EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

OPINION ON HOMOSALATE

COLIPA n° S12

The SCCP adopted this opinion at its 11th plenary on 21 March 2007

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, Ramon Grimalt, 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 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

© European Commission 2007
(ISSN)

The opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The opinions are published by the European Commission in their original language only.

http://ec.europa.eu/health/ph_risk/risk_en.htm

ACKNOWLEDGMENTS

Dr. C. Chambers
Prof. G. Degen (rapporteur)
Dr. B. Jazwiec-Kanyion
Prof. V. Kapoulas
Prof. C. Lidén
Prof. J.-P. Marty
Prof. T. Platzek
Dr. S.C. Rastogi
Prof. J. Revuz
Prof. V. Rogiers
Prof. T. Sanner (chairman)
Dr. J. van Engelen
Dr. I.R. White

Keywords: SCCP, scientific opinion, UV filter, homosalate, S12, Directive 768/76/EEC, CAS 118-56-9, EINECS 204-260-8

Opinion to be cited as: opinion of the SCCP on homosalate

TABLE OF CONTENTS

1. BACKGROUND

Submission I on the UV-filter Homosalate with the chemical name 3,3,5-trimethylcyclohexylsalicylate was submitted in December 2005 by COLIPA[1].

Homosalate is proposed for continued use in sunscreen products at a maximum concentration at 10% weight/weight.

The substance is currently regulated in the Cosmetics Directive (76/768/EEC) in annex VII, part 1 (list of permitted UV filters) under entry 3.

A re-evaluation of the substance on EU level was asked for by the Member states.

2. TERMS OF REFERENCE

1. *Does the SCCP consider the continued use of Homosalate safe for the consumers, when used as an UV-filter in a concentration up to 10% w/w in cosmetic products taken into consideration the provided scientific data?*

2. *Does the SCCP consider the use of homosalate in a concentration up to 10% w/w in other products than sunscreen products safe for the consumer?*

3. *Does the SCCP foresee any other restrictions to the safe use of Homosalate?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Homosalate (INCI)

Ref.: 28, 63, 64

3.1.1.2. Chemical names

Benzoic acid, 2-hydroxy-, 3,3,5-trimethylcyclohexyl ester (EC inventory)
Cyclohexanol, 3,3,5-trimethyl-, salicylate
Homomenthyl salicylate
m-Homomenthyl salicylate
Metahomomenthyl salicylate
Salicylic acid, 3,3,5-trimethylcyclohexyl ester
Salicylic acid, m-homomenthyl ester
3,3,5-Trimethylcyclohexyl 2-hydroxybenzoate

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

3,3,5-Trimethylcyclohexyl salicylate

Ref.: 28, 63, 64

3.1.1.3. Trade names and abbreviations

Caswell No. 482B
CCRIS 4885
Eusolex HMS
Filtersol ''A''
COLIPA n° S12
Neo Heliopan® HMS
NSC 164918
Uniderm Homosal
(8CI)

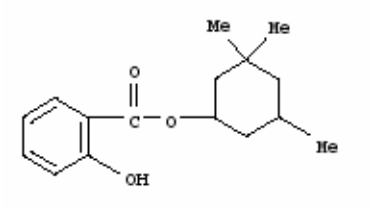
Ref.: 28, 63, 64

3.1.1.4. CAS / EINECS number

CAS: 118-56-9
EINECS: 204-260-8

Ref.: 28, 63, 64

3.1.1.5. Structural formula



Ref.: 55, 64

3.1.1.6. Empirical formula

Formula: $C_{16}H_{22}O_3$

Ref.: 63, 64

3.1.2. Physical form

Clear, colourless to pale yellow liquid

3.1.3. Molecular weight

Molecular weight: 262.02 g / mol

Ref.: 63, 64

3.1.4. Purity, composition and substance codes

Assay (GC):	98.0% min
UV absorbance (E 1%/1cm):	170-180 (at 305 nm)
Content (GLC, sum 2 isomers):	> 98.0 area %
Ash:	<0.05%
Sulphated ash:	<0.1%
Water:	0.01%
Additives:	no preservatives, no antioxidants, no solvents

Ref.: 63, 64

3.1.5. Impurities / accompanying contaminants

Heavy metals:	Arsenic not detectable (<0.01 ppm) Lead not detectable (<0.50 ppm) Mercury: not detectable (<0.10 ppm) Cadmium: not detectable (<0.01 ppm) Nickel: not detectable (<0.50 ppm) Iron 1 ppm
Microbiological information:	<10/ml (detection limit)

Ref.: 63, 64

3.1.6. Solubility

Paraffin oil (at 20 °C):	miscible
Isopropyl myristate (at 20 °C):	miscible
Ethanol (at 20 °C):	miscible
Water (at 20 °C):	immiscible
Propylene glycol (at 20 °C):	immiscible

Ref.: 63, 64

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 5.82 and 6.16 (calculated)

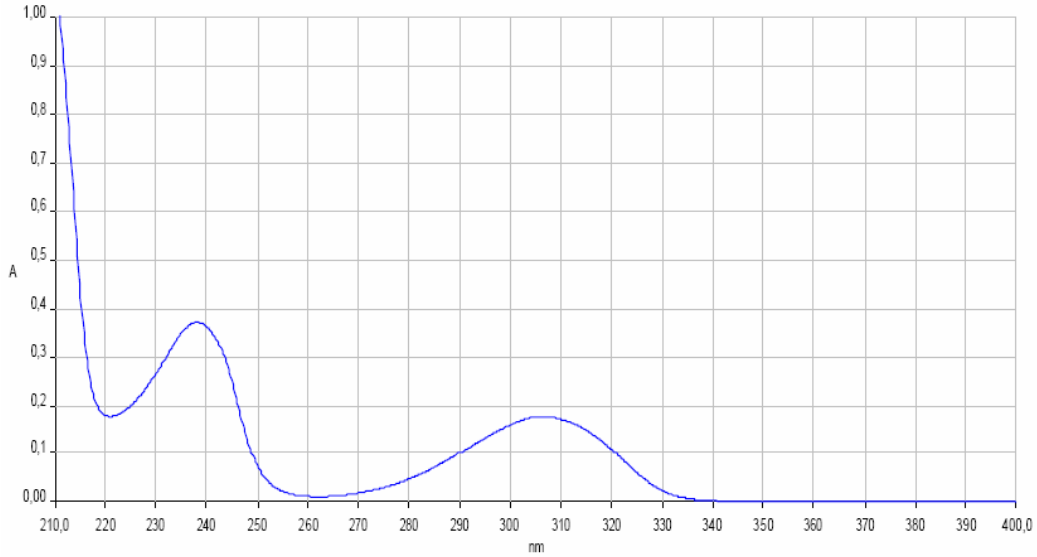
Ref.: 55

3.1.8. Additional physical and chemical specifications

Organoleptic properties:	slight mint odour
Melting point:	/
Boiling point:	/
Flash point:	> 100
Vapour pressure:	/
Relative density (D 20/4):	1.0512 (1.050-1.053)
Specific gravity (D 25/25):	1.049 –1.053
Viscosity:	/
pKa:	/
Acid value (potentiometric filtration, mg KOH/g):	0.0 - 1.0 max
Refractive index (n 20/D, 20 °C):	1.516–1.519
Extinction	170 –180

(UV/VIS spectrum in methanol; 0.10 mg/ml cuvette 0.1 cm 305 nm)

UV spectrum
The UV spectrum of Homosalate was determined using an amount of 10 mg dissolved in ethanol. Two peaks were observed, one at 238.18 nm, the other at 306.39 nm.



Ref.: 47, 63, 66

3.1.9.	Stability and Photo-stability

Shelf life: at least 2 –3 years

Ref.: 63, 67

Photo-stability
The photo-stability of Homosalate was examined in the presence of a photo-labile UV-A absorbing research material using the Suntest CPS Heraeus Xenon lamp (irradiance: 40 W/m² (24 min = 1 MED)). A 30 mg emulsion containing 5% Homosalate was spread on a glass plate with an area of 10 cm², dried for 30 minutes and exposed to 5, 10, 15 and 20 MED under cooling (20 °C). The samples were immersed in 25 ml ethanol and analyzed UV spectrophotometrically and by chromatography (HPLC). The decrease in Homosalate content ranged between 0 – 2.7% und thus, Homosalate was shown to stable under these conditions.

Ref.: 30, 65, 68

In addition, dilute solutions in isopropanol and cyclohexane as well as in mineral oil and ethanol/water were shown to be photo-stable.

Ref.: 61

General Comments to physico-chemical characterisation

- Log P_{ow}: calculated values cannot be accepted as estimates of the true physical constants without justification, indicating that the reported values are realistic.
- the stability of the test substance in the marketed product (and in the test solutions) was not reported.

3.2. Function and uses

Homosalate is used as a broad-band UV filter in concentrations of up to 10% in the EU or 15% depending upon where the product is used (e.g. in the USA) in sunscreen products alone or in combination with other UV absorbers to protect the skin against harmful effects of the UV radiation.

Ref.: 63, 64

3.3. Toxicological Evaluation

Introductory remarks

Homosalate has a long history of use as broad-spectrum UV filter in sunscreens alone or in combination with other UV filters.

The safety of Homosalate for its usage in sunscreen drug products for over the counter (OTC) human drugs was first peer reviewed by the US FDA in 1978 (reference 19). Based on the data available at that time the FDA expert panel classified Homosalate as safe and effective.

In the subsequent sections the most reliable and valid studies available for the respective endpoint, were described in detail. Other studies covering the same endpoint or studies with only minor or questionable relevance were cited only in a short form for completeness sake.

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline: /
Species/strain: Rat/FDRL
Group size: 3 males and 2 females per dose level
Test substance: Homosalate (Homomethyl salicylate)
Batch: R-5269-D
Purity: /
Doses: 0.5; 1.0; 2.0; 4.0; 8.0 ml/ kg bw
Observation: 14 days
GLP: /

The acute oral toxicity was determined in rats employing the procedure recommended in Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, Association of the Food and Drug Officials of the US

Homosalate was administered orally by gavage to each 3 male and 2 female FDRL rats at dose levels of 0.5; 1.0; 2.0; 4.0; 8.0 ml/kg bw with the test substance as received. Body weights were determined on days 0 and 14. The animals were observed for treatment-related effects for a 14-day observation period. Gross pathology was performed in rats that died and in survivors sacrificed at 14 days.

Results

There were no mortalities at any dose level and no clinical signs were noted at dose levels between 0.5 –2.0 ml/kg bw. All animals gained weight. One out of 5 rats at 4.0 ml/kg bw showed soft faeces and at 8.0 ml/kg bw, 1/5 had diarrhoea, was cold, emaciated and showed urinary incontinence. At necropsy only unspecific findings were observed in single rats at each dose level.

Conclusion
The acute oral toxicity (LD50) of Homosalate was >8.0 ml/kg for male and female FDRL rats.

Ref.: 48

Guideline: /
Species/strain: Rat / no information on strain
Group size: 10 animals
Test substance: Homosalate (Homomethyl salicylate)
Batch: no information available
Purity: /
Doses: 5000 mg / kg bw
Observation: no information available (more than 6 days, vide infra)
GLP: /

Homosalate was administered orally at a single dose level of 5000 mg/kg bw. The animals were observed for treatment-related effects and gross pathology was performed.

Results
During the observation period 1 of 10 animals died on day 6. No further details were recorded. There were no mortalities at any dose level and no clinical signs were noted at dose levels. At necropsy only non-specific findings of acute intoxication were noted in single rats.

Conclusion
The acute oral toxicity (LD50) of Homosalate was > 5000 mg/kg in rats.

Ref.: 54

3.3.1.2. Acute dermal toxicity

Guideline: /
Species/strain: Rabbit/albino (strain not cited)
Group size: 10 animals
Test substance: Homosalate (Homomenthyl salicylate)
Batch: no information available
Purity: no information available
Doses: 5000 mg/kg bw dermal
Observation: no information available
GLP: no (study performed prior to implementation of GLP)

Homosalate was administered dermally to 10 rabbits at a single dose level of 5000 mg/kg bw.
The animals were observed for treatment-related effects and gross pathology was performed.

