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

CONCERNING

CHOLINE CHLORIDE

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 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.

Choline chloride is currently listed in Annex II – list of substances which must not form part of the composition of cosmetic products – to Directive 76/768/EEC (entry n° 168 : choline salts an their esters, e.g. choline chloride) and is therefore banned for use in any cosmetic product.

The European Commission was requested to amend Annex II and Annex III in order to allow the use of choline chloride in certain cosmetic products.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Does the safety profile documented in the attached submission support the use of choline chloride in cosmetic products?

* Does the SCCNFP propose any restrictions or conditions for the use of choline chloride 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

The information in the dossier submitted was derived mainly from literature.

2.1.1. Primary name

Choline chloride (INCI name)

2.1.2. Chemical names

Ethanaminium, 2-hydroxy-N,N,N-trimethyl-,chloride

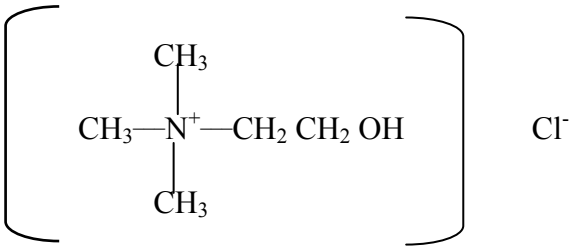
2.1.3. Trade names and abbreviations

(2-hydroxyethyl)trimethylammonium chloride
Choline hydrochloride
Cholinium chloride aqueous solution
Hepacholine
Lipotril

2.1.4. CAS n° / EINECS n°

CAS No : 67-48-1
EINECS n° : 200-655-4

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_5H_{14}NOCl$
Mol weight : 139.63

2.1.7. Purity, composition and substance codes

Purity : /

2.1.8. Physical properties

Appearance	:	white crystals
Melting point	:	303-305 °C (decomposes)
Boiling point	:	not applicable
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{ow}	:	-5.16

2.1.9. Solubility

Soluble in water and ethanol, soluble in acetone and chloroform, insoluble in ether and benzene.

2.2. Function and uses

Choline has humectant properties. Request for use in personal care rinse-off formulations, such as soap bars and liquid body soaps, at a final concentration up to 5%.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

Members of the Life Sciences Research Office agreed that 16-20 g/day of choline chloride approximated the highest tolerable dose. Orally administered choline above this dose is limited by the occurrence of gastrointestinal side effects.

Ref. : 6

In the USA, an adequate intake of 550 mg daily for men and 425 mg daily for women has been determined for choline. The tolerable upper intake level for adults is 3500 mg daily.

Ref. : A

Adequate daily intakes (ADI) of choline have been established. A summary of these for various age groups is presented in the table below. All of the above values are for the choline base. Values for choline salts are higher. For example, values for choline chloride would be 1.4 times as high.

Infants	0-5 months	125 mg/day or 8 mg/kg
	6-11 months	150 mg/day or 17 mg/kg.
Children	1-3 years	200 mg/day
	4-8 years	250 mg/day
	9-13 years	375 mg/day
Males	14 and over	550 mg/day
Females	14-18 years	450 mg/day
	19 and over	425 mg/day
Pregnant Women		450 mg/day

Lactating Women	All ages	550 mg/day

Ref. : B

The safety margin between the daily requirement (for rats : 16 to 42 mg/kg bw) and the toxic concentration (LD50 : 280 to 750 mg/kg bw/day) is relatively narrow with choline.

Ref. : C

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

A 21-Day Cumulative Irritation study on 25 subjects with self-perceived sensitive skin was conducted. The materials evaluated were 0.5 % choline chloride aqueous solution, a soap bar containing 5 % choline chloride and a liquid body soap containing 5 % choline chloride. The soap bar and liquid body soap formulas (both 1.0 % w/v aqueous solutions) and 0.5 % choline chloride aqueous solution and vehicle control (water) were evaluated. The positive control was 0.75 % (w/v) Sodium Lauryl Sulfate. The controls were the respective choline chloride-free samples: water, choline -free soap bar, and choline-free liquid body soap.
Test samples were applied to the back of volunteer subjects under semi-occlusive patch conditions. Twenty-four hours after application, the patches were removed, the sites evaluated for signs of irritation, and identical patches applied to the same sites. This procedure was repeated daily for a period of 21 consecutive days, although patches applied on a Friday were not removed until the next Monday.

Results
Statistical analysis of the cumulative irritancy demonstrated no significant differences between the samples containing choline chloride and their respective choline chloride free controls. Based on these results, the choline chloride-containing soap bar, liquid body soap and solution as well as the choline chloride-free counterparts were classified as not significantly irritating.

There was no study report provided. The provided Ref 21 Colgate-Palmolive Study No. DCR-2000-117-TKL. Study is for a baby powder.

Ref. : 21

2.4.2. Irritation (mucous membranes)

No data

2.5. Sensitisation

A Human Repeated Insult Patch Test was done on two hundred two subjects. The test concentration was 0.5 % (w/v) choline chloride aqueous solution during the induction phase and 0.2 % (w/v) aqueous solution during the challenge phase. The vehicle (distilled water) was used as the control. In addition, 0.1 % (w/v) Sodium Lauryl Sulfate was used as an internal control to assess subject compliance. The patch conditions were occlusive patch.

