EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

OPINION ON

Camphor benzalkonium methosulfate

COLIPA n° S57

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

The SCCP adopted this opinion at its 19th plenary of 21 January 2009

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.

Scientific Committee members
Claire Chambers, Gisela Degen, Ruta Dubakiene, 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 R. 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. C. Chambers
Prof. G. Degen
Dr. B. Jazwiec-Kanyion
Prof. V. Kapoulas
Prof. J.-P. Marty
Prof. T. Platzek
Dr. S.C. Rastogi
Prof. J. Revuz (rapporteur)
Prof. V. Rogiers
Prof. T. Sanner (chairman)
Dr. J. van Engelen
Dr. I.R. White

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1. BACKGROUND

Submission I on the UV-filter camphor benzalkonium methosulfate (INCI) with the substance name N,N,N-Trimethyl-4-(2-oxoborn-3-ylidenemethyl) anilinium methyl sulphate in the Cosmetic Directive was submitted by COLIPA[1] in January 2006.

SCCP adopted at its 10th plenary meeting on 19 of December 2006 an opinion (SCCP/1015/06) on Camphor benzalkonium methosulfate with the conclusion:

"Based on the information provided, the SCCP is of the opinion that the use of camphor benzalkonium methosulphate as a UV-filter at a maximum concentration of 6.0% in the cosmetic sun protection preparations does not pose a risk to the health of the consumer.
Because of its borderline Margin of Safety, its use in other types of cosmetic products is not recommended. However, this figure does include the outlier which may or may not be relevant.
A new study on dermal absorption following the relevant SCCNFP opinions and in accordance with its Notes of Guidance is required before any reconsideration of the opinion."

The substance is currently regulated in the Cosmetics Directive in Annex VII, part 1 ("List of permitted UV filters which cosmetic products may contain") in a concentration up to maximum 6%.

By the current response, submitted in January 2008, the applicants apply for a maximum authorized concentration up to 3%.

2. TERMS OF REFERENCE

1. *Does the SCCP consider camphor benzalkonium methosulphate safe for use as an UV-filter in cosmetic products in a concentration up 3.0% taken into account the data provided?*

2. *And/or does the SCCP have any further scientific concerns with regard to the use of camphor benzalkonium methosulphate as a UV-filter in cosmetic products?*

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

3. OPINION

Taken from SCCP/1015/06

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Camphor benzalkonium methosulfate (INCI name)

Comment
The evaluation studies are performed on the 30% aqueous solution

3.1.1.2. Chemical names

N, N, N-Trimethyl-4-[(4, 7, 7-trimethyl-3-oxobicyclo [2.2.1]hept-2-ylidene)methyl]-benzenaminium methylsulphate in 30% aqueous solution
4-(2-oxo 3-bornylidenemethyl) phenyl trimethylammonium methylsulphate

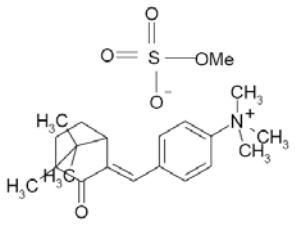
3.1.1.3. Trade names and abbreviations

Mexoryl SO (30% aqueous solution of camphor benzalkonium methosulfate)
52368 (other code)

3.1.1.4. CAS / EINECS number

CAS: 52793-97-2
EINECS: 258-190-8

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{20}H_{28}NO.\ CH_3O_4S$

3.1.2. Physical form

Yellow to yellow green liquid (aqueous solution at 30%)

3.1.3. Molecular weight

Molecular weight: 409.55

3.1.4. Purity, composition and substance codes

Analytical study carried out on three batches: 0123383, 0126402 and 0127132

UV Purity: > 99, > 99, >95, respectively for batches 0123383, 0126402, 0127132

Comment
Other batches have been used in the evaluation studies: 0106845; M171; op565-566; CFQ 14151; No data are provided on these batches in the analytical file.

In the summary of submission the following data are given:

- Code CFQ14151 Batch 1 (active ingredient content 30%, radiochemical purity >98.1%)
- Batch op 565-566 (active ingredient content 95.7%, relative purity >99%)
- Batch M171 (active ingredient content 29.4%)

The analytical certificates are present in the respective studies

3.1.5. Impurities / accompanying contaminants

Gas chromatography
Camphor: < 0.1 g/100g in the three batches
Detected in batch 01264 02, Not detected in the other batches)

HPLC

Impurity A: 4-Dimethylaminobenzoic acid < 0.1 g/100g (detected in batch 0126402; not detected in the other batches)

Impurity B: 4- Dimethylaminobenzaldehyde < 0.1 g/100g (not detected in the three batches)

Impurity C: N, N-dimethyl-4-[(E)-(-4,7,7-trimethyl-3-oxobicyclo [2,2,1]hept-2-ylidene) methyl] aminobenzene
< 0.1 g/100g in batch 0123383 (detected)
0.18 g/100g in batch 0126402
0.56 g/100g in batch 0127132

Impurities content are expressed against active material

Residual solvent impurities GC (µg/g) in the three batches

Methanol	< 500 (D)	< 500 (D)	500
Methyl ethyl ketone	< 500 (D)	500	2300
Isopropanol	< 500 (D)	< 500 (D)	< 500 (D)
Toluene	< 500 (ND)	< 500 (ND)	< 500 (ND)

D: detected
ND: not detected

3.1.6. Solubility

Solubility (g/100 ml - 23°C after 24 h) [concerns the 30% solution].