Results
No mortality was noted but signs of skin irritation occurred in form of slight or moderate redness in 4/10 or 6/10 as well as slight or moderate oedema in 7/10 or 3/10 rabbits, respectively. At necropsy only non-specific findings of acute intoxication were noted in single animals.

Conclusion
The acute oral toxicity (LD50) of Homosalate was > 5000 mg/kg for rabbits.

Ref.: 54

3.3.1.3. Acute inhalation toxicity

No data submitted

Applicant conclusion on acute toxicity
The toxicity of Homosalate is very low. The LD50 values for acute oral and acute dermal toxicity are significant above the current limit values for testing and classification of >2000 mg/kg bw/d, i.e. LD50 acute oral (rat) >5000 mg/kg bw or >8.0 ml/kg bw and LD50 acute dermal (rabbit) >5000 mg/kg bw.
Although these studies were performed prior to the implementation of specific EU/OECD testing guidelines or GLP requirements, they can be regarded as scientifically valid considering the date when they were performed. Further, also in respect to animal welfare, there is no need for further testing.

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

There is no guideline conform study available in respect to the skin irritation potential of Homosalate in experimental animals.
However, the skin irritative property of Homosalate was tested within a combined study according to a modified Harber et al. (1982, 1987) protocol in male and female guinea pigs as well as in a combined and optimized mice ear swelling study in female BALB/C mice. The methods investigated and the results are described in detail in section 3.3.10.1 (Photosensitization *in vivo*). In these investigations it was shown that Homosalate revealed no skin irritation potential in guinea pigs or mice (References: 25, 26).
The studies on the skin irritation potential of Homosalate in humans are described and assessed in section 3.3.3 (Sensitisation).

3.3.2.2. Mucous membrane irritation

Guideline:	Eye irritation study according to the method of Draize et al. (1959) comparable to OECD 405
Species:	Rabbit/New Zealand White
Group:	3 male animals
Substance:	sunscreen containing 12% Homosalate
Batch:	No information available
Purity:	Neat sunscreen
Dose:	0.1 ml instillation in the conjunctival sac of the right eye
GLP:	in compliance

The potential irritant effect of a sunscreen containing 12% homosalate was investigated in 3 male New Zealand White rabbits. 0.1 ml of the unchanged sunscreen was placed into the conjunctival sac of the right eye of each of the animal. The left eye was not treated and served as control. The test substance was not washed out. Thereafter, the eyes were examined with the aid of an auxiliary light source for signs of irritation covering the cornea, iris and the conjunctiva of each animal at 1, 24, 48 and 72 hours. The findings were scored according to Draize et al. (1959). Following the observation at 24 hour, all test and control eyes were examined using fluorescein solution and any residual test material was gently rinsed out with physiological saline.

Results
Treatment had no effect on the cornea of the rabbits at any time-point. Exposure to the sunscreen resulted in iritis in 2/3 rabbits at the 1-hour scoring interval but recovered completely within 24 hours. Conjunctivitis consisting of redness, swelling and/or

discharge was recorded in all rabbits at the 1-hour interval, improved in incidence and severity subsequently. At 72 hours no conjunctival reaction was noted.

Conclusion
It was shown that the sunscreen containing 12% homosalate formulation led to slight eye irritation under the conditions of the study.

Ref.: 62

For completeness sake it has to be mentioned that two alternative *in vitro* eye irritation investigations with a Homosalate containing cream (product 285679, batch VN01D, no information about composition and Homosalate content) are available. The agarose diffusion method showed slight cytotoxicity and the hen's egg test–chorioallantoic membrane (HETCAM) revealed an irritative effect. However, these data are considered as not reliable for the assessment of the irritation potential of Homosalate.

Ref.: 16, 18

Applicant conclusion on irritation
There are only limited data available on the irritative potential of Homosalate to the skin and the eyes in experimental animals. However, the existing data derived from the combined study either in male and female guinea pigs or female mice did not indicate an irritation potential to the skin or the mucous membranes.
In addition, numerous clinical studies in human with different types of sunscreens and other cosmetic products containing Homosalate up to 15% and performed under controlled and standardized conditions including GLP/GCP and under supervision or participation of a certified dermatologist revealed no irritative potential, even not under enhanced condition.
Therefore, it is considered that Homosalate poses no risk in respect to eye or skin irritation for the consumer from the usage of sunscreens and other Homosalate containing cosmetic products under intended use conditions.

3.3.3. Skin sensitisation

There is no guideline conform study available in respect to the skin sensitizing potential of Homosalate in experimental animals.
However, the skin sensitizing property of Homosalate was tested within a combined study according to a modified Harber et al. (1982, 1987) protocol in male and female guinea pigs as well as in a combined and optimized mice ear swelling study in female BALB/C mice. The details of the methodology and results are described below in section 3.3.10.1 (Photosensitization *in vivo*). In these specific studies it was shown that Homosalate revealed no specific or selective skin sensitizing potential in guinea pigs or mice.

Ref.: 25, 26

In addition, there exists a personal communication from Maibach in a publication on the quantitative structure-toxicity relationship (QSTR) that Homosalate (homomenthyl salicylate, CAS 118-56-9) showed no sensitizing potential in the guinea pig maximization test. The model calculations per se predicted Homosalate as a substance with weak/moderate sensitizing potential according to the specific QSTR model of the authors.

Ref.: 17

Human data, maximization test

Guideline/Method:	Human Maximization test according to Draize, 1966
Species:	Human
Group size:	25 volunteers: 7 males, 18 females
Test substance:	Homosalate

Batch:	not cited
Route:	Occlusive epicutaneous application
Scoring system:	Scoring scale of Draize
GLP:	not in compliance

Homosalate was tested for potential sensitization on Human skin in a maximization test in 25 healthy volunteers. Prior to the main study, the test substance was occlusively applied for 48 hours to aid the decision whether sodium lauryl sulfate (SLS) pre-treatment can be applied. As no irritation was observed, SLS pre-treatment was decided for the main test.
In the main phase of the study, the neat test substance was applied for five 48-hour periods under an occlusive dressing, each time at the same site. Prior to each application the exposure site was pre-treated occlusively with 2.5% aqueous SLS for 24 hours. After a rest period of 10 days, a challenge patch was applied to a different site for 48 hours under occlusive conditions. Each challenge location was occlusively pre-treated for one hour with 5% - 10% aqueous SLS. The challenge sites were read and scored after removal of the patch and 24 hours afterwards.

Results
The 25 volunteers revealed no signs of skin irritation or sensitization at any challenge readings.

Conclusion
Homosalate was shown to cause no signs of sensitization under the conditions of the Maximization test in male and female Human volunteers.

Ref.: 42

In the subsequent sections recent reliable and representative data on Human studies were supplied which were not performed with Homosalate per se but with representative products (mostly sunscreens) with a varying concentration of Homosalate. The main purpose of these studies was to investigate the safe usage of these products under enhanced and comprehensive use conditions.

Human data, repeated insult patch test (RIPT)

Guideline/Method:	Approved study protocol and standard operating procedures by the New England Institutional Review Board (NEIRB) 2005
Species:	Human
Group size:	a) –c) 236 induced volunteers and 209 completed
Test substance:	a) SPF-30 sunscreen (formula #769-187, Homosalate content: 10%) b) SPF-45 sunscreen (formula #769-190, Homosalate content: 15%) c) SPF-30 sunscreen (formula #769-193, Homosalate content: 10%)
Batch:	a) batch #0015C-P (white cream) b) batch #0015C-V (white cream) c) batch #0015C-M (white cream)
Route:	Semi-occlusive epicutaneous application
Scoring system:	Modified scoring scale of the International Contact Dermatitis Research Group System (Fisher, Alexander A., Contact Dermatitis, Lea & Febiger, 1986, 26)
GLP:	in compliance

A repeated insult patch test (RIPT) according to the approved NEIRB study protocol was performed with 3 different sunscreens containing 10% or 15% Homosalate among other

substances on a panel of 236 male and female volunteers under GLP conditions.

Induction period: During the induction phase, approximately 0.2 g of the test material was applied to the dry wiped skin on the left side of the back of each volunteer. The webril/adhesive patch was semiocclusively covered and remained on the skin for 24 hours. Thereafter, the patches were removed and the skin was scored. The patch removal was followed by a rest period of 24 hours for workdays or 48 hours for weekend. A series of 9 induction patches was completed over a period of 3 weeks.
Rest period: The last induction patching was followed by a rest period of two weeks with no application.
Challenge period: After the rest period, a webril/adhesive patch was applied with 0.2 g of the test material and fixed semi-occlusively on the virgin, right side of the back of each volunteer for 24 hours. After removal, the application sites were scored at about 24, 48, 72 and 96 hours post-patching. The complete test was conducted under the supervision of a Board-Certified Dermatologist, which participated also in the scorings of the volunteers.

Results
209 volunteers completed the study and 27 discontinued but not due to test material reaction. During the induction phase each one volunteers showed a transient and negligible erythema after application of sunscreen SPF-30 (a) or 45 (b) on single readings, while no skin findings were observed on the tested skin areas of any of the volunteers at any time challenge tested with these materials. With sunscreen SPF-30 (c), no skin finding was noted during induction in any of the volunteers, while one subject showed a low level reaction on the 48 hour reading but not at 24, 72 or 96 hours readings.

Conclusion
With none of the tested sunscreen products containing 10% or 15% Homosalate there was an indication for an irritative or sensitizing potential under the conditions of the RIPT study in male and female Human volunteers.

Ref.: 35, 38, 41

Beside these recent RIPTs (all performed in 2005) there are numerous other Human repeat insult patch tests available, which were preformed during 2000-2003 with different products (sunscreen, creams, lotions) following the same or a comparable test procedure with occlusive and semi-occlusive application. The investigated cosmetic products contained Homosalate in a range between 10% - 15%. In none of these studies a clinically relevant potential for dermal irritation or sensitization was observed.

Ref.: 6, 7, 21, 22, 23, 29, 46, 51, 52, 69

In addition, a comparative cumulative irritation test was performed recently with a total of 16 different cosmetic products including several sunscreens containing 10% or 15 % Homosalate among other ingredients in male and female volunteers. 28 persons were induced and 26 completed the test.
The test materials were applied occlusively to the same site on the back with a frequency of 3 times/week for 6 applications within a 14 day period. Approximately 48 hours after each patching (exception: 72 hours on weekends) the patches were removed at the test laboratory and the sites were scored and graded for skin finding.
Isolated cases of minimal skin reactions were recorded transiently in few sunscreens. The total grand scores were 0 or 1.0 at maximum of a potential maximum total score of 628. Thus, the sunscreens exhibited no potential for cumulative irritation.

