial hyperplasia was observed in males and females.

Under the conditions of this dermal carcinogenicity study there was no evidence of carcinogenic activity of benzethonium chloride in male or female B6C3F1 mice up to the highest dose applied, 1.5 mg/kg bw/day.

Ref. : 29

2.10. Special investigations

Several subcutaneous injection studies have been reported in rats and mice. In one study in rats, a dose-related increase in the incidence of granulomatous reactions (mainly fibrosarcomas) occurred at the injection site.

Concentrations as low as 0.002 % inhibited the motility of the isolated ileum of rats and rabbits. Blood pressure measurements in the dog indicated nearly complete blockage of sympathetic ganglia at an i.v. dose of 2 mg/kg.

2.11. Safety evaluation**Determination of the NOAEL**

In summary, 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 administrated 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

The results obtained concerning the skin absorption of benzethonium chloride are controversial :

- Experimental data on living animals (rabbits and rats) have shown very slight absorption of benzethonium chloride.
- Absorption has also been investigated in human volunteers according to two methods which have not been entirely validated; nevertheless, according to the authors, they have shown deposition of benzethonium chloride on the stratum corneum and its transfer to the viable tissues circa $0.25 \mu\text{g cm}^{-2} \text{ h}^{-1}$.
- An *in vitro* test using an 0.5 % aqueous emulsion of benzethonium chloride on excised abdominal skin did not indicated any significant absorption.

The results obtained from the above absorption studies are not convincing as they are quite different according to the method undertaken: no penetration has been demonstrated in a passive form study (*in vitro*), slight penetration has been obtained by a more active but controversial system in an *in vivo* assay.

- In a recent *in vitro* study with human and rat dermatomed skin membranes, the max penetration of $10 \mu\text{l/cm}^2$ of a 1% formulation of benzethonium chloride through human skin was 0.29% ($0.29 \mu\text{g benzethonium chloride/cm}^2/24\text{h}$) and the total amount absorbed was 4.14% ($4.14 \mu\text{g benzethonium chloride/cm}^2/24\text{h}$).

Competition may exist between the local reaction induced on skin by benzethonium chloride and its absorption through the skin.

Calculated safety margin

According to the all given data, the NOAEL in oral repeated dose studies 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 a recently conducted *in vitro* skin penetration study the following calculations can be made.

Percutaneous absorption	:	$0.29 \mu\text{g Benzethonium Chloride/cm}^2/\text{day}$	
Typical body weight	:	60 kg	
Skin area surface	:	18000 cm^2	
Systemic exposure	:	$\frac{18000 \text{ cm}^2 \times 0.29 \mu\text{g/cm}^2}{60 \text{ kg} \times 1000}$	= 0.09 mg/kg bw/day

NOAEL : 3.6 mg/kg BW/day
 MOS : 40

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

2.12. Opinion

The data provided in the submitted dossier does not support the requested use of Benzethonium chloride as a preservative in leave-on products.

Its present use as a preservative in rinse-off products at a maximum authorised concentration of 0.1 % is considered safe.

2.13. References

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