- Water: S ≥ 20
- Ethanol: S ≥ 20
- Isopropanol: S ≥ 20
- DMSO: S ≥ 20

3.1.7. Partition coefficient (Log P_{ow})

Log $P_{o/w}$: 0.28 (Calculated, CLOGP v.4.2 – C. Hansch)
Log $P_{o/w}$ *Experimental*: Not done

Mexoryl SO reacting as a surfactant, it was not possible to apply the EEC method A8 (HPLC method or shake-flask method) to evaluate experimentally the Log $P_{O/W}$ value.

3.1.8. Additional physical and chemical specifications

Heavy metals content
Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Pd, Se, Sn, Ti, V, Zn: each < 1mg/kg
Hg: < 0.1 mg/kg

Water content
- water content of batch 0123383: 70.3 g/100g
- water content of batch 0126402: 69.4 g/100g
- water content of batch 0127132: 70.2 g/100g

Acid value (IA)
mg KOH /g of active ingredient:
- batch 0123383 1.2
- batch 0126402 2.4
- batch 0127132 1.4

General Comments on Physico-chemical characterisation

* Batches 0106845; M171; op565-566; CFQ 14151 were used but not characterised.
* A UV-spectrum was not submitted

3.2. Function and uses

Camphor benzalkonium methosulfate is used up to a maximum concentration of 3.0% as a UV-filter in cosmetic products.

3.3. Toxicological Evaluation

Taken from SCCP/1015/06

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	OECD 401
Species/strain:	albino rats Sprague Dawley OPA
Group size:	5 males, 5 females; fasted
Test substance:	Mexoryl SO (30% aqueous solution of camphor benzalkonium methosulfate)
Batch:	M 171
Purity:	/
Dose:	2000 mg/kg (600 mg/kg active ingredient)
Observation:	14 days
GLP:	in compliance

There were no deaths, no clinical signs or macroscopic findings. The LD50 (camphor benzalkonium methosulfate) is > 600 mg/kg bw

Ref.: 1

3.3.1.2. Acute dermal toxicity

Guideline:	OECD 402
Species/strain:	Sprague Dawley SD
Group size:	5 males, 5 females; fasted
Test substance:	Mexoryl SO (30% aqueous solution of camphor benzalkonium methosulfate)
Dose:	2000 mg/kg (600 mg/kg of active ingredient)
Batch:	0123383
Purity:	> 99% (of active ingredient)
Observation:	14 days
GLP:	in compliance

No mortality occurred following cutaneous application in male or female animals. No clinical signs were noted in male animals. Erythema and/or oedema were observed from day 2 to day 9 at the application site in the majority of females. Desquamation of the skin at the application site was also observed in a single female on days 4 and 5.

All animals had fully recovered by day 10. Decrease in body weight or body weight gain were noted in females from day 1 to 3 or from day 3 to 8, whereas body weight changes in males were within the expected range. There were no necropsy findings.

Ref.: 2

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline:	OECD 404
Species/strain:	New-Zealand albino rabbit
Group size:	3 males,
Test substance:	Mexoryl SO (30% aqueous solution of camphor benzalkonium methosulfate)
Batch:	M 171
Purity:	/
Dose:	0.5 ml undiluted covered with gauze during 4 hours (semi occlusion)
Observation:	1, 24, 48, 72 hours; 14 days
GLP:	in compliance

Results
Mild erythema, only visible one hour after removal.

Ref.: 3

Guideline:	OECD 404
Species/strain:	New-Zealand albino rabbit
Group size:	3 males
Test substance:	Mexoryl SO (30% aqueous solution of camphor benzalkonium methosulfate)
Batch:	0106845
Purity:	/
Dose:	0.5 ml undiluted covered with gauze during 4 hours (semi occlusion)
Observation:	1, 24, 48, 72 hours
GLP:	in compliance

Results
No reaction was observed.

Ref.: 4

Comment
Mexoryl SO (30% aqueous solution of camphor benzalkonium methosulfate) has minimal irritant potential to rabbit skin following a single application.

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405
Species/strain:	New Zealand albino rabbit
Group size:	3 males
Test substance:	Mexoryl SO (30% aqueous solution of camphor benzalkonium methosulfate)
Batch:	M 171(30% solution)
Purity:	/
Dose:	0.1ml/ eye
Observation:	1,24,48,72 hours
GLP:	in compliance

Results

Important eye irritation in all animals: redness, discharge and chemosis in bulbar and palpebral conjunctivae and iris congestion. Reaction increased up to 24 hours and faded by 72h.

Conclusion
Mexoryl SO batch M171 is irritating to the eyes.

Ref.: 5

Guideline:	OECD 405
Species/strain:	New Zealand albino rabbit
Group size:	3 males
Test substance:	Mexoryl SO
Batch:	0106845 (30% solution)
Purity:	/
Dose:	0.1ml/ eye
Observation:	1,24,48,72 hours
GLP:	in compliance

Results
Mild conjunctival reactions were observed in all animals from day 1; these reactions persisted up to day 8 at the latest. A mild irritant reaction was noted in 2/3 animals on day 1, persisted in one animal on day 3. Mild corneal opacity was recorded in all animals on day 2 and 3.