Ref.: 32

Table 1: Summary of human data

Test	Test substance	No. of volunteers	Application Remark	Results Conclusion	Ref.
RIPT	SPF-45 sunscreen (769-190, 15% H)	236 induced, 209 completed	Semi-occlusive Induction 1 low level transient reaction Challenge: no reaction	No potential for dermal irritation or sensitization	35
RIPT	SPF-30 sunscreen (769-187, 10% H)	236 induced, 209 completed	Semi-occlusive Induction: 1 low level transient reaction Challenge: no reaction	No potential for dermal irritation or sensitization	38
RIPT	SPF-30 sunscreen (769-193, 10% H)	236 induced, 209 completed	Semi-occlusive Induction: no reaction Challenge: 1 low level transient reaction	No potential for dermal irritation or sensitization	41
RIPT	MT#2101420 (10% H)	112 inducted 102 completed	Semi-occlusive Induction and challenge no reaction	No potential for dermal irritation or sensitization	6
RIPT	Lotion 3 (12% H)	203 inducted 202 completed	Occlusive Induction: 1 patchy erythema during last induction Challenge: no reaction	No potential for dermal irritation or sensitization	7
RIPT	U02195.05 (283221, 10% H)	240 inducted 210 completed	Semi-occlusive Induction: 1 low level transient reaction Challenge: no reaction	No potential for dermal irritation or sensitization	21, 29
RIPT	MT# 2047459	600 inducted 600 completed	No information on occlusion Induction and challenge: no reaction application	No potential for dermal irritation or sensitization	46
RIPT	U03036.01 (283267, 10% H)	219 inducted 211 completed	Occlusive Induction: 1 with transient redness grade 2, 1 with transient redness/edema grade 3 Challenge: no reaction	Reactions were considered incidental as artefacts No potential for dermal irritation or sensitization	22, 51
RIPT	U03036.03 (283273, 10% H)	219 inducted 211 completed	Occlusive Induction: 1 with transient redness grade 2, 1 with transient redness/edema grade 3 Challenge: no reaction	Moderate cumulative irritation in 2 volunteers during induction only. No clinically relevant potential for dermal irritation or sensitization	23, 52

Test	Test substance	No. of volunteers	Application Remark	Results Conclusion	Ref.
RIPT	2 Sunscreen lotions (each 15% H)	221 induced 215 completed	Occlusive Induction: few transient low level reactions Challenge: 1 showed low level reaction	No relevant potential for dermal irritation or sensitization	69
CIT	SPF-30 sunscreen (769-187, 10% H) SPF-30 sunscreen (769-193, 10% H) SPF-45 sunscreen (769-190, 15% H)	28 induced 26 completed	6 x occlusive (48 h/72 h week/weekend) within 14 days (3x/week)	No potential for cumulative irritation	32
H = Homosalate; CIT = cumulative irritation test; RIPT = repeated insult patch test					

Applicant conclusion on sensitisation
Although only limited information on the skin sensitizing potential is available in experimental animals, the existing data obtained in guinea pigs and mice exhibited no sensitizing potential of Homosalate. Furthermore, recent clinical studies in human with different types of sunscreens and other cosmetic products containing Homosalate up to 15% and performed under controlled and standardized conditions including GLP/GCP and under supervision or participation of a certified dermatologist revealed no skin sensitizing potential, not even under enhanced condition. Therefore, it is considered that Homosalate is of no sensitization risk for the consumer from the usage in sunscreens at intended use conditions.

Comment
The SCCP does not consider the recent RIPT studies as ethical.
These data appears to be generated in the USA. Within Europe, such studies are not regarded as ethical.

3.3.4. Dermal / percutaneous absorption

3.3.4.1. Percutaneous absorption in vitro

Human skin

Guideline: OECD 428 (Draft, 2000); OECD Guidance Document 28 (2004); Basic criteria for in vitro assessment of cosmetic ingredients (SCCNFP/0750/03, October 2003); Diembeck et al., 1999
Test System: Human skin
Substance: 10% Homosalate in a standard sun screen
Batch: Non labelled: 4095213 (purity: 99.88% (GLC))
Radiolabelled: CFQ 14329, specific activity: 54 mCi/mmol
Purity: Non labelled: 99.88% (GLC)
radiochemical purity: 99.8% (HPLC)
Dose: approx. 3.4 mg dose formulation/0.64 cm^2 (corresponding to approx. 0.5 mg Homosalate/cm^2)
Skin preparation: Fresh dermatomed human skin from abdominal surgery from 3 female donors
Mean thickness (n=6): Donor 1: 397±30 µm
Donor 2: 357±13 µm
Donor 3: 519±90 µm
Skin temperature: 32 °C

Test chamber:	Flow-through automated diffusion cells (PermeGear Inc, Riegelsville, PA/USA)
Receptor fluid:	DMEM and Ham's F 12 culture medium (3:1) supplemented with hEGF, hydrocortisone, gentamycin, glutamine and 10% FCS
Solubility:	12 µg/ml in receptor fluid
Route:	topical application
Exposure time:	24 h
GLP:	in compliance

Homosalate was investigated for its skin penetration in vitro as a 10% standard sunscreen formulation. Fresh dermatomed human skin from surgery was processed and put on the flow through automated diffusion cells. The temperature was checked regularly and was about 32 °C at ambient humidity. The receptor fluid was pumped at a speed of about 1.6 ml/h. The complete formulation was prepared one day prior the start. Homogeneity and concentration of radioactivity in the formulation were analyzed. A total amount of approx. 3.4 mg dose sunscreen formulation/0.64 cm^2 (corresponding to approx. 0.5 mg Homosalate/cm^2) was applied. Exposure duration was 24 h. During exposure receptor fluid samples were collected at regular intervals. After 24 h exposure, the skin surface was washed using a mild soap solution and cotton swamps. Each skin was 10 times tape stripped using Dsquame. The tape strips containing pieces of epidermis were pooled. The mass balance was determined using receptor fluid, skin surface washes, receptor and donor compartment washes, tape strips and digested skin. Radioactivity was determined using LBK/Wallac S1414 scintillation counter.

Results
The results of dermal absorption in human skin were as follows:

Table 2: In vitro percutaneous penetration of Homosalate in a standard sunscreen through viable human skin

Group	**A**	**B**	**C**
Homosalate in formulation (%)	10.1	10.1	10.1
Dose (µg/cm²)	544.9	548.0	541.2
N° of biopsies	6	6	6
Penetration into the receptor fluid after 24h	1.36 µg/cm² 0.25 % of dose	0.87 µg/cm² 0.16 % of dose	0.66 µg/cm² 0.12 % of dose
Flux constant (µg x cm²/h)	0.077	0.057	0.039
Lag time (h)	6.5	7.0	7.6
Total absorption (% of dose)	1.4	0.9	0.9
Total absorption # (µg/cm²)	7.63	4.93	4.87
# total absorption as amount in receptor fluid including wash and skin membrane excluding tape strips			

Conclusion
The mean flux constant for the absorption of Homosalate after application of a 10% Homosalate containing standard sunscreen formulation was 0.058 µg/cm^2. The mean total absorption was 1.1% of the applied dose corresponding to 5.81 µg/cm^2 in human skin. The mean recovery was 92.4%. The highest absorption was found in group A: 1.4 ± 0.4% (7.63 ± 2.18 µg/cm^2) with the highest absorption 2.0% (10.9 µg/cm^2).

Ref.: 13

Rat skin

Guideline:	OECD 428 (Draft, 2000); OECD Guidance Document 28 (2004); Basic criteria for in vitro assessment of cosmetic ingredients (SCCNFP/0750/03, October 2003); Diembeck et al., 1999
Test System:	Rat skin (Sprague-Dawley)
Substance:	10% Homosalate in a standard sun screen
Batch:	Non labelled: 4095213 (purity: 99.88% (GLC))

	Radiolabelled: CFQ 14329, specific activity: 54 mCi/mmol
Purity:	Non labelled: 99.88% (GLC)
	radiochemical purity: 99.8% (HPLC)
Dose:	approx. 3.4 mg dose formulation/0.64 cm^2 (corresponding to approx. 0.5 mg Homosalate/cm^2)
Skin preparation:	Fresh punched out rat skin from 3 female Sprague-Dawley rats
Mean thickness (n=6):	Rat 1: 669±47 µm
	Rat 2: 755±73 µm
	Rat 3: 763±89 µm
Skin temperature:	32 °C
Test chamber:	Flow-through automated diffusion cells (PermeGear Inc, Riegelsville, PA/USA)
Route:	topical application
Receptor fluid:	MEM (Minimal Essential Medium) supplemented with gentamycin, glutamine and 10% FCS
Solubility:	12 µg/ml in receptor fluid
Exposure time:	24 h
GLP:	in compliance

The same 10% Homosalate containing standard sunscreen formulation was also tested in rats. Freshly punched out skin samples from 3 female Sprague-Dawley rats were investigated according to the same procedure as described above for human with the exception that the receptor fluid consisted of MEM (Minimal Essential Medium) supplemented with gentamycin, glutamine and 10% FCS.

Results
The results of dermal absorption in viable rat skin were as follows:

Table 3: In vitro percutaneous penetration of Homosalate in a standard sunscreen through viable rat skin

Group	**D**	**E**	**F**
Homosalate in formulation (%)	10.1	10.1	10.1
Dose (µg/cm²)	535.9	535.9	535.9
N° of biopsies	6	6	6
Penetration into the receptor fluid after 24h	7.12 µg/cm² 1.33 % of dose	19.37 µg/cm² 3.62 % of dose	18.50 µg/cm² 3.45 % of dose
Flux constant (µg x cm²/h)	0.412	0.997	1.012
Lag time (h)	6.8	4.6	5.7
Total absorption (% of dose)	7.4	7.7	11.0
Total absorption # (µg/cm²)	39.66	41.26	58.95
# total absorption as amount in receptor fluid including wash and skin membrane excluding tape strips			

Conclusion
The results of dermal absorption in viable rat skin were as follows:
The mean flux constant for the absorption of Homosalate after application of a 10% Homosalate containing standard sunscreen formulation was 0.807 µg/cm^2. The mean total absorption was 8.7% of the applied dose corresponding to 46.62 µg/cm^2 in rat skin. The mean recovery was 93.1%.

Ref.: 13

For completeness sake it has to be mentioned that skin penetration *in vitro* was also determined with two sunscreen formulations containing 5% Homosalate and other sunscreens prepared as an O/W emulsion gel or petrolatum jelly preparation. Human full-thickness skin obtained from 3 female breast or abdominal surgery donors was mounted on static Franz diffusion cells. An amount of 3.0 mg/cm^2 of each sunscreen formulation was applied for 30 min. or 6 h and penetration in the epidermis and dermis was determined.

The amount of Homosalate measured after 30 min in the epidermis was 0.4 µg/cm (0.2% of dose) independent from formulation and amounted to 0.3 µg/cm^2 (0.2% of dose) tested as an emulsion gel or 0.6 µg/cm^2 (0.3% of dose) when applied in petrolatum. No Homosalate could be determined after 30 min or 6 h in the dermis. Thus, only adsorption in the epidermis was noted and no penetration through the skin.
Ref.: 5

A published study showed that pre-treatment of freshly excised full-thickness dorsal skin from female hairless mice with an ethanol (80%) solution containing 5% Homosalate led to enhanced transdermal penetration of a pesticide (2,4-dichlorophenoxyacetic acid). However, this study was considered as not valid and of no relevance for the assessment of the percutaneous absorption of Homosalate *in vitro*.

Ref.: 50

3.3.4.2. Percutaneous absorption *in vivo*

There exists currently no scientific or regulatory valid *in vivo* skin penetration study. Only few studies with Homosalate were published and are available in the open literature. The tape stripping methodology was applied by Chatelain et al. (2003) and Sarveiya et al. (2004). In both studies it was shown that penetration through the skin was minimal and the vast majority was retained by the stratum corneum. In addition, Chatelain et al. (2003) observed a difference in respect to the applied formulation. The total amount penetrating into the stratum corneum was higher from the O/W emulsion gel than from the petrolatum jelly formulation.
Finally, no quantitative conclusion for skin penetration is possible but qualitatively, it can be stated that –as to the *in vitro* results –the stratum corneum adsorbed the greatest fraction and only small amounts can be considered as absorbed and systemically bioavailable. In addition, the type of preparation/formulation had an influence on the proportion of adsorption.

Ref.: 5, 19, 56

Applicant conclusion on dermal/percutaneous absorption
The recent comparative rat versus human *in vitro* percutaneous absorption study performed under current guideline requirements and under GLP conditions showed that application of a 10% Homosalate containing sunscreen led to mean absorption of 8.7% (corresponding to 46.62 µg/cm^2) in rats and to 1.1% (corresponding to 5.81 µg/cm^2) in human using freshly dermatomed skin. The mean recovery was 92.4%. The highest absorption was found in group A: 1.4 ± 0.4% (7.63 ± 2.18 µg/cm^2) with the highest absorption 2.0% (10.9 µg/cm^2). Further, it was demonstrated that based on total absorption, human skin was about 8-fold less permeable than rat skin.
Comment
Beside this valid investigation, there are few *in vitro* and *in vivo* studies available with topical application of Homosalate as constituent of preparations in varying concentrations dealing with different parts and aspects of dermal adsorption, absorption or penetration. The majority did not meet current testing guidelines, has methodological and reporting deficiencies and has therefore to be regarded as not suitable for the final assessment of dermal absorption. As a qualitative conclusion, it can be stated that the greatest proportion of the applied homosalate is adsorbed in the stratum corneum. Only minor proportions are systemically (bio)available. Also, the solvent used for the formulation was shown to influence absorption.