During the induction phase, the test material was patched for 24 hours on the back of the volunteer subjects; 48 h post-application sites were evaluated and identical patches applied.

Sites patched on Friday were evaluated the following Monday, though. A rest period of two weeks followed the induction phase. During the challenge phase, the test material was patched for 24 h to previously unexposed sites and the sites were evaluated 48- and 72-h post application.

The results of the study showed no evidence of dermal sensitisation reactions elicited by choline chloride.

Ref. : 22

Patch testing was done on a woman who developed acute dermatitis from working with indoor plants sprayed with insecticides and Cycocel, a growth inhibitor. The growth inhibiting substances in Cycocel are reported to be chlormequat (2chloroethyl-trimethyl-ammonium chloride) and choline chloride (2-hydroxyethyltrimethylammonium chloride). Repeat patch tests with choline chloride were also done.

Patch test were done with Cycocel (1 % pet.) and Cycocel 10 %. Repeat patch test were done with choline chloride (1 % in water and in pet.), and 1 % chlormequat.

Patch test of Cycocel (1% pet.) was negative, but Cycocel 10% pet. was positive. Repeat patch tests with choline chloride were positive (++ to +++ reactions) whereas 1 % chlormequat was negative. Control tests with the 2 substances in 10 patients were negative.

These results were interpreted by the submission authors to indicate a relatively low irritancy potential of choline chloride.

Ref. : 23

Comment
In the HRIP Test, the age range and sex of the volunteers was not provided. The test concentration was 0.5%, whilst the submission has asked for concentrations up to 5% to be considered. The case history showed mild to strong positive reactions suggesting that 1% choline chloride has an irritancy potential.

2.6. Teratogenicity

No data

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Percutaneous absorption *in vitro*

Guideline	:	OECD draft 428
Tissue	:	Human epidermal skin (3 donors)
Method	:	Franz diffusion cells
Test material	:	Choline chloride (Aldrich)
Batch No	:	MI 11612PU, purity: 99 %
Dose level	:	10µl/cm^2 of 50 mg/ml choline in water
Receptor fluid	:	PBS saline (pH 7.4)
Replicate cells	:	12 occluded, 12 non-occluded, 3 control
Analytical method	:	Liquid scintillation counter
GLP	:	In compliance

Skin absorption was determined using radiolabelled (50 mCi of 1,2-^{14}C) choline chloride by liquid scintillation. Full thickness skin membranes were prepared from human abdominal and breast skin obtained from three donors. Skin samples were mounted on Franz-type diffusion cells, and ^{14}C-choline chloride was applied to the surface of the skin for 24 hours. Samples were evaluated under occluded and non-occluded conditions.

A single application of choline (5 %) was administered to the surface of the skin. The average volume that was applied to occluded cells was 9.26 µl. The average volume that was applied to unoccluded cells was 8.26 µl.

The surface area in contact with the substance was approximately 1.0 cm ^{2} with a nominal receptor chamber volume of 3 ml. The exact area and volume was measured for each diffusion cell. 200 µl samples were taken at 1, 2, 6, 12, and 24 hours. The amount of choline that penetrated into the receptor fluid was 0.457 µg/cm^2 in the occluded cells and 0.383 µg/cm^2 in the unoccluded cells. This corresponds to 0.127 % and 0.110 %, respectively of the applied dose.
Total absorption was determined from radiolabelled choline levels in the epidermis, dermis and receptor fluid. Under the conditions of this study, 7.42µg/cm^2 and 13.86µg/cm^2 (1.9 % and 3.43 %) of the applied dose was absorbed under occluded and non-occluded conditions respectively, with most remaining in the epidermis (5.90µg/cm^2 and 10.7 µg/cm^2) and dermis (1.06 µg/cm^2 and 2.40 µg/cm^2) . There was no statistically significant difference between the data obtained under occluded versus non-occluded conditions. Under the conditions of this study, choline chloride is expected to have a low potential for percutaneous absorption.

Ref. : 24

2.8. Mutagenicity/Genotoxicity

Data not applicable

2.9. Carcinogenicity

Data not applicable

2.10. Special investigations

Data not applicable

2.11. Safety evaluation

Not applicable. The amount absorbed dermally is negligible compared with exposure orally.

2.13. References

Only the references for the irritation (21), sensitisation (22 &23) and percutaneous absorption (24) were considered pertinent for this evaluation.

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3. Opinion of the SCCNFP

In view of the extensive oral exposure from food, the SCCNFP is of the opinion that dermal exposure to choline chloride, in rinse off products at 5%, is not anticipated to pose any serious risk.

Since it is a quaternary ammonium derivative, it may be a potential irritant.

Before any further consideration, the following information is required :

* data on irritation (skin and mucous membrane) at the intended use concentrations and in accordance with the Notes of Guidance.

4. Other considerations

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5. Minority opinions

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