Conclusion
Mexoryl SO was irritant to rabbit eyes under the experimental conditions.

Ref.: 6

Guideline:	OECD 405
Species/strain:	New Zealand albino rabbit
Group size:	3 females
Test substance:	Mexoryl SO (30% aqueous solution of camphor benzalkonium methosulfate)
Batch:	0123383
Purity:	> 99%
Dose:	20% aqueous solution of Mexoryl SO = 6% solution of camphor benzalkonium methosulfate; dose: 0.1ml/ eye
Observ. period:	1, 24, 48, 72 hours
GLP:	in compliance

Results
A slight to well defined conjunctival redness, chemosis and ocular discharge were observed in all animals 1-hour following instillation of the test item. A slight conjunctival redness was still present in 2/3 animals at the 24-hour reading. Full recovery had occurred in all animals at the 48-hour examination.

Conclusion
Under the conditions of the test, a 20% aqueous solution of Mexoryl SO (6% active ingredient) was irritating to rabbit eyes.

Ref.: 7

3.3.3. Skin sensitisation

Magnusson Kligman Guinea Pig Maximisation test

Guideline:	OECD 406
Species/strain:	Hartley guinea pigs.
Group size:	15 males, 15 females (10 verum, 5 controls)
Test substance:	Mexoryl SO (30% aqueous solution of camphor benzalkonium methosulfate)
Batch:	0106845
Purity:	/
Concentrations:	The following concentrations were administered in 0.9% NaCl (vehicle): intra-dermal induction at 1%, topical induction at 50%, topical challenges at 50% and 100%.
GLP:	in compliance

The concentrations used were determined during a preliminary ranging study. Freund's adjuvant was used during the induction phase.
On day 1, six intra-dermal injections were performed in the inter-scapular region. On day 7, as the test substance was non irritating, 10% Sodium Lauryl Sulfate was applied on the administration site to induce local irritation. On day 8, either the vehicle or 50% (w/w) Mexoryl SO in NaCl was topically applied to the site of the previous intra-dermal injection and held in place for 48 hours under occlusive conditions. On day 22, all animals were challenged by cutaneous applications of Mexoryl SO at 100% and 50% (w/w) on the posterior right and left flanks, respectively.
The treatments were held in place for 24 hours under occlusive conditions and skin reactions were evaluated 24 and 48 hours after removal of the dressing.

Results
No deaths, clinical signs or compound-related body weight changes were observed during the study. No cutaneous reactions (except skin dryness on 1/20 animals of the treated group at the 48-hour reading) were noted after the challenge application.

Conclusion
Mexoryl SO was non-sensitising in this Guinea Pig Maximisation Test.

Ref: 8

3.3.4. Dermal / percutaneous absorption

Human Dermatomed Skin, *in vitro*

Guideline:	OECD 428
Group size:	Human skin samples (abdomen and breast) were obtained from six different female donors following plastic surgery
Test Material:	batches 0123383 (29.7%, Mexoryl SO content; relative purity >99%) CFQ14151 batch 1 (active ingredient content of 29.7%, radiochemical purity ≥ 98.4% of [^{14}C]-Mexoryl SO
GLP:	in compliance

Skin samples were allowed to thaw at ambient temperature, dermatomed (390-400 µm in thickness) and mounted in flow-through diffusion cells, using calcium and magnesium free phosphate-buffered saline (PBS) as the receptor fluid (flow rate 1.5 mL/h). The integrity of the skin was verified by measuring the permeability coefficient for tritiated water (Kp < 2.5 x 10-3 cm/h for all selected membranes) prior to application of the sunscreen formulation. The skin was maintained at approximately 32°C.

A typical sunscreen formulation containing 20% Mexoryl SO (corresponding to 6% camphor benzalkonium methosulphate) was applied to the skin surface at about 5 mg/cm² (corresponding to exactly 341.26 µg/cm² of camphor benzalkonium methosulfate). Twenty four (24) hours after application, the remaining formulation on the skin surface was removed using a standardized washing procedure. Then, the percutaneous absorption of camphor benzalkonium methosulfate was estimated by measuring its concentration by liquid scintillation counting in the following compartments: dislodgeable dose, *stratum corneum* (isolated by tape strippings), skin (living epidermis/dermis) and receptor fluid.

Results
All twelve samples yielded data that could be analysed. At the end of the 24-hour exposure period, most of the camphor benzalkonium methosulfate applied on the skin surface was removed with the washing procedure, yielding a total dislodgeable dose of 100.11% of the applied dose for a total recovery rate of 102.67%. Approximately 2.24% of the applied dose was retained by the stratum corneum and 0.17% was located in the skin.
The absorbed dose (amount found in the receptor fluid) was 0.05 ± 0.04 µgeq/cm² (0.02 ± 0.01% of the applied dose).
The dermal delivery (sum of the amounts measured in the living epidermis/dermis and receptor fluid) represented 0.65 ± 1.04 µgeq/cm² (0.19 ± 0.31% of the applied dose).

Conclusion
The dermal absorption (sum of the amounts measured in the living epidermis/dermis and receptor fluid) of camphor benzalkonium methosulfate from a typical sunscreen formulation containing Mexoryl SO at 20% (corresponding to 6% camphor benzalkonium methosulfate) was estimated to be 0.65 ± 1.04 µgeq/cm² as a mean; range: (0.06 – 3.72 µgeq/cm²) under exaggerated use conditions.