A 2.0% absorption 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

Range-finding study

Guideline: /
Species/strain: Rat
Group size: 5 animals/sex/group
Test substance: Homosalate
Batch: /
Purity: /
Doses: 0, 100, 300, 1000 mg/kg bw
Route of exposure: gavage
Observation: 2 weeks exposure period
GLP: not in compliance

Homosalate was investigated for its subacute toxicity in a 2-week range-finding study in male and female rats. Each 5 male and 5 female rats received the test substance at dose levels of 0, 100, 300 and 1000 mg/kg bw orally by gavage for 2-weeks. Clinical examinations covering clinical signs, mortality, body weight and food consumption, haematology and clinical chemistry including coagulation were performed. At termination of treatment, all animals were sacrificed and macroscopically examined.

Results
Wet fur and/or salivation were observed at 100, 300 and 1000 mg/kg bw (males: 2/5, 5/5, 5/5, females 0/5, 5/5, 5/5, respectively). However, this is not considered as a toxic effect but as an indication of a bad taste of the test substance preparation.
With the exception of a slight retarded body weight gain in males animals and a corresponding reduction of food efficiency at 1000 mg/kg bw, there was no relevant effect body weight data, food consumption or food efficiency in the other groups. Haematology and gross pathology revealed no treatment-related findings at any dose level. Increases in APTT and/or PT were observed in males at ≥300 mg/kg bw and in females at 1000 mg/kg bw. Bilirubin was reduced at ≥100 mg/kg bw in males and at ≥300 mg/kg bw in females, while triglycerides were increased in both sexes at 1000 mg/kg bw. However, these effects were considered as not adverse (Bilirubin) or only potentially adverse (triglycerides) by the author (no data or further information supplied).

Conclusion
The author assumed a No Adverse Effect Level (NOAEL) of 100 mg/kg bw for repeated application in rats over a period of 14 days due to the effects on coagulation in males at ≥300 mg/kg bw and in females at 1000 mg/kg bw.

Ref.: 31

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

No data submitted

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

Applicant conclusion on repeated dose toxicity
Based on the limited data supplied and derived from the 14 day range-finding study in

male and female rats, no final conclusion in respect to the toxicological profile after repeated application can be drawn. However, the initial data can be considered as an indication that systemic toxicity of Homosalate might be not severe. After 14-day repeated oral application a preliminary NOAEL of 100 mg/kg bw was derived. This value will be used in the MOS calculation.
Furthermore, the systemic toxicity of Homosalate and probable metabolites after repeated dermal application was separately evaluated and assessed. The respective opinion prepared by Roberts (2005, Reference: 55) is enclosed in the references and main aspects of this expert evaluation are used and provided for the overall safety evaluation in section 3.3.14.

3.3.6. Mutagenicity / Genotoxicity

Guideline/method:	OECD 471 (Ninth Addendum, 21 July 1997)
Test system:	*Salmonella typhimurium*, strain TA98, TA100, TA102, TA1535, TA1537
Replicates:	triplicate plates, two independent assays
Test substance:	Homosalate
Batch:	4095213
Purity:	99.88 area % salicylic acid-3,3,5-trimethyl-cyclohexylester
Concentrations:	Range-finding experiment (±S9 mix): 3, 10, 33, 100, 333, 1000, 2500, 5000 µg/plate Experiment I - S9 mix: 33, 100, 333, 1000, 2500, 5000 µg/plate +S9 mix: 3, 10, 33, 100, 333, 1000, 2500, 5000 µg/plate Experiment II (±S9 mix): 10, 33, 100, 333, 1000, 2500, 5000 µg/plate
Solvent:	DMSO
Positive Controls:	- S9 mix: TA 100, TA 1535: sodium azide, 10 µg/plate TA98, TA1537: 4-nitro-o-phenylene-diamine, 10 µg/plate in TA98, 50 µg/plate in TA1537, TA102: methyl methane sulfonate, 5 µl/plate + S9 mix: all strains: 2-aminoanthracene, 2.5 µg/plate (TA98, TA100, TA1535, TA537), 10 µg/plate TA 102
GLP:	in compliance

The test substance was tested for mutagenicity in the reverse mutation assay on bacteria both, with and without metabolic activation (S9 mix prepared from phenobarbital/ß-naphthoflavone induced male Wistar rat liver) according to the plate incorporation test (experiment I) and the pre-incubation assay (experiment II). The Salmonella typhimurium strains TA97, TA98, TA100, TA102, TA1535 and TA1537 were exposed to the test substance (dissolved in DMSO) at concentrations ranging from 3 µg/plate to 5000 µg/plate.
For control purposes the solvent (DMSO) and positive controls (sodium azide, 4-nitro-o-phenylene-diamine, methyl methane sulfonate, 2-aminoanthracene) were also investigated.

Results
Bacteriotoxicity in form of reduced background growth was observed in the presence of metabolic activation at 5000 µg/plate in strain TA98 and at ≥2500 µg/plate in strain TA100 in experiment I as well as in form of a reduction in the number of revertants in strains TA1537 and TA100 at ≥2500 µg/plate (+S9 mix) and in strain TA100 at 1000-5000 µg/plate (+S9 mix) or at 5000 µg/plate (-S9 mix).
The test substance did not induce an increase in revertant colony numbers in the bacterial strains at any concentration tested in the presence or absence of metabolic activation. The sensitivity and validity of the test system used was demonstrated by the expected induction of a significantly increased number of revertants with the positive controls.

Conclusion
Homosalate did not induce gene mutations by base pair changes or frame shifts in the genome of the bacterial strains used in the presence and absence of S9-mix up to bacteriotoxic concentrations. Thus, it was shown to be non-mutagenic in this Salmonella typhimurium test.

Ref.: 15

Within the frame-work of the US National Toxicology Program (NTP) Homosalate was investigated for its mutagenic potential in Salmonella typhimurium strains TA97, TA98, TA100 and TA1535 in the presence and absence of metabolic activation (10% S9 mix of Aroclor 1254- induced male Sprague-Dawley rat or Syrian hamster livers) according to the preincubation assay described by Harworth et al., 1983. The test substance was dissolved in DMSO and concentrations of 0, 10, 33, 100, 333, 1000, 3333 and 10000 µg/plate were examined. Precipitation was observed occasionally at 10000 µg/plate. Homosalate exhibited no mutagenic potential in any of the tested strains at any concentration in this Salmonella typhimurium test.

Ref.: 45, 70

In a published standard plate incorporation test according to Ames et al. (1975) with Homosalate (citation only, no data; source: Rockes) dissolved in DMSO using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 with and without metabolic activation with S-9 mix prepared from liver homogenate of Aroclor 1254-pretreated male Sprague-Dawley rats, no gene mutations were detected.

Ref.: 4

Chromosome aberration test in Chinese hamster ovary (CHO) cells

Guideline/method:	OECD 473 (ninth addendum, 21 July 1997)
Test system:	Chinese Hamster V79 cell line
Test substance:	Homosalate
Batch:	4095213
Purity:	99.88 area % salicylic acid-3,3,5-trimethyl-cyclohexylester
Concentrations:	Experiment I - S9 mix: 4 h exposure, 18 h harvest: 5.0; 10.0; 20.0 µg/ml +S9 mix: 4 h exposure, 18 h harvest: 12.5; 25.0; 50.0 µg/ml Experiment II - S9 mix: 18 h exposure, 18 h harvest: 1.6; 3.1; 6.3 µg/ml - S9 mix: 28 h exposure, 28 h harvest: 6.3 µg/ml +S9 mix: 4 h exposure, 28 h harvest: 6.3; 12.5; 25.0 µg/ml
Solvent:	Ethanol
Positive controls:	Without S9 mix: Ethylmethane sulfonate, 200 - 300 µg/ml With S9 mix: Cyclophosphamide, 1.4 µg/ml
GLP:	in compliance

Homosalate was assessed for its potential to induce structural chromosome aberrations in Chinese hamster V79 cell line *in vitro*. The test substance was tested in the presence and absence of metabolic activation (S9 mix prepared from phenobarbital/β-naphthoflavone induced male Wistar rat liver). The test article was dissolved in ethanol. The cultures of cells were exposed to the test substance for 4, 18 or 28 h in the absence of metabolic activation and to 4 h in the presence of S9 mix. In each experimental group, two parallel cultures were set up. Colcemid was added to the cultures 15.5 h and 25.5 h, respectively after the start of the treatment. The cells on the slides were treated 2.5 h later. The cells were fixed with a mixture of methanol and glacial acetic acid and two slides per group were prepared per experiment. After preparation the cells were stained

with Giemsa. 100 well spread metaphase plates per culture were scored for cytogenetic damage on coded slides, except for the positive control in Experiment II, at the 28 hrs preparation interval without metabolic activation, where only 50 metaphase plates were scored due to strong genotoxicity. In addition, the number of polyploid cells in 500 metaphase plates per culture was determined.
In a range finding pre-test on toxicity cell numbers 24 hrs after start of treatment were scored as an indicator for cytotoxicity. Concentrations between 19.5 and 2500 µg/ml were applied.
Ethylmethane sulfonate (200 –300 µg/ml) for the non-activation set and cyclophosphamide (1.4 µg/ml) requiring activation served as positive control substances. A solvent control (ethanol) was also included in the test.

Results
In the range-finding part, clear toxic effects were observed after 4 h treatment with 19.5 µg/ml and above in the absence of S9 mix and with 78.1 µg/ml and above in the presence of S9 mix. In addition, 24 h continuous treatment with 19.5 µg/m and above in the absence of S9 mix induced strong toxic effects. Precipitation of the test item in culture medium was observed after treatment with 156.3 µg/ml and above in the absence of S9 mix and with 312.5 µg/ml and above in the presence of S9 mix. No relevant influence of the test item on the pH value or osmolarity was observed.
In the main study, precipitation of the test substance 4 h after start of treatment was observed in the presence of S9 mix in experiment I at preparation interval 18 h with 50 µg/ml and above. In all other experimental parts, no precipitation occurred after treatment with the test item.
Cytotoxicity indicated by clearly reduced cell numbers and/or mitotic indices of about or below 50% of control was observed in all experimental parts in the presence and absence of metabolic activation. However, in experiment II in the absence of S9 mix after 18 h and 28 h continuous treatment concentrations showing clear cytotoxicity were not scorable for cytogenetic damage.
In both independent experiments, no biologically relevant increase in the number of cells with structural chromosomal aberrations was observed. Two significant increases (2.5% and 2%, respectively) were observed in the absence of S9 mix in experiment II but these were within the historical control range (0.0 –4.0 % aberrant cells, exclusive gaps) and were considered as not relevant. No relevant increase in the frequencies of polyploid metaphases was found as compared to the frequencies of the controls.
The sensitivity of the test system was shown since the vehicle control led to no findings but the positive control substances led to statistically expected increases in the proportion of cells with chromosomal aberrations.

Conclusion
Under the conditions of the assay described, Homosalate showed no clastogenic potential in the absence or presence of metabolic activation in Chinese hamster V79 cells when tested up to cytotoxic concentrations.

Ref.: 11

Applicant conclusion on mutagenicity/genotoxicity in vitro
Homosalate was tested in bacterial and mammalian test systems *in vitro*. No genotoxic/mutagenic potential was noted in three bacterial gene mutation assays in *Salmonella typhimurium* strains in the presence or absence of metabolic activation.
In mammalian cell systems, Homosalate showed no clastogenic potential with or without metabolic activation.
Finally, Homosalate is considered to be of no genotoxic/mutagenic risk to humans.

