EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

OPINION ON

Choline chloride

Scientific Committees
on consumer products
on emerging and newly identified health risks
on health and environmental risks

The SCCP adopted this opinion at its 15th plenary of 15 April 2008

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.

They are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

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.

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TABLE OF CONTENTS

1. BACKGROUND

Choline chloride with the chemical name (2-hydroxyethyl)trimethylammonium chloride is currently banned in the Cosmetic Directive 76/768/EEC, Annex II, entry 168.

However, the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers adopted during the 26th Plenary Meeting of 9th December 2003 an opinion (SCCNFP/0672/03) on choline chloride with the conclusion:

"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."*

Choline chloride has humectant properties. The request is to use choline chloride in personal care rinse-off formulations, such as soap bars and liquid body soaps up to a maximum concentration of 5%.

2. TERMS OF REFERENCE

Does SCCP consider the use of Choline chloride as a humectant in cosmetic rinse-off products safe for the consumers when used in a maximum concentration of 5%, taking into accounts the new provided data on skin and mucous membrane irritation?

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Choline chloride (INCI name)

3.1.1.2. Chemical names

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

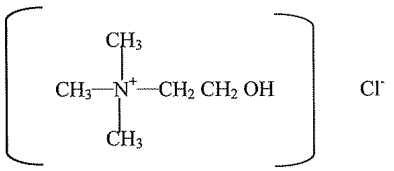
3.1.1.3. Trade names and abbreviations

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

3.1.1.4. CAS / EINECS number

CAS: 67-48-1
EINECS: 200-655-4

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_5H_{14}NO\ Cl$

3.1.2. Physical form

White crystals

3.1.3. Molecular weight

Molecular weight: 139.63

3.1.4. Purity, composition and substance codes

≥ 98%

3.1.5. Impurities / accompanying contaminants

Trimethylamine:	max. 500 ppm
Ethylene glycol:	max. 500 ppm
Organic impurities (TMA + glycol + chloroetheanol):	max. 1500 ppm
Heavy metals as lead:	max. 20 ppm

3.1.6. Solubility

Soluble in water and ethanol, acetone and chloroform
Insoluble in ether and benzene

3.1.7. Partition coefficient (Log P_{ow})

Log Kow: - 3.77 (measured, solution 75% w/w in water at 25 °C)
Log K_{oc}: 0.37 (calculated)

3.1.8. Additional physical and chemical specifications

Melting point:	247 °C
Boiling point:	decomposition on heating
Relative density:	1.1 g/cm³ at 20 °C (70% choline chloride in water)
Relative vapour density:	/
Vapour Pressure:	6.57 x 10^{-10} hPa at 25 °C (calculated)

3.1.9. Stability

Practically unlimited storage at 20-30°C

3.2. 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%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from opinion SCCNFP/0672/03

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

Acceptable 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 18 mg/kg (bw = 7 kg)
	6-11 months	150 mg/day or 15 mg/kg (bw = 10 kg)
Children	1-3 years	200 mg/day or 13 mg/kg (bw = 15 kg)
	4-8 years	250 mg/day or 9 mg/kg (bw = 27 kg)
	9-13 years	375 mg/day
Males	14 and over	550 mg/day or 9 mg/kg (bw = 60 kg)
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 (LD_{50}: 280 to 750 mg/kg bw/day) is relatively narrow with choline.

Ref.: C

3.3.1.2. 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

Taken from opinion SCCNFP/0672/03

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

Comment
The correct study report for this test has now been provided with submission II.

Present submission

Split-arm exaggerated arm wash study

A split-arm exaggerated arm wash study was conducted to assess the skin irritation potential of a choline chloride containing liquid cleanser and a choline chloride containing soap bar versus the appropriate placebo. Twenty-nine subjects were enrolled into this study. Three out of the twenty-nine panellists terminated the study prior to the end because they had reached an end-point of 3 (marked erythema) for one of the test products (placebo or choline chloride containing).

Results
Clinical observations
- Soap bar: at termination, there was no statistical difference in the level of dryness and erythema induced by the soap bar with choline chloride and the placebo soap bar.
- liquid cleanser: at termination, there was no statistical difference in the level of dryness and erythema induced by the liquid cleanser with choline chloride and the placebo liquid cleanser. Both induced low levels of dryness and erythema.

Instrumental measurements
Skin redness (a* values, Minolta Chromameter)
- Soap bar: at termination, the soap bar with choline chloride induced statistically less redness as measured by the Minolta a* values than the placebo soap bar.
- Liquid cleanser: at termination, there was no statistically significant difference between the liquid cleanser with choline chloride and the placebo liquid cleanser in skin redness.

Transepidermal water loss
- soap bar: at termination, the placebo soap bar induced statistically significantly more barrier damage as seen by the greater increase in TEWL values than the soap bar with choline chloride.

- liquid cleanser: at termination, there was no statistically significant difference between the liquid cleanser with choline chloride and the placebo liquid cleanser with respect to barrier damage.

Conclusion
The results of this study show that choline chloride applied topically in rinse-off formulations is not an irritant.

Ref.: 30

Comment
While the dossier states a choline chloride content of 5% for the test products, the study report contains no information on choline chloride information.

3.3.2.2. Mucous membrane irritation

In vitro Bovine Corneal Opacity and Permeability Assay (BCOP)

Guideline:	/
Species/strain:	11 isolated bovine corneas
Test substance:	choline chloride
Test article:	5.0 mg/ml choline chloride (pH 5.3)
Positive control:	ethanol
Negative control:	sterile deionised water
Batch:	014K0185
Purity:	99.7%
GLP:	in compliance
Date:	12 January – 29 March 2005

The BCOP assay was used to assess the potential ocular irritancy of the test article to isolated bovine corneas. An *in vitro* score was determined for the test article based on the induction of opacity and permeability (to fluorescein) in isolated bovine corneas.