Ref.: 20

Comment
The mean dermal absorption + 2 standard deviations (0.65 + 2 x 1.04) or 2.73 µg equiv/cm² will be used for the calculation of the Margin of Safety.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

No data submitted

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Guideline:	OECD 401
Species/strain:	Sprague Dawley rats Crl: OFA.SD. (IOPS Caw).
Group size:	Sixty eight (68) Sprague-Dawley (SD) rats per sex
Test substance:	Mexoryl SO
Dose:	0, 100, 300 and 1000 mg/kg/day
Batch:	0123383
Purity:	> 99% (active ingredient content of 29.7%)
Observation:	13 consecutive weeks gavage followed by a 4 week treatment-free period
GLP:	in compliance

Results
There were three deaths in the main high dose group and three among the different satellite groups. These deaths were considered not related to treatment.
The only treatment-related clinical sign was excessive salivation observed immediately after treatment in some animals given 300 mg/kg/day and most animals given 1000 mg/kg/day

from week 5/6. There were no ophthalmological findings. There were no treatment-related adverse effects on body weight in either sex at any dose-level.
Food consumption was not affected by treatment at any dose-level.
There were no treatment-related changes in haematology, coagulation or serum clinical chemistry parameters, apart from a minimal increase in reticulocyte count in females given 1000 mg/kg/day. Minimally but statistically significantly increased mean creatinine concentrations for treated females relative to controls (no dose-related increase) were considered to be incidental.
At necropsy, mean adrenal gland weight was lower and spleen weight was minimally higher in females given 100 mg/kg/day relative to controls. In the absence of any histopathological correlate, these minimal and non-dose related changes were considered to bear no toxicological significance.
Several animals at 300 or 1000 mg/kg/day showed discolouration of the digestive tract. At histopathological examination, erosions in gastric and/or duodenal glandular mucosa were observed with a slightly higher incidence and severity at 1000 mg/kg/day than in control group. Given the incidences and severity observed, these minor changes were considered to be related to treatment with Mexoryl SO at 1000 mg/kg/day. No changes were seen in gastric or duodenal mucosa of animals killed at the end of the treatment-free period.
Toxicokinetic analysis showed that several animals given 100 or 300 mg/kg/day had plasma concentrations below the limit of quantification (2 ng/ml). All rats given 1000 mg/kg/day had detectable but highly variable amounts of Camphor Benzalkonium Methosulfate in plasma after one administration as well in week 13, with Cmax reached between 0.5 and 2 hours after administration and t1/2 ranging from 0.91 to 11.42 hours. On the first day of dosing, few AUC0-4h could be calculated and no linearity or proportionality could be determined; at 1000 mg/kg/day, AUC0-4h were 52.1 and 92.7 ng/ml, for males and females, respectively. A marked sex-related difference was observed in AUC values and was confirmed by Cmax values.

Conclusion
According to the authors, the repeated daily oral administration to rats of Mexoryl SO at levels up to 1000 mg/kg/day for 13 weeks was well tolerated. At necropsy, discolouration in the digestive tract was seen at 300 and 1000 mg/kg/day, and erosion of the mucosa was observed in the stomach or duodenum from some rats given 1000 mg/kg/day, at a low incidence. Accordingly, under the conditions of the study the No Observed Adverse Effect Level (NOAEL), was considered to be 300 mg/kg/day.

Ref.: 11

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline:	OECD 401
Species/strain	Five strains of *Salmonella typhimurium* (TA 1535, TA 1537, TA 98, TA 100 and TA 102)
Test substance:	Mexoryl SO
Batch:	0106845 (active ingredient content of 30.3%)
Purity:	/
Concentration:	312.5, 625, 1250, 2500 and 5000 µg/plate both in the presence and the absence of S9
GLP:	in compliance

Two independent experiments in the absence and the presence of metabolic activation (S9 mix prepared from the livers of rats given Aroclor 1254). The experiments were conducted according to the direct plating incorporation method, apart from the second test with S9 which was performed according to the pre-incubation method. Known mutagens were used as positive controls, and cultures treated with DMSO (solvent) were used as negative controls. Three plates per treatment condition were used.

Results
The test substance did not induce any significant increase in the number of revertants, both in the presence or the absence of S9 mix in any of the five strains.

Conclusion
Mexoryl SO was not mutagenic to Salmonella typhimurium strains TA 1535, TA 1537, TA 98, TA 100 and TA 102 either in the absence or the presence of metabolic activation.

Ref.: 12

Chromosome Aberration Test in Cultured Human Lymphocytes

Guideline:	OECD 473
Test substance:	Mexoryl SO both in the presence and the absence of S9.
Batch:	0123383 (active ingredient content of 29.7%)
Purity:	>99%
GLP:	in compliance

This study was conducted on cultured human peripheral blood lymphocytes from two healthy, male and female volunteers.