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

There are no studies available with Homosalate *per se* in respect to reproductive performance (reproduction and fertility) or pre-/postnatal developmental toxicity including teratogenicity.
However, based on the suggested metabolic fate of Homosalate as pointed out by Roberts (2005) and following his conclusions, it can be stated that the metabolite salicylic acid is comprehensively investigated in respect to teratogenicity. Isophorone, which is also metabolized to trimethylcyclohexanol, was tested for teratogenicity in multiple species and was negative. Menthol, which is structurally similar to trimethylcyclohexanol was investigated for reproductive toxicity and teratogenicity and revealed no adverse effects. Consequently , it is considered that there is currently no need for further testing.

Ref.: 55

3.3.9. Toxicokinetics

No toxicokinetics study with Homosalate *per se* is available.
However, based on his evaluation Roberts (2005) assumed rapid and complete metabolism of Homosalate by esterases in the skin, plasma, liver and other body tissues to salicylic acid and trimethylcyclohexanol, both compounds with a complete and comprehensive data base.

Ref.: 55

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

Neutral red uptake (NRU) phototoxicity test

Guideline:	OECD 432 (2004)	
Test system:	Balb/c 3T3 fibroblasts (ATCC, Manassas, VA, USA)	
Test substance:	Homosalate	
Batch:	No information supplied	
Dose levels:	Range-finding:	0.291 –1000 µg/ml (8 dose levels, approx. 1/2 steps)
	Main assay:	1.63 - 100 µg/ml (8 dose levels, approx. 1/4 steps)
Positive control:	Chlorpromazine:	
	Range-finding:	0.156 - 100 µg/ml (12 dose levels, approx. 1/2 steps)
	Main assay:	0.156 –9.53 µg/ml (8 dose levels, approx. 1/2 steps)
Solvent:	first DMSO for stock solutions, afterwards with HBSS for dilution	
Irradiation:	UV-A 1.7 mW/cm^2 for 50 minutes resulting in UV-A dose of 5 J/cm^2	
GLP:	in compliance	

For the assay, Balb/c 3T3 cells were subcultured in 96-well microtiter plates, when the culture flask were 50% - 80% confluent. Prior to treatment the culture medium (DMEM) was removed and the cells were washed in pre-warmed Hank's Balanced Salt Solution (HBSS).Eight concentrations of the test substance were diluted from stock solution with HBSS and added to the cells. After 60 minutes of incubation, the cells were exposed to

the sun simulator (Dermalight SOL 3) for 50 minutes (UV-A irradiance: 1.7 mW/cm^2, UV-A dose: 5 J/cm^2). After irradiation, the cells were washed and the uptake of Neutral red was determined 24 hours later in a plate reader at 550 nm (OD550). Prediction of the phototoxic potential was achieved by calculation of the photoinhibition factor (PIF) and the mean photo effect (MPE). The respective IC50 values were defined as the concentration of the test material which causes a 50% reduction of NRU compared of untreated control cultures.

Results
The results obtained with Homosalate and the positive control substance (Chlorpromazine) are summarized below:

Table 4: Summary of IC50 results

Test substance	IC_{50} (µg/ml)			pH in HBBS (highest dose tested)
Homosalate	Range finding	Phototox (+UVA)	13.554	7.5
		Cytotox (-UVA)	15.825	
	Trial 2#	Phototox (+UVA)	13.077	7.0
		Cytotox (-UVA)	16.742	
	Trial 3#	Phototox (+UVA)	11.874	7.5
		Cytotox (-UVA)	13.098	
Chlorpromazine	Range finding	Phototox (+UVA)	1.3948	7.5
		Cytotox (-UVA)	22.983	
	Trial 2#	Phototox (+UVA)	1.6485	7.0
		Cytotox (-UVA)	22.711	
	Trial 3#	Phototox (+UVA)	1.4614	7.5
		Cytotox (-UVA)	25.315	
#Trial 1 was repeated and not reported since the results obtained with the positive control were considered as not valid				

Table 5: Summary of mean photo effects (MPE) and photo-irritancy factor (PIF) IC50 results

Test substance	MPE [1]			PIF [2]		
	Range finding	**Trial2**	**Trial 3**	**Range finding**	**Trial2**	**Trial 3**
Homosalate	0.011	0.014	0.005	1.169	1.281	1.103
Chlorpromazine	0.539	0.529	0.464	16.483	13.782	17.336
Mean photo effect:	MPE <0.1 predicts no phototoxicity 0.1 ≤ MPE ≤ 0.150 predicts probable phototoxicity MPE ≥ 0.150 predicts phototoxicity					
Photo irritancy factor	PIF < 2.0 predicts no phototoxicity 2.0 ≤ PIF ≤ 5.0 predicts probable phototoxicity PIF ≥ 5.0 predicts phototoxicity					

In each trial the results for Homosalate in respect to the PIF and MPE values were below the respective cut-off criteria for phototoxicity.

Conclusion
Homosalate was shown to have no phototoxic potential in the presence of artificial sunlight in murine Balb/c 3T3 fibroblasts.

Ref.: 14

Photosensitization *in vivo*

Combined phototoxicity and photoallergy study in guinea pigs

Guideline/method:	Modified method according to the Harber et al. (1982, 1987)
Species/strain:	Guinea pig/Dunkin Hartley
Group size:	5 –20 animals/group
Test substance:	Homosalate (source: Humco Chemical, Memphis, TN, USA)
Purity:	>95% (HPLC)
Batch:	No information available
Route:	Occlusive epicutaneous induction and challenge in Hill Top Chambers®
Carrier:	Induction: Methanol Challenge: Acetone
Dose level:	Induction: 1% in methanol Challenge: 1% in acetone
Light source:	Bank of 6 fluorescent black light lamps (Sylvania F20T12/BL)
Irradiation:	10 J/cm^2 UV-A
Positive control:	Musk Ambrette; tetrachlorosalicylanilide)
GLP:	Not in compliance

The contact photosensitizing property of the test substance was evaluated according to a modified Harber et al. (1982, 1987) protocol using male and female albino Hartley guinea pigs. This specific study design investigated not only photoallergy but also phototoxicity and skin sensitization.
Prior to the main study, a primary irritation study was performed to determine the non-irritation level on skin in the presence or absence of UV-A irradiation. Four concentrations (2/animal) were tested using patches Hill Top Chambers® on the left and right site of the lumbar region. The patches were occluded with a rubber dental dam for 2 hours. Thereafter, the patches were removed and a hole was cut in the right dental dam for exposure to UV-A (10 J/cm^2). The patch sites were graded on a scale from 0 –3 at 24 h and 48 h after irradiation.
Within the main study, induction was performed 3 times a week for 2 weeks for a total of 6 inductions on the depilated skin in nuchal region. On the first time of exposure, the animals received 4 intradermal injections with Freund's complete adjuvant. Thereafter, the areas were stripped with a cellophane taped followed by the application of the Hill Top Chambers® with test substance, vehicle or nothing (sham = empty patch). The patches were occluded for 2 hours, followed by removal of the occlusive dressing and irradiation (10 J/cm^2) of the substance, vehicle or sham treated site. After the 6th induction exposure, a rest period of 10 –14 days followed.
For challenge, all animals were treated with the test substance or vehicle with and without irradiation on the depilated skin in the lumbar region. The patch sites on the right and left site were occluded for 2 hours followed by removal, hole cutting in the right site of the dental dam and irradiation (10 J/cm^2). At 24 hours and 48 hours after challenge the skin was graded.

Results
The results of the primary irritation screen showed the test substance slightly irritating at the induction concentration of 1% in methanol but the challenge concentration of 1% in acetone was not.

The results obtained with Homosalate are summarized below:

Table 6: Results of the combined sensitization, photoirritation and photoallergy study with Homosalate in guinea pigs

Treatment		Reaction/result	Severity grade [d]	
Induction a	**Challenge b**	**Incidence c**	**24h**	**48h**
H + UV-A	H + UV-A	0/20	0.1	0.0
H + UV-A	H	0/20	0.1	0.0
Sham	H + UV-A	0/5	0.2	0.1
Sham	H	0/5	0.0	0.0
Sham	Acetone + UV-A	0/5	0.0	0.0
Sham	Acetone	0/5	0.0	0.0
Methanol+ UV-A	Acetone + UV-A	0/10	0.0	0.0
Methanol + UV-A	Acetone	0/10	0.0	0.0

H = Homosalate; UV-A = 10 J/cm² per exposure

a = 1% in methanol or methanol alone; b = 1% in acetone or acetone alone; c = number of responders (≥ 1) per total tested at 24/48h; d = sum of skin grades divided by total of animals tested

Along with homosalate, the potential of two other sunscreens (p-aminobenzoic acid and 4-isopropyldibenzoylmethane) and 2 known human photoallergens (musk ambrette, MA and tetrachlorosalicylanilide, TCSA) to cause photoallergy, phototoxicity and/or contact sensitization was also determined. As expected, a photoallergic response was achieved with both TCSA and MA. The results of studies conducted with the sunscreens showed that p-aminobenzoic acid was photoallergenic whereas homosalate and 4-isopropyldibenzoylmethane were not.

Conclusion
Homosalate was shown to have no photoallergic, contact allergic, phototoxic or irritant potential under the conditions tested in this modified Harber et al. (1982, 1987) study in male and female guinea pigs.

Ref.: 25

Mouse ear-swelling photoallergy test

Guideline/method:	Optimized method according to Brown et al. (1986), Granstein et al. (1983), Maguire and Kaidbey (1982) and Takigawa and Miyachi (1982)
Species/strain:	Mouse/BALB/c
Group size:	6 - 8 animals/group
Test substance:	Homosalate (source: Witco Corp., Humco Chemical Division, Memphis, TN, USA, purity: >95%)
Batch:	No information available
Route:	Epicutaneous induction on the back and epicutaneous challenge on right/left ear
Carrier:	Acetone or acetone/corn oil (4:1) but not specified for Homosalate
Dose level:	Induction: 10% Challenge: 5%
Light source:	UV-A: Bank of 8 fluorescent black light lamps (Sylvania F40/350BL) UV-B: Bank of 8 fluorescent light lamps (Philips F40UVB)
Irradiation:	UV-A: 10 J/cm^2 UV-B: 30 J/cm^2

Ear thickness measmt: Hand-held dial micrometer (Oditest gauge, model D-10000 The Dyer Co., Lancaster PA, USA)
GLP: Not in compliance

The photoallergic potential of Homosalate was investigated according to an optimized method (Brown et al. (1986), Granstein et al. (1983), Maguire and Kaidbey (1982) and Takigawa and Miyachi (1982)) using female BALB/c mice. This study was also designed to investigate the contact sensitizing and phototoxic potential of the test material. Prior to the main study, a phototoxicity/irritation screen was performed in some of the investigated materials. However, no information was supplied for Homosalate. For application and measurement of ear thickness a specially designed restrainer was used to restrict mice movement.
Induction: 6 –8 animals per group were intraperitoneally injected with cyclophosphamide (200 mg/kg bw). On the day of first induction, the dorsal surface of each mouse was clipped. The animals received induction treatments on days 0, 1 and 2 as 50 µl of the test substance or vehicle to the clipped skin area in the photoallergy, contact allergy control group and vehicle/irradiation control groups. Mice of the phototoxicity group received no treatment during the induction period. Thereafter, the mice were placed in the irradiation boxes. 30 –60 minutes after application, the treated sites were wiped clean. The contact allergy mice were returned to the cages. The animals in the photoallergy and vehicle/irradiations control groups were irradiated first with UV-A (10 J/cm^2) followed by UV-B (30 J/cm^2).
Challenge: Baseline ear thickness measurements were performed on each mouse 7 days after the first induction. For challenge, 8 µl of the test substance in vehicle or the solvent alone was applied to each side of both ears for a total of 16 µl. 30 –60 minutes thereafter, the ears were wiped followed by irradiation with UV-A (10 J/cm^2) and UV-B (30 J/cm^2). Ear measurements of the ear thickness were performed with the hand-held dial micrometer at 24 hours after challenge and the change from baseline was determined.