The isolated corneas were mounted in the holders and the 2 chambers were filled with Minimum Essential Medium (EMEM) without phenol red, containing 1% foetal bovine serum and 2 mM L-glutamine (complete MEM). The corneal holders were incubated at 32 °C for a minimum of 1 hour. After incubation, the medium was replaced with fresh Complete MEM. The initial opacity was determined for each cornea using a Spectro Designs OP-KIT opacitometer.

Choline chloride was tested as a 10% (v/v) dilution of a 5% (w/v) initial dilution in sterile, deionised water. An aliquot of 750 µl of the test article, positive or negative control was introduced into the anterior chamber. Five corneas were incubated in the presence of the test article at 32 °C for 10 minutes. Positive and negative control: each 3 corneas.
After incubation, the test article and the controls were removed. The epithelial side of the corneas was washed with Complete MEM (containing phenol red) to ensure total removal of the test article and the controls. After a final rinse with Complete MEM (without phenol red), the anterior chamber was filled with fresh Complete MEM and an opacity measurement was performed.
After the opacity measurement, the medium was replaced: fresh Complete MEM in the posterior chamber and 1 ml of a 4 mg/ml fluorescein solution in the anterior chamber. After 90 minutes of incubation, 360 µl aliquots from the posterior chambers were placed into designated wells on a 96-well plate. The optical density at 490 nm (OD_{490}) was determined using a Molecular Devices *V*max kinetic microplate reader.

Results

	Exposure time	Mean opacity value	Mean OD_{490} value	*In vitro* score
Test substance	10 min	-0.2	0.001	-0.2
Positive control	10 min	43.7	1.023	59.0

In vitro score: 0 – 25 = mild irritant
25.1 – 55 = moderate irritant
55.1 and above = severe irritant

Conclusion
Choline chloride, when used at 0.5%, is not considered an irritant for the eyes in the test conditions.

Ref.: 31

Comment
Choline chloride was tested at only 0.5% instead of the requested use concentration of 5%. The BCOP (Bovine Cornea Opacity Permeability) is not a validated alternative method for eye irritation testing. It is a screening method for hazard identification. It is not suitable for risk characterisation.

In an old, non-GLP study, conducted broadly to OECD test guideline 405, only slight irritation was observed; however, the degree of irritation would not be classifiable under GHS. A 70% aqueous solution of the test substance was applied to one eye of one female and one male rabbit, the left eyes served as controls, to which saline was applied. After ten minutes, reddening of the eyes and tear secretion were observed. Slight reddening persisted up to three hours after application. No eye irritation or effects on the cornea were detectable after one day observation period. Post application readings were done after 1 and 3 hours, 1, and 8 days.

Ref.: D

3.3.3. Skin sensitisation

Taken from opinion SCCNFP/0672/03

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.

3.3.4. Dermal / percutaneous absorption

Taken from opinion SCCNFP/0672/03

Percutaneous absorption *in vitro*

Guideline:	OECD draft 428
Tissue:	Human epidermal skin (3 donors)
Method:	Franz diffusion cells
Test material:	Choline chloride (Aldrich)
Batch:	MI 11612PU
Purity:	99 %
Dose level:	10µl/cm² 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 cm² 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/cm² in the occluded cells and 0.383 µg/cm² 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, 7.42 µg/cm² and 13.86 µg/cm² (1.9% and 3.43%) of the applied dose was absorbed under occluded and non-occluded conditions respectively, with most remaining in the epidermis (5.90 µg/cm² and 10.7 µg/cm²) and dermis (1.06 µg/cm² and 2.40 µg/cm². There was no statistically significant difference between the data obtained under occluded versus non-occluded conditions.

Conclusion
Under the conditions of this study, choline chloride is expected to have a low potential for percutaneous absorption.

Ref.: 24

3.3.5. Repeated dose toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

Taken from opinion SCCNFP/0672/03

Data not applicable

3.3.7. Carcinogenicity

Taken from opinion SCCNFP/0672/03

Data not applicable

3.3.8. Reproductive toxicity

No data submitted

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

Taken from opinion SCCNFP/0672/03

Not applicable.

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

(Choline chloride)

Not applicable.

The amount absorbed dermally is negligible compared with oral exposure.

3.3.14. Discussion

Skin Irritation
Choline chloride applied topically in rinse off formulations (soap bar and liquid cleanser at a concentration of 5%) was not an irritant.

Mucous membrane irritation
Eye irritation was tested in a BCOP (Bovine Cornea Opacity Permeability) test, which is not a validated alternative method for eye irritation testing. It is a screening method for hazard identification, but is not suitable for risk characterisation. Moreover, Choline chloride was tested at only 0.5% instead of the requested use concentration of 5%. At 0.5% concentration, Choline chloride did not show eye irritation in this test.

An old non-GLP and non-OECD-guideline eye irritation study with 70% choline chloride was considered inappropriate for risk assessment.

A new study at the requested use concentration up to current standards and in accordance with the SCCP notes of Guidance is required.

4. CONCLUSION

The SCCP is of the opinion that the study included in the present submission is not sufficient to answer the concerns about mucous membrane irritation.

Before any further consideration, the following information is required:

* a study on mucous membrane irritation at the intended use concentration of 5%, in accordance with the SCCP Notes of Guidance.

5. MINORITY OPINION

Not applicable

6. REFERENCES

Taken from opinion SCCNFP/0672/03

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

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