Mexoryl SO was evaluated in two independent experiments in the absence and presence of metabolic activation (S9 mix prepared from the livers of Aroclor 1254-treated rats). The highest concentration tested in the first experiment was selected on the basis of pH, osmolarity and solubility. For selection of the concentrations used in the second experiment, any toxicity indicated by the reduction of mitotic index (MI) in the first experiment was also taken into account.
For each culture, heparinized whole blood was added to culture medium containing a mitogen (phytohemagglutinin) and incubated at 37°C, for 48 hrs.
In both experiments, lymphocyte cultures were exposed to each concentration of Mexoryl SO or with known clastogens in the presence (cyclophosphamide, CPA) or absence (mitomycin C, MMC) of S9 mix for 3 hours or 20 hours (second experiment without S9 mix), then rinsed.
Solvent-treated cultures (culture medium) were used as negative controls.
Cells were harvested 20 hours after the beginning of treatment (corresponding approximately to 1.5 normal cell cycle) in both experiments. One and a half hour prior to harvest, cell cultures were treated with a colcemid solution (10 µg/ml) to block them in metaphase.
Chromosome preparations were stained and examined microscopically for mitotic index and for aberrations when selected. Two hundred well spread metaphases per concentration were evaluated blind.

Results
The test item was freely soluble in culture medium. At 5000 µg/ml, the pH and osmolarity values were equivalent to those of the vehicle control culture. The dose levels selected for metaphase analysis were 1250, 2500 and 5000 µg/ml for the 3-hour treatment in the presence and the absence of S9 mix (first experiment) and 2500, 3750 and 5000 µg/ml for the 3-hour treatment in the presence of S9 mix and the 20-hour treatment in the absence of S9 mix (second experiment).

When compared to concurrent controls, treatment of cultures with MMC and CPA resulted in statistically significant increases in the number of cells bearing structural aberrations, showing the adequate sensitivity of the test system and procedure used.
In experiments 1 and 2, no significant increase in the frequency of cells with structural chromosomal aberration was noted.

Conclusion
Under the conditions of the study, Mexoryl SO did not produce chromosome aberrations in cultured human lymphocytes both in the presence and the absence of metabolic activation.

Ref.: 13

3.3.6.2. Mutagenicity / Genotoxicity *in vivo*

Bone Marrow Micronucleus Test by the Oral Route in Rats

Guideline:	OECD 474,
Species/strain:	Sprague-Dawley rats
Group size:	76 rats, 38 males and 38 females
Test substance:	Mexoryl SO
Batch:	0123383 (active ingredient content of 30.3%)
Purity:	> 99% (area%, UV)
GLP:	in compliance

A preliminary toxicity test was performed to define the dose-levels to be used for the cytogenetic study.
In the main study, four groups of five male and five female Sprague-Dawley rats received a single oral administration of the test item at the dose-levels of 500, 1000 and 2000 mg/kg.
Two groups of five males and five females received the vehicle (water for injections) under the same experimental conditions, and acted as control groups.
One group of five males and five females received the positive control test item (cyclophosphamide) once by oral route at the dose-level of 15 mg/kg.
The animals of the treated and vehicle control groups were killed 24 or 48 hours after treatment and the animals of the positive control group were killed 24 hours after treatment. Bone marrow smears were then prepared.
For each animal, the number of the micronucleated polychromatic erythrocytes (MPE) was counted in 2000 polychromatic erythrocytes. The polychromatic (PE) and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes (PE + NE).

Results
Since no toxic effects were observed in the preliminary test, the top dose-level was 2000 mg/kg. The two other selected dose-levels were 500 and 1000 mg/kg.
No clinical signs and no mortality were observed in the animals of both sexes given 500 and 1000 mg/kg. At the dose-level of 2000 mg/kg, 1/5 male died following the treatment and 2/5 females were found dead 24 hours following the treatment. No clinical signs were noted in the surviving animals.
The mean values of MPE as well as the PE/NE ratio in the groups treated with the test item were equivalent to those of the vehicle group.
Cyclophosphamide induced a highly significant increase ($p < 0.001$) in the frequency of MPE, indicating the sensitivity of the test system under our experimental conditions. The study was therefore considered valid.

Conclusion
Mexoryl SO did not induce damage to the chromosomes or the mitotic apparatus of rat bone marrow cells after a single oral administration at the dose-levels of 500, 1000 and 2000 mg/kg.

Ref.: 14

Comment
Batch 0123383 is said to have an active ingredient content of 30.3%; in the previous experiment (Chromosome Aberration Test in Cultured Human Lymphocytes) it is said to have an active ingredient content of 29.7%

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

Estrogenic activity (uterotrophic assay)

Guideline:	OECD Protocol and Guidance for the conduct of the rodent uterotrophic assay (Draft of 20 April 2000).
Species/strain:	Sprague-Dawley rats
Group size:	36 juvenile female rats, taken from 4 litters of pups, were supplied with their mothers/foster mothers.
Test substance:	Mexoryl SO
Batch:	0123383
Purity:	> 99% (HPLC)
GLP:	in compliance

30 pre-pubertal female Sprague-Dawley rats (20 days old at the start of treatment) were divided into five groups and received the reference item, test item or vehicle, subcutaneously, once daily for 3 days; reference item: 17-α-Ethinyloestradiol (EE), at 0.3 µg/kg day; Mexoryl SO, at 10, 30 or 100 mg/kg/day. The control group received the vehicle alone (purified water).
On completion of the treatment period, *i.e.* on day 4 (approximately 24 hours after the last dosing), the animals were weighed and sacrificed. The uterus (without ovaries) was weighed immediately after sacrifice, with (full uterus) and without (empty uterus) the uterine fluid. A complete macroscopic *post-mortem* examination of the abdominal cavity was performed, focusing on the reproductive tract. The uterus, ovaries and vagina were preserved.