Results
The results of the combined sensitization, photoirritation and photoallergy study with Homosalate in female BALB/c mice after an induction with a 10% preparation and challenge with a 5% preparation and UV-A (10 J/cm^2) and UV-B (30 J/cm^2) are indicated in form of ear swelling as change from baseline in mm x 10^{-2} at 24 hour post challenge.

Contact photoallergy group: -0.1 ± 0.6 mm x 10^{-2}
Contact allergy group: -0.3 ± 0.4 mm x 10^{-2}
Vehicle radiation group: -0.1 ± 0.4 mm x 10^{-2}
Phototoxicity group: -0.2 ± 0.5 mm x 10^{-2}

Conclusion
Homosalate was shown to have no photoallergic, contact allergic or phototoxic potential under the conditions of the experiment. Several other currently tested compounds did elicit photoallergy.

Ref.: 26

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

The phototoxic potential of Homosalate was tested within a combined study according to modified Harber et al. (1982, 1987) protocol in male and female guinea pigs. The details of the methodology and results are described above in section 3.3.10.1 (Photosensitization *in vivo*). Homosalate tested at 1% in methanol or acetone in the presence and absence of UV-A irradiation (10 J/cm^2) showed no sings of irritation and no phototoxic potential under the conditions of the study.

Ref.: 25

In addition, the phototoxic potential of Homosalate was also investigated within a combined and optimized mice ear swelling study in female BALB/C mice. The details of the methodology and results are described above in section 3.3.10.1 (Photosensitization *in vivo*). In the presence of UV-A (10 /cm^2) and UV-B (30 J/cm^2) irradiation no phototoxic potential was detected for Homosalate under the conditions of this study.

Ref.: 26

Photomutagenicity in a *Salmonella typhimurium* Reverse Mutation Assay

Guideline:	OECD 473 (Ninth Addendum, 21 July 1997)	
Test system:	*Salmonella typhimurium* TA1537, TA98, TA100, TA102	
Replicates:	triplicate plates, two independent experiments	
Test substance:	Homosalate	
Batch:	batch 4095213 (purity: 99.88 area % salicylic acid-3,3,5-trimethyl-cyclohexylester)	
Concentrations:	Exp. I and II: 33; 100; 333; 1000; 2500 and 5000 µg/plate in DMSO	
Irradiation:		
Source of light:	Xenon-lamp (Suntest CPS, ATLAS) with emission of a continuous spectrum of simulated sunlight.	
Intensity of irradiation:	0.1 –0.3 mW/cm^2	
Bacterial Strains	UVA (mJ/cm^2)	UVB (mJ/cm^2)
TA 1537 (0.3 mW/cm^2)	40.5	1.4 (Exp. I)/1.5 Exp. II)
TA 98 (0.3 mW/cm²)	15	0.5 (Exp. I)/0.6 Exp. II)
TA 100 (0.1 mW/cm²)	4	0.14 (Exp. I)/0.15 Exp. II)
TA 102 (0.3 mW/cm²)	72	2.8 (Exp. I)/2.8 Exp. II)
Positive Controls:	Without Irradiation TA 100: sodium azide, NaN3, 10 µg/plate TA1537, TA98 4-nitro-o-phenylene-diamine, 10 µg/plate in TA98, 50 µg/plate in TA1537 TA 102 methyl methane sulfonate, 4 µl/plate With Irradiation: TA102 8-Methoxypsoralen, 125 µg/plate	
GLP:	in compliance	

Homosalate was investigated for its potential to induce gene mutations under irradiation with artificial sunlight according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the Salmonella typhimurium strains TA1537, TA98, TA100 and TA102. The test was performed in two independent experiments. Each concentration, including the controls, was tested in triplicate and concentrations of 33; 100; 333; 1000; 2500 and 5000 µg/plate were tested in experiment I and II. The test substance was dissolved in DMSO.

Results
No toxic effects, evident as a reduction in the number of revertants, were observed (without irradiation) and in both experiments. The plates incubated with the test item showed normal background growth up to 5000 µg/plate in all strains used.
No substantial increase in revertant colony numbers of any of the four tester strains was observed following treatment with Homosalate under irradiation with artificial sunlight at any dose level. There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. The sensitivity and validity of the test system used was demonstrated by the expected induction of a significantly increased number of revertants with the appropriate positive controls.

Conclusion
Homosalate did not induce gene mutations by base pair changes or frameshifts in the genome of the bacterial strains used and was therefore shown to be non-mutagenic in

this Salmonella typhimurium photomutagenicity test.

Ref.: 12

Chromosome Aberration Test *in vitro*: Photo-mutagenicity in Chinese Hamster V79 Cells

Guideline:	OECD 473 (Ninth Addendum, 21 July 1997)				
Test system:	Chinese Hamster V79 cell line				
Test substance:	Homosalate				
Batch:	4095213 (purity: 99.88 area % salicylic acid-3,3,5-trimethyl-cyclohexylester)				
Concentrations:	Experiment I: 0.31; 0.63; 1.25; 2.50 µg/ml dissolved in ethanol Experiment II: 0.63; 1.25; 2.50 µg/ml dissolved in ethanol				
Irradiation / Light source:	Xenon-lamp (Suntest CPS, ATLAS) with an additional special filter glass emitting visible and UVA/UV B light >290 nm				
UV doses:					
	With irradiation			Without irradiation	
	Exp. I	Exp. II	Exp. II	Exp. I	Exp. II
Pre-incubation of the cells with the test item	30 min	30 min	30 min	30 min	30 min
Intensity of irradiation (UVA)	0.3 / 0.3 /		0.3 mW/cm^2		
Dosage UVA/UVB [mJ/cm^2]	125/6	125/3	200/5	0/0	0/0
Total exposure period:	3 h	3 h	3 h	3 h	3 h
Recovery	15 h	25 h	25 h	15 h	25 h
Preparation interval	18 h	28 h	28 h	18 h	28 h
Positive controls:	with irradiation: 8-Methoxypsoralene, 0.5 µg/ml without irradiation: Ethylmethane sulfonate, 400 µg/ml				
GLP:	in compliance				

Homosalate was investigated for its potential to induce structural chromosomal aberrations in V79 Chinese Hamster cells in the absence and the presence of artificial sunlight in two independent experiments. The cultures were pre-incubated with the test item for 30 min. After pre-incubation, the cultures were exposed to
125 mJ/cm^2 UVA (Exp. I and II) or 200 mJ/cm^2 UVA (Exp. II). Three hours after start of treatment, the cultures were washed. Corresponding cultures with the test item were kept in the dark for the 3 hrs exposure period. The chromosomes were prepared 18 hrs (Exp. I) and 28 hrs (Exp. II) after start of treatment. Two parallel cultures were investigated and at least 100 metaphase plates were scored for structural chromosome aberrations in each culture, except for the positive controls, where only 50 metaphase plates were scored.

Results
In the absence and the presence of irradiation, toxic effects were observed in both experiments as indicated by clearly reduced mitotic indices or cell numbers of below 50% of control.
In both experiments (I and II), in the absence and the presence of irradiation, no biologically relevant increase in the number of cells carrying structural chromosomal aberrations was observed after treatment.

No relevant increase in the frequencies of polyploid metaphases was found after treatment with the test item when compared to the controls and the range of the historical control data. The sensitivity of the system was demonstrated since the positive

controls induced statistically significant increases in cells showing structural chromosome aberrations.

Conclusion
In conclusion, Homosalate did not induce structural chromosome aberrations in the absence or presence of artificial sunlight as determined by the chromosomal aberration test in V79 Chinese Hamster cells and was thus shown to be non-clastogenic in this chromosomal aberration photomutagenicity test when tested up to cytotoxic concentrations.

Ref.: 10

Applicant conclusion on photo-induced toxicity
Phototoxicity/photoirritation *in vitro* and *in vivo*
In vitro Homosalate was proven to be not phototoxic in the NRU assay using murine BALB/c fibroblasts.
In vivo there exists also no indication for a phototoxic potential in experimental animals as indicated by the results of a combined phototoxicity and photoallergy study in male and female guinea pigs as well as in the ear swelling photoallergy test in female mice.
Photosensitization *in vivo*
Homosalate was shown to have no photoallergic potential in male and female guinea pigs and in the optimized ear swelling study in female BALB/c mice.
Photomutagenicity/photoclastogenicity
Homosalate was tested in bacterial and mammalian test systems *in vitro* according to valid testing guidelines and under GLP conditions with the characterized test material. No photogenotoxic/ mutagenic potential was noted in the bacterial gene mutation assays in *Salmonella typhimurium* strains and no photo-clastogenic potential was recorded so far in the chromosome aberration test in Chinese hamster V79 cells, both with and without irradiation.
Finally, Homosalate can be regarded to be of no concern in respect to photo-induced toxicity for humans.

3.3.11. Human data

Phototoxicity test

Guideline/Method:	Approved study protocol and standard operating procedures by the New England Institutional Review Board (NEIRB) 2005
Species:	Human
Group size:	a) - c) 20 volunteers
Test substance:	a) SPF-30 sunscreen (formula #769-187, Homosalate content: 10%) b) SPF-45 sunscreen (formula #769-190, Homosalate content: 15%) c) SPF-30 sunscreen (formula #769-193, Homosalate content: 10%)
Batch:	a) batch #0015C-P (white cream) b) batch #0015C-V (white cream) c) batch #0015C-M (white cream)
Route:	Semi-occlusive epicutaneous application
Light source:	UV-A irradiation by four Philips F40BL fluorescent tubes with a peak output at 369 nm and half-power bandwidth of 15 –16 nm.
UV dosage:	UV-A intensity: 3.3 –4.4 mW/cm^2; duration: about 17 min (total dose of 4.0 ± 0.4 J).
Scoring system:	Modified scoring scale of the International Contact Dermatitis Research Group System (Fisher, Alexander A., Contact Dermatitis, Lea & Febiger, 1986, 26).
GLP:	in compliance

The potential to induce a phototoxic response was evaluated with 3 different sunscreens containing 10% or 15% Homosalate among other substances on a panel of 20 male and female volunteers under GLP conditions and according to the approved NEIRB study protocol.
A webril/adhesive patch was used semi-occlusively. Approximately 0.2 g of the test material was applied to each patch. The skin was wiped clean prior to patching the volunteers.
The patch of the irradiated site was applied on the back and the non-irradiated test site was
placed on the opposite side of the back and was protected from the light source by the clothing or patch. In addition, during the whole testing period, the volunteers protected the non-irradiated testing sites from exposure to sunlight.
Testing schedule: On day 1 the volunteers were patched with duplicate patches for about 24 hours. On day 2 the patches were removed after about 24 hours post patching from the sites to be irradiated, were read and scored. These sites were irradiated with UV-A (details see above) and afterwards scored. The non-irradiated sites were protected and were read and scored after the irradiation procedure. On days 3 –4 the skin reactions on the irradiated and non-irradiated testing sites were read and scored about 48 and 72 hours post patching. The complete test was conducted under the supervision of a Board-Certified Dermatologist, which participated also in the scorings of the volunteers.

Results
All 20 volunteers completed the study. No skin reactions were noted either on the irradiated or the non-irradiated contact sites of the tested sunscreens or on the untreated but irradiated control sites.

Conclusion
In this phototoxicity test none of the sunscreen products containing 10% or 15% Homosalate did induce a dermal phototoxic response under the conditions of the study.
Ref.: 33, 36, 40

Comment
The dose of UVA was low for a phototoxicity test.