Results
No unscheduled deaths occurred during the study period in any group. No clinical signs were observed for any study animals. Body weight and body weight change were unaffected by the test item-treatment.
A slight decrease was noted in the uterus weight, reaching a statistical significance level at 100 mg/kg/day Mexoryl SO. In the group receiving EE, the uterus weights were significantly higher than the control group values.
No treatment related necropsy findings were noted. There were no remarkable morphological differences which might have explained the trend in decreased uterus weights in the females given Mexoryl SO at 100 mg/kg/day.

Ref.: 18

Comment
The minimal and non-dose related changes in uterus weight are considered to bear no toxicological significance.

3.3.8.1. One generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Embryo-foetal Development Toxicity Study by the Oral Route in Rats

Guideline:	OECD 414
Species/strain:	Sprague-Dawley rats
Group size:	100 mated females
Test substance:	Mexoryl SO
Batch:	0123383 (active ingredient content of 29.7%)
Purity:	>99%
GLP:	in compliance

Daily oral gavage at 0, 100, 300 or 1000 mg/kg/day was given to mated SD rats (25/group) from day 6 through day 19 of gestation. The dams were killed on GD 20.

Results
No mortality and no treatment-related clinical signs occurred during the study. The body gain of the pregnant rats was similar in the treated and control groups throughout the study.
Food consumption during the first three days of treatment was slightly lower in the 1000 mg/kg/day group than in the control group (-10 % on average, $p<0.01$). The other dose groups were not affected. No abnormalities were found at the terminal necropsy examination of the adult females given the test item.
There were 23/25 to 25/25 pregnant females in each treated group except in the control group where 21/25 females were pregnant. All pregnant females had viable foetuses at term.
Caesarean data, including pre- and post- implantation losses, foetal weight, gravid uterus weight and foetal sex ratio, did not show any treatment-related changes.
Examination of the foetuses did not reveal any treatment-related abnormalities. There was one malformed foetus in each of the control and 300 mg/kg/day groups (with meningocele or displaced testes, respectively). The incidences of foetuses with other anomalies (i.e., slightly dilated renal pelves and/or convoluted urethras) were slightly (but not statistically significant and with no dose relationship) higher in the 300 and 1000 mg/kg/day groups than for controls. However, these slight changes did not indicate any adverse effects of Mexoryl SO, as the observed incidences were within the historical control values.

Conclusion
There were no teratogenic effects under the conditions of the study. For maternal toxicity, the No Adverse Effect Level (NOAEL) and the No Effect Level (NOEL) were 1000 mg/kg/day (characterised by a minor transient depression in food consumption) and 300 mg/kg/day, respectively. For developmental toxicity, the No Effect Level (NOEL) was 1000 mg/kg/day in this study.

Ref.: 19

3.3.9. Toxicokinetics

See 3.3.5.2. toxicokinetics data in Sprague-Dawley rats

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

Phototoxicity on BALB C 3T3 cells

Guideline:	OECD 432 (draft March 2002)
Species/strain:	BALB/C 3T3 clone 31
Test substance:	Mexoryl SO
Batch:	0123383 (active ingredient content of 29.7%)
Purity:	>99%
GLP:	in compliance

An *in vitro* Neutral Red Uptake Photo-Toxicity test was conducted with Mexoryl SO on Balb/c 3T3 mouse fibroblasts. The concentrations selected were 1000, 500, 250, 125, 62.5, 31.3, 15.6 and 7.81 µg/mL, and the treated cells were exposed to UVA.

Results
In both experiments, a Photo Irritation Factor (PIF) could not be determined as no cytotoxicity was observed either with or without UVA irradiation after treatment up to the highest concentration assayed.

Conclusion
Mexoryl SO was not photo-toxic *in vitro*.

Ref.: 9

Photo-irritation and Photo-sensitisation Study by the Dermal Route in Guinea-pigs

Guideline:	OECD 432 (draft March 2002)
Species/strain:	Dunkin Hartley guinea pigs
Group size:	Twenty-Five (25) female and male
Test substance:	Mexoryl SO
Batch:	0123383 (active ingredient content of 29.7%)
Purity:	>99%
GLP:	in compliance

The concentration of 5% was determined as the maximum non-irritant concentration on flanks of clipped and shaved guinea pigs during the preliminary study, and irradiation doses of UVA and UVB were infra erythematogenic.
During a 8 day-induction period, six topical applications and/or (UVA + UVB) irradiation were performed. This induction period was followed by a rest period of 13 days. On day 22, a challenge was performed by topical application and/or (UVA + UVB) irradiation.

Results
No clinical signs and no deaths were noted during the study. No cutaneous reactions which could be attributed to a photo-irritant effect or a photo-sensitising effect of the test item were observed.

Conclusion
Topical applications of Mexoryl SO followed by UV irradiation did not induce any photo-irritant or photo-sensitising reactions in guinea pigs.

Ref.: 10

Comments

Undiluted Mexoryl has irritant potential to New Zealand albino rabbit (see 3.3.2.1); on shaved and clipped Guinea pig it was irritant at a concentration of 10%.

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

Bacterial Reverse Mutation Test in the Presence of UV Light

Guideline: /
Species/strain: Cultures of *Escherichia coli* strain WP2
Group size:
Test substance: Mexoryl SO
Batch: op 565-566 (active ingredient content of 95.7%)
Purity: > 99%
GLP: in compliance

The study was performed according to SCC/803-5/90.

Mexoryl SO was evaluated in two independent experiments that were performed in a darkened laboratory.
The experiments were conducted according to the direct plating incorporation method. Once bacteria were plated, plates were exposed to various doses of unfiltered UVA + UVB light (UVA/UVB doses in mJ/cm²: 5.6/1.8 and 11.3/3.6 in experiment 1, and 5.5/1.8 and 11.0/3.5 in experiment 2) or glass-filtered UVA light (230 and 460 mJ/cm² UVA), then incubated at 37°C in the dark for three days. Revertant colonies were then scored. Since the test substance was freely soluble in ethanol, the highest dose level was 5000 µg/plate despite the slight toxicity noted at this maximum dose in the range-finder study. The dose levels used in main experiments were as follows: 8, 40, 200, 1000 and 5000 µg/plate.
The following known strain specific bacterial (photo-) mutagens were used as positive controls: 8-Methoxypsoralen (8-MOP) in the presence of light and 4-Nitroquinoline-N-oxide (NQO) without irradiation, respectively. Cultures treated with ethanol (solvent) with and without irradiation, and 8-MOP without irradiation were used as negative controls. Triplicate platings were performed for each treatment condition and quintuplicate platings were performed for solvent and NQO controls.

Results
Mean solvent (with and without irradiation) and 8-MOP (without irradiation) control mutation counts were comparable and consistently low. The positive control 8-MOP in the presence of UV irradiation induced large increases in revertant numbers compared to concurrent irradiated solvent controls. Though highly toxic in experiment 1, the positive control NQO induced large increases in revertants number in experiment 2. The study was therefore considered to be valid.
Following Mexoryl SO treatments up to 5000 µg/plate in the presence of unfiltered UVA+UVB or glass-filtered UVA light, no increases in revertant numbers were observed in *Escherichia coli* strain WP2.

Conclusion
The test substance was not mutagenic to *Escherichia coli* strain WP2 in the presence of UV light.

Ref.: 15, 17

Comment
The test substance was called Mexoryl SO. However, it is not clear whether it was in fact the neat compound, namely camphor benzalkonium methosulfate.

Chromosome Aberration Test in Chinese Hamster Ovary Cells in the Presence of UV light

Guideline: /
Species/strain: Chinese hamster ovary cells
Test substance: Mexoryl SO
Batch: op 565-566 (active ingredient content of 95.7%)
Purity: >99% (active ingredient)
GLP: in compliance

The study was performed according to SCC/803-5/90.

In the cytotoxicity range-finder experiment, Mexoryl SO was tested at 78.13, 156.3, 312.5,625, 1250, 2500 and 5000 µg/ml in ethanol. Cultures were incubated for approximately 2 hours at 37°C, rinsed, and then incubated for a further 18 hours before harvesting. The highest concentration tested in the main experiment was selected on the basis of osmolality, solubility, and toxicity indicated by the reduction of mitotic index (MI). Since Mexoryl SO was freely soluble in ethanol, osmolality values were equivalent to those of the vehicle control culture, and no mitotic inhibition was observed, the dose levels selected for metaphase analysis were 1250, 2500 and 5000 µg/ml both in the absence and the presence of UV light.
In the main experiment, cultures were treated with three concentrations of Mexoryl SO or with known clastogens (methyl methanesulphonate (MMS) in the absence of light and 8-methoxypsoralen (8-MOP) in the presence of light). Solvent-treated cultures (ethanol) were used as negative controls. Cultures were treated in triplicate for Mexoryl SO and solvent solutions, and in duplicate for the chemical controls. Thereafter, cultures were irradiated (200 mJ/cm² unfiltered UVA + 34.8 mJ/cm² unfiltered UVB, or 700 mJ/cm² glass-filtered UVA) at least 15 minutes after treatment to ensure equilibration of chemicals into the cells and within 2 hours to ensure rinsing occurred on time, and then incubated for a further 18 hours before harvesting.
Two hours prior to harvest, cell cultures were treated with a colchicine solution (1 µg/ml) to block them in metaphase. Chromosome preparations were stained and examined microscopically for mitotic index and for aberrations when selected. Two hundred well spread metaphases per concentration were evaluated blind.

Results
The non-irradiated solvent control cultures showed frequencies of structural aberrations within historical control ranges, as did the non-irradiated 8-MPO control, UV-irradiated solvent controls and the non-irradiated test chemical cultures. The positive control treatments (MMS, irradiated 8-MOP) induced large increases in the incidence of chromosome aberration indicating the adequate sensitivity of the test system and procedure used.
No increases in the frequency of structural or numerical aberrations, which exceeded the historical normal range, were observed in cultures treated with Mexoryl SO up to 5000 µg/ml in the presence of UV light.

Conclusion
Mexoryl SO did not induce chromosome aberrations in Chinese hamster ovary cells both in the presence and the absence of UV light.

Ref: 16, 17

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS) for the 6% concentration

In calculating the Margin of Safety, the mean + 2 standard deviations of the dermal absorption through human dermatomed skin (ref. 20) is used. The maximum dermal absorption observed was 3.73 µg/cm²; it appears to be an outlier.