These results are in line with a further phototoxicity study performed with a sunscreen containing 10% Homosalate. This product was tested under usage of an occlusive dressing but otherwise similar experimental condition in 24 male and female volunteers. Again, there was no induction of phototoxicity.
Ref.: 9, 24

Photoallergy test

Guideline/Method:	Approved study protocol and standard operating procedures by the New England Institutional Review Board (NEIRB) 2005
Species:	Human
Group size:	a) - c) 29 volunteers induced, 28 volunteers completed
Test substance:	a) SPF-30 sunscreen (formula #769-187, Homosalate content: 10%) b) SPF-45 sunscreen (formula #769-190, Homosalate content: 15%) c) SPF-30 sunscreen (formula #769-193, Homosalate content: 10%)
Batch:	a) batch #0015C-P (white cream) b) batch #0015C-V (white cream) c) batch #0015C-M (white cream)

Route:	Semi-occlusive epicutaneous application
Irradiation:	UV-A and UV-B for induction and UV-A for challenge, each irradiation about 24 hours post-patching
Light source:	UV-A irradiation by four Philips F40BL fluorescent tubes with a peak output at 369 nm and half-power bandwidth of 15 –16 nm. UV-B irradiation by custom made light source with a peak output at 313 nm and half-power bandwidth of 30 nm.
UV dosage:	UV-A intensity: 3.3 –4.4 mW/cm^2; duration: about 17 min (total dose of 4.0 ± 0.4 J). UV-B intensity: 1.0 –0.2 mW/cm², The UV-B irradiation was based on each volunteers skin type and minimal erythema dose (MED) as determined prior the first irradiation.
Scoring system:	Modified scoring scale of the International Contact Dermatitis Research Group System (Fisher, Alexander A., Contact Dermatitis, Lea & Febiger, 1986, 26).
GLP:	in compliance

Three sunscreens, containing either 10% or 15% Homosalate among other substances were investigated for its potential to induce photoallergy in human. A panel of male and female volunteers was examined under GLP conditions and according to the approved NEIRB study protocol.
Induction period: A webril/adhesive patch was used semi-occlusively. Approximately 0.2 g of the test material was applied to each patch. The skin was wiped prior to patching the volunteers.
The patch of the irradiated site was applied on the upper back. An additional site served as irradiated but with no test material applied control site. The non-irradiated test site was placed on the opposite side of the back and was protected from the light source by the clothing or cloak. In addition, during the whole testing period, the volunteers protected the non-irradiated testing sites from exposure to sunlight.
Twice a week, for the first three weeks, the patches were applied to identical sites for a total of six induction patching. The patches remained on the skin for about 24 hours. After removal, the skin was read and scored and irradiated with UV-A and UV-B (details see above) and afterwards scored again. The non-irradiated sites were protected and were read and scored after the irradiation procedure. Each UV irradiation took place about 24 hours after each patching.
Rest period: The last induction patching was followed by a rest period of two weeks with no application.
Challenge period: After the rest period, the volunteers were queried for any reactions. For challenge, the lower back was used as the virgin site for the challenge testing site and for the irradiated control site. The non-irradiated challenge patch was applied on the opposite site. The challenge patches were applied to virgin sites only for about 24 hours. Thereafter, the patches were removed, the sites scored and irradiated with UV-A followed by skin examination and scoring. During irradiation, the non-irradiated sites were protected and scored after the irradiation procedure took place. In total, the application sites were scored at about 24, 48, 72 and 96 hours post-patching.

Results
28 volunteers completed the study and 1 discontinued but not due test material reaction. During the induction phase only single volunteers exhibited low level and transient skin reactions on the irradiated and test substance treated sites, but these skin reactions were also noted on the irradiated control sites, which received no substance application. No skin reactions were noted on the non-irradiated test substance contact sites. At challenge, no skin reactions were noted either on the irradiated or the non-irradiated contact sites of the tested sunscreens or on the untreated but irradiated control sites.

Conclusion
All three sunscreen products containing 10% or 15% Homosalate exhibited no

photoallergic or dermal sensitizing potential under the conditions of the study in male and female human volunteers.

Ref.: 34, 37, 39

These results are in line with a further photoallergy study performed with a sunscreen containing 10% Homosalate. This product was tested under usage of an occlusive dressing but otherwise similar experimental condition with initially 28 male and female volunteers for induction and 27 subjects completing the study. There was no induction of photoallergy or dermal sensitization.

Ref.: 8, 24

There also exists a paper on photopatch testing of 118 patients (77 females, 41 males) with suspected photoallergic contact dermatitis towards a set of cosmetic ingredients with UV-A (5 J/cm^2) and UV-B (10 mJ/cm^2). Within this panel 7/118 patients showed photoallergic reactions but none reacted to Homosalate tested as 5% preparation in petrolatum.

Ref.: 49

For completeness sake, it should also be mentioned that although Homosalate is widely used and has a long history of usage in sunscreens and various other cosmetic products, there are only isolated cases reported concerning induction of skin sensitization or photoallergic reactions (e.g., 2/70 Mayo clinic patients with a positive photopatch reaction to 2% Homosalate in petrolatum, 1/34 scratch-chamber tested Swedish patients tested positive). This is a further indication that Homosalate has a negligible potential to induce adverse skin reactions in the human population.

Ref.: 20, 27, 44, 53

Applicant conclusion on human data
There is a comprehensive data base on human studies performed with homosalate in different concentrations in representative sunscreen formulations or other cosmetic products covering photo-induced toxicity in respect to photo-irritation and photo-allergy.
Although homosalate has extensive use in various broad-spectrum sunscreens and a variety of other cosmetic formulations and a long history of usage during the last decades, there are only isolated reports on adverse skin reactions in patients.
Whenever the clinical studies were performed under controlled and standardized conditions including GLP and under supervision or participation of a certified dermatologist, homosalate was proven not to be photoirritant and possess no photo-allergic potential even under enhanced condition.

Table 7: Summary of human data

Test	Test substance	No. of volunteers	Application Remark	Results Conclusion	Ref.
PT	SPF-30 sunscreen (769-187, 10% H)	20	Semi-occlusive	No induction of phototoxicity	33
PT	SPF-45 sunscreen (769-190, 15% H)	20	Semi-occlusive	No induction of phototoxicity	36
PT	SPF-30 sunscreen (769-193, 10% H)	20	Semi-occlusive	No induction of phototoxicity	40
PT	U03127.03 (283285/1, 10% H)	24	Occlusive	No induction of phototoxicity	9, 24
PA	SPF-30 sunscreen (769-187, 10% H)	29 induced 28 completed	Semi-occlusive	No induction of photoallergy or dermal sensitization	34
PA	SPF-45 sunscreen (769-190, 15% H)	29 induced 28 completed	Semi-occlusive	No induction of photoallergy or dermal sensitization	37
PA	SPF-30 sunscreen (769-193, 10% H)	29 induced 28 completed	Semi-occlusive	No induction of photoallergy or dermal sensitization	39

Test	Test substance	No. of volunteers	Application Remark	Results Conclusion	Ref.
PA	U03127.03 (283285/1, 10% H)	28 inducted 27 completed	Occlusive No irritant or cumulative irritant reaction. 1/27 exhibited incidentally preexisting hyperirritability	No induction of photoallergy or dermal sensitization	8, 24
PT = phototoxicity test; PA = photoallergy test					

Comment
The SCCP does not consider the recent photo-allergy studies as ethical. These data appears to be generated in the USA. Within Europe, such studies are not regarded as ethical.

3.3.12. Special investigations

Estrogenic potential *in vitro*

Guideline/method: Mechanistic study on oestrogen receptor binding properties according to a modified protocol of Bosel and Shain (1974)
Test system: Human recombinant oestrogen receptor (ER), α-subtype (PanVera, Madison, WI; USA)
Replicates: Two separate experiments with triplicate concentration levels
Test substance: Homosalate
Batch: 50446454
Purity: 99.6%
Concentrations: 100, 1000, 10000, 100000 nM
Solvent: DMSO
Positive Controls: Genistein: 0.03 –100 nM
Estradiol: 10 - 10000 nM
GLP: Not in compliance

The potential interaction of Homosalate with the oestrogen receptor (ER) was examined in a receptor binding assay with human recombinant ER of the α-subtype as receptor and radiolabelled estradiol as ligand. Estradiol (0.03 –100 nM) with strong ER affinity and genistein (10 –10000 nM) with weak ER affinity were used as positive control substances. A modified method according to Boesel and Shain (1974) was applied. 100 µl assay buffer containing BSA and 2 % DMSO (± test compounds) and 50µl of 4.8 nM solution of radiolabelled estradiol in assay buffer were mixed in microtiter plate wells. Following incubation at 4°C overnight under continuous shaking, charcoal suspension in assay buffer was added. After mixing the samples charcoal was sedimented by centrifugation and 50 µl aliquots of the clear supernatant were analyzed by liquid scintillation counting (^{3}H activity). Aliquots (50 µl) containing the ER-ligand complex were mixed with 200 µl of the scintillation cocktail and radioactivity was counted for 10 min in the reader with 1 hour delay allowing samples to equilibrate. Receptor binding was corrected for unspecific binding. Binding of radiolabelled estradiol in the presence of test compound was related to binding in its absence. IC_{50} values were calculated, if possible. Two separate experiments were conducted each with triplicate concentrations and nine fold incubations of vehicle (DMSO) alone.

Results
No affinity of Homosalate to the oestrogen receptor (ER) at the maximum applicable concentration of 100000 nM was observed. The quantity of radiolabelled ligand estradiol binding in the presence of the test compound was comparable to that of the control. The sensitivity of the test system was shown as the positive controls estradiol and genistein displaced the radiolabelled estradiol from the ER with IC50 values of 1.7 nM and 1.85 nM

and 165 nM and 145 nM, respectively.

Conclusion
Homosalate showed no affinity to the human recombinant oestrogen receptor up to the highest concentration technically applicable of 100000 nM.

Ref.: 3

Estrogenic potential *in vivo*

Guideline/method:	Uterotrophic assay in immature rats according to OECD Validation Work and OECD Protocol (Draft, 2000)
Species/strain:	Rat/Wistar ((HsdCpb:WU)
Group size:	6 animals/sex/group
Test substance:	Homosalate
Batch:	507 57115
Purity:	89.64%
Dose levels:	0, 200, 1000 mg/kg bw
Vehicle:	Corn oil
Positive control:	Ethinylestradiol (EE); 0.3 and 1.0 µg/kg bw
Route:	Subcutaneous (sc.)
Exposure period:	3 consecutive days
GLP:	in compliance

Homosalate was investigated for its estrogenic potential in the uterotrophic assay in immature rats. Each 6 juvenile female Wistar rats received the test substance dissolved in corn oil at dose levels of 200 and 1000 mg/kg bw by subcutaneous injections, once a day on three consecutive days. Treatment started after an acclimatization period of three days when the juvenile rats were 19 days old. For control purposes, each 6 rats remained untreated and one additional group of 6 rats received the carrier (corn oil). Ethinylestradiol was selected as positive control substance and each 6 rats were subcutaneously injected with 0.3 and 1.0 µg/kg bw according to the same schedule. Clinical examinations covering clinical signs, mortality, body weight and food consumption were performed. At termination of treatment, all animals were sacrificed, macroscopically examined and the uterus weight (wet and blotted) was determined.

Results
There was no mortality and no effect on the general state of health. Body weights and food consumption was comparable to the control groups. No effects on the uterus weights after sc. treatment with Homosalate at 200 and 1000 mg/kg bw was observed. The sensitivity of the juvenile female rats was demonstrated as the positive control caused an enlargement of the uterus accompanied by an increase in uterus weight.

Conclusion
The repeated subcutaneous injection of Homosalate at dose levels up to 1000 mg/kg bw to juvenile female Wistar rats on three consecutive days revealed no estrogenic potential in the uterotrophic assay.

Ref.: 1

For completeness sake it has to be mentioned that Schlumpf et al. (undated, 2001) investigated the estrogenic potential of Homosalate among other sunscreens *in vitro* using MCF- 7 human breast cancer cells and *in vivo* in the uterotrophic assay in immature Long-Evans rats. The MCF-7 cells were exposed to concentrations the range between $1x10^{-7}$ –$5x10^{-5}$ M, while the immature rats received dietary dose levels of 491 and 892 mg/kg bw during post natal days 21 – 25. In the *in vitro* assay induction of proliferation in the MCF-7 cells was noted with an EC50 value of 1.56 µM and this was

interpreted by the authors as a positive result. In contrast, *in vivo* no estrogenic effect of Homosalate was noted.
The SCCP concluded that a number of important technical shortcomings in the study of Schlumpf et al. were detected. The final conclusion was that based on the actual scientific knowledge, the SCCNFP is of the opinion that the organic UV-filters used in cosmetic sunscreen products, allowed in the EU market today, have no estrogenic effects that could potentially affect human health.