CALCULATION OF THE MARGIN OF SAFETY

Maximum absorption through the skin	**A (µg/cm^2)**	**=**	**2.73 µg/cm²**
Skin Area surface	**SAS (cm^2)**	**=**	**18000 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**49.14 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.819 mg/kg**

NOAEL (rat, 90-day, oral) of 300 mg/kg corresponding to 89.1 mg/kg bw/day of active ingredient

= 89.1 mg/kg bw

Margin of Safety	**NOAEL / SED**	**=**	**109 ***

* The Margin of Safety has been calculated on the basis of the dermal absorption from a typical sunscreen formulation containing 6% Camphor Benzalkonium Methosulphate. No specific dermal absorption study has been performed with the 3% concentration; so a direct calculation of the MOS for a 3% concentration cannot be done.

3.3.14. Discussion

In its previous opinion on camphor benzalkonium methosulphate as a UV-filter, the SCCP stated that the use at a maximum concentration of 6.0% in the cosmetic sun protection preparations does not pose a risk to the health of the consumer. However, because of its borderline Margin of Safety (109), its use in other types of cosmetic products was not recommended.
In the new submission the applicant has chosen the strategy to propose use at half the concentration (3% instead of 6%), in all products, without submitting a new dermal absorption study.
For dermal absorption, a linear extrapolation from 6.0 to 3.0% cannot be made. Therefore, no MOS for the 3% concentration can be calculated. However, as the skin absorption of a substance decrease when the applied concentration is lowered, the amount absorbed from a 3% concentration would decrease in comparison to the 6% concentration.
Taking into account the decreased absorption at lower concentration and considering the fact that the MoS calculation was based on a conservative NOAEL (local effects in the GI tract), the use of camphor benzalkonium methosulfate at the reduced maximum concentration of 3% in the final products may be considered as safe for all types of cosmetic products.

Camphor benzalkonium methosulfate (Mexoryl SO ; S57) is irritating to the eyes at the concentration of 6%. No study of eye irritation has been conducted with a 3% concentration

Physico-chemical properties
Camphor Benzalkonium Methosulfate is used up to a maximum concentration of 6.0% in sun screen formulations (provided by Mexoryl SO at a maximum concentration of 20%). Batches 0106845; M171; op565-566; CFQ 14151 were used but not characterised.
An UV-spectrum was not submitted

General toxicity
The LD50 (camphor benzalkonium methosulfate) is > 600 mg/kg bw.
In a 90-day study, the NOAEL was considered to be 300 mg/kg/day.
For maternal toxicity, the NOAEL and the NOEL were 1000 mg/kg/day (characterised by a minor transient depression in food consumption) and 300 mg/kg/day, respectively. For developmental toxicity, the NOEL was 1000 mg/kg/day.

Irritation / sensitisation
Mexoryl SO is not irritating to the skin; it has a mild irritating potential to the eye when used undiluted or as a 20% aqueous solution.
Mexoryl SO was a non-sensitiser in a Guinea Pig Maximisation Test.

Percutaneous absorption
The dermal absorption (sum of the amounts measured in the living epidermis/dermis and receptor fluid) of camphor benzalkonium methosulfate from a typical sunscreen formulation containing Mexoryl SO at 20% (corresponding to 6% camphor benzalkonium methosulfate) was estimated to be 0.65 ± 1.04 µgeq/cm² as a mean; range: (0.06 – 3.72 µgeq/cm²) under exaggerated use conditions.
The mean dermal absorption + 2 standard deviations (0.65 + 2 x 1.04) or 2.73 µg equiv/cm² measured with a 6% concentration, will be used for the calculation of the Margin of Safety

Mutagenicity/Genotoxicity
Mexoryl SO was not mutagenic to *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100 and TA 102 either in the absence or the presence of metabolic activation. It did not produce chromosome aberrations in cultured human lymphocytes both in the presence and the absence of metabolic activation.
Mexoryl SO did not induce damage to the chromosomes or the mitotic apparatus of rat bone marrow cells after a single oral administration at the dose-levels of 500, 1000 and 2000 mg/kg.

Photo-toxicity/photo-irritation/photo-sensitisation
Mexoryl SO was not photo-toxic in vitro.
Topical applications of Mexoryl SO followed by UV irradiation did not induce any photo-irritant or photo-sensitising reactions in guinea pigs.

Photo-mutagenicity
The test substance was not mutagenic to *Escherichia coli* strain WP2 in the presence of UV light.
Mexoryl SO did not induce chromosome aberrations in Chinese hamster ovary cells both in the presence and the absence of UV light.

Carcinogenicity
No data submitted

4. CONCLUSION

In response to opinion SCCP/1015/06 of 19 December 2006, the applicant preferred not to perform a new dermal absorption study as requested by the SCCP. Instead, a reduction of maximum authorised concentration from 6.0 to 3.0% as a UV filter in cosmetic products was proposed.

On the basis of the available data, a reduced maximum concentration of 3% as a UV filter in cosmetic products is considered safe.

Camphor benzalkonium methosulfate is irritating to the eyes at the concentration of 6%. No study of eye irritation has been conducted with a 3% concentration.

The inhalation exposure to camphor benzalkonium methosulfate from spray products (e.g. deodorants) was not assessed.

5. MINORITY OPINION

Not applicable

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