Ref.: 57, 58, 59

A recent publication on estrogenic activity *in vitro* and *in vivo* showed that Homosalate among other sunscreens is able to affect the oestrogen receptor when tested *in vitro* for effects on the gene expression in stable oestrogen receptor α and β (ER α, ER β) reporter cell lines (HEK293). In contrast, in a transgenic Zebra fish assay *in vivo*, no estrogenic activity was demonstrated. The authors stated that one should be aware of over-interpretation when predicting *in vivo* effects from weak *in vitro* data.

Ref.: 60

Androgenic potential *in vitro*

Guideline/method:	Mechanistic study on androgen receptor binding properties according to a modified protocol of Bosel and Shain (1974)
Test system:	Rat recombinant fusion protein to thioredoxin containing the hinge region and ligand binding domain of the androgen receptor (AR, PanVera, Madison, WI; USA)
Replicates:	Two separate experiments with triplicate concentration levels
Test substance:	Homosalate
Batch:	50446454
Purity:	99.6%
Concentrations:	100, 1000, 10000, 100000 nM
Solvent:	DMSO
Positive Controls.	Dihydrotestosterone: 0.1 - 300 nM
Androstendione:	30 –100000 nM
GLP:	not in compliance
Published:	No

Homosalate was tested for its potential to interact with the androgen receptor (AR) in a receptor binding assay with rat recombinant fusion protein containing the hinge region and ligand binding domain of the androgen receptor as receptor source and radiolabelled methyltrienolone (R 1881) as ligand. Dihydrotestosterone (0.1 - 300 nM) with strong AR affinity and androstendione (30 – 100000 nM) with weak AR affinity were used as positive control substances. A modified method according to Boesel and Shain (1974) was applied. 100 µl assay buffer containing γ-globulin and 2 % DMSO (± test compounds), 50µl of 8 nM solution of radiolabelled R1881 in assay buffer were mixed in microtiter plate wells. Following incubation at 4 °C overnight under continuous shaking, charcoal suspension in assay buffer was added. After mixing the samples charcoal was sedimented by centrifugation and 50 µl aliquots of the clear supernatant were analyzed by liquid scintillation counting (^{3}H activity). Aliquots (50 µl) containing the AR-ligand complex were mixed with 200 µl the scintillation cocktail and radioactivity was counted for 10 min in the reader with one hour delay allowing samples to equilibrate. Receptor binding was corrected for unspecific binding. Binding of radiolabelled R1881 in the presence of test compound was related to binding in the absence. IC50 values were calculated, if possible. Two separate experiments were conducted each with triplicate concentrations and six fold incubations of vehicle (DMSO) alone.

Results
Homosalate showed a weak affinity to the androgen receptor (AR) but the concentration-response relationships were flat and even the highest technically achievable concentration of 100000 nM revealed a displacement of 32% or 41% in the two experiments. Therefore, no IC50 value could be calculated. The sensitivity of the test system was shown as the positive controls dihydrotestosterone and androstendione displaced the radiolabelled methyltrienolone from the androgen receptor. In contrast to Homosalate, the concentration-response curves were steep and parallel. The IC50 values were 5.2 nM or 4.4 nM for dihydrotestosterone and 2.5 µM or 1.8 µM for androstendione, respectively.

Conclusion
As Homosalate showed only a weak affinity to the rat androgen receptor with a flat concentration-response relationship up to the highest concentration technically applicable of 100000 nM in contrast to the reference androgens, this result is not considered as an indication for a specific interaction with the androgen binding domain of the androgen receptor.

Ref.: 2

The human breast carcinoma cell line MDA-kb2 cell was used to screen several UV filters including homosalate *in vitro* for its potential to influence the androgen receptor. In this specific *in vitro* assay, Homosalate was found to antagonize dihydrotestosterone induced androgen activation in concentrations below cytotoxicity as an indication for anti-androgenic activity *in vitro*. No agonistic activity was observed. However, these preliminary results of a screening assay were considered as of no relevance for the *in vivo* situation.

Ref.: 43

3.3.13. Safety evaluation (including calculation of the MoS)

The calculation of the systemic exposure dose (SED) was carried out according to the recent SCCP principles and procedures as laid down in the SCCP Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation, 6th revision, adopted during the 10th plenary meeting of 19 December 2006 (SCCP/1005/06).

CALCULATION OF THE SYSTEMIC EXPOSURE DOSE

The safety calculation is only considering dermal exposure.

Maximum dermal absorption of test substance reported was 2.0% (dermatomed human skin, in vitro).

NOAEL based on haematological effects in a 2 week oral toxicity study was 100 mg/kg bw/d.

Amount of sunscreen applied g/day	A (g/day)	= 18.0
Concentration in the finished product	C (%)	= 10%
Dermal absorption	DAp (%)	= 2.0%
Default human body weight		= 60 kg
Systemic exposure dose (SED) mg/kg	A x 1000 mg/g x C/100 x DAp/100/60	= 0.60

Margin of Safety	NOAEL / SED = 100/0.60	=	167

Although the NOAEL was derived from a 14-day range-finding study in male and female rats and although no toxicokinetic data are available, the SCCP considers it not ethical to request a new 90-day oral toxicity study.

3.3.14. Discussion

General toxicity
The acute oral and dermal toxicity of Homosalate is very low. The respective LD50 values for the acute oral toxicity in rats and for the acute dermal toxicity in rabbits are far above >2000 mg/kg bw.
Initial data from a subacute oral 14-day range finding studying male and female rats performed with Homosalate per se can be considered as an indication that systemic toxicity might not be severe. From this study, a preliminary NOAEL of 100 mg/kg bw was derived. A NOAEL of 100 mg/kg bw/d is used in calculation of MOS. Furthermore, Roberts (2005, Reference: 55) suggested that Homosalate is rapidly metabolized to salicylic acid and trimethylcyclohexanol. Both compounds are comprehensively toxicologically characterized and clear NOAELs covering all relevant endpoints for these compounds are available. The same is true for isophorone, which also has a trimethylcyclohexanol metabolite and for menthol as a compound with a similar structure to trimethylcyclohexanol. Thus, based on the assumed metabolism of Homosalate and the comprehensive data base of the metabolites and in respect to structure relationship evaluations, it is considered that there is currently no need for further testing.
There are no studies available with Homosalate per se in respect to reproductive performance or pre-/postnatal developmental toxicity including teratogenicity. However, based on the suggested metabolic fate of Homosalate as pointed out by Roberts (2005) and following his conclusions it can be stated that the metabolite salicylic acid is comprehensively investigated in respect to teratogenicity. Isophorone, which is also metabolized to trimethylcyclohexanol was tested for teratogenicity in multiple species and was negative. Menthol, which is structurally similar to trimethylcyclohexanol was investigated for reproductive toxicity and teratogenicity and revealed no adverse effects. Finally, it is considered that there is currently no need for further investigations in respect to reproductive performance and developmental toxicity.

Photo-toxicity
In vitro Homosalate was proven to be not phototoxic in the NRU assay using murine BALB/c fibroblasts**.** *In vivo* there exists also no indication for a phototoxic potential in experimental animals.

Irritation / Sensitisation
The limited data in experimental animals in respect to the irritative potential of Homosalate did not indicate an irritation potential to the skin or the mucous membranes. In addition, numerous clinical studies in human revealed no irritative potential, not even under enhanced condition.
The existing data obtained in guinea pigs and mice exhibited no sensitizing potential of Homosalate and numerous clinical studies in human revealed no skin sensitizing potential.

Photo-irritation/sensitisation
No photosensitization was found after topical treatment in male and female guinea pigs and female mice.

Dermal absorption
The recent comparative rat versus human *in vitro* percutaneous absorption study performed under current guideline requirements and under GLP conditions showed that application of a 10% Homosalate containing sunscreen led to mean absorption of 8.7% (corresponding to 46.62 µg/cm^2) in rats and to 1.1% (corresponding to 5.81 µg/cm^2) in human using freshly dermatomed skin. The mean recovery was 92.4%. The highest absorption found was 1.4 ± 0.4% (7.63 ± 2.18 µg/cm^2) with the highest absorption 2.0% (10.9 µg/cm^2). 2% absorption is used in calculation of MOS. Beside this valid investigation, there are few *in vitro* and *in vivo* studies available with topical application of Homosalate as constituent of preparations in varying concentrations dealing with different parts and aspects of dermal adsorption, absorption or penetration. The majority did not meet current testing guidelines and mainly qualitative but no quantitative conclusions could be drawn.

Mutagenicity / Genotoxicity
No genotoxic/mutagenic potential was noted in three bacterial gene mutation assays in *Salmonella typhimurium* strains in the presence or absence of metabolic activation. In mammalian cells systems, Homosalate showed no clastogenic potential with or without metabolic activation.

Photo-mutagenicity/genotoxicity
No photo-genotoxic/mutagenic potential was noted in the bacterial gene mutation assays in *Salmonella typhimurium* strains and no photo-clastogenic potential was recorded in the chromosome aberration test in Chinese hamster V79 cells, both with and without irradiation.

Human data
The human data can be summarized as follows; unchanged Homosalate caused no signs of sensitization in the human Maximization test in male and female volunteers. Further, whenever studies with different sunscreens and cosmetic products were performed under controlled and standardized conditions including GLP/GCP and under supervision or participation of a certified dermatologist, Homosalate was shown to have no irritative or sensitization potential and was proven to be not photoirritant and posses no photo-allergic potential even under enhanced condition. Although Homosalate is widely used and has a long history of usage, only very isolated cases of allergic/photoallergic reactions are available in the open literature. This is considered as further indication that Homosalate has a negligible potential to induce adverse skin reactions in the human population.

Estrogenic / Androgenic potential
The uterotrophic assay in juvenile rats performed according to the OECD draft guideline under GLP with repeated subcutaneous injection of Homosalate at dose levels of up to 1000 mg/kg bw revealed no estrogenic potential and an oestrogen receptor binding study *in vitro* performed under standardized conditions showed no affinity to the human oestrogen receptor when tested up to the highest technically feasible concentration. This supports the conclusion of the SCCNFP that based on recent scientific knowledge Homosalate as well as other organic UV-filters has no estrogenic effect that could potentially affect human health.
For completeness sake it has to be mentioned that under standardized experimental *in vitro* conditions Homosalate showed a weak affinity to the rat androgen receptor but this result is not considered as an indication for a specific interaction with the androgen binding domain of the androgen receptor. A published *in vitro* screening revealed anti-

androgenic activity but these preliminary results were considered as of no for the *in vivo* situation.

Additional considerations on the systemic toxicity of homosalate given topically
An opinion by Roberts (2005, Reference: 55) reviewed also toxicity data on Homosalate metabolites which can be formed in the skin, namely salicylic acid and trimethylcyclohexanol. When Homosalate after topical application (based on 2% absorption) is assumed to have undergone 100% metabolism to salicylic acid and trimethylcyclohexanol, the estimated SED for salicylic acid is 0.3 mg/kg/day. The SCCNFP 2002 opinion on salicylic acid used a NOAEL of 75 mg/kg for the risk assessment, based on rat oral teratogenicity data. An MoS of 250 can be calculated for salicylate formed as homosalate metabolite. Accordingly, the estimated SED for trimethylcyclohexanol is about 0.31 mg/kg/day. Trimethylcyclohexanol does inhibit HMG CoA reductase. Based on a NOAEL of 43 mg/kg/day (estimated from a LOAEL of 426 mg/kg and an uncertainty factor of 10), an MoS of 143 is calculated for trimethylcyclohexanol. In conclusion, both metabolites of homosalate when formed in skin do not alter SCCP's conclusions on the systemic toxicity of the compound since MoS for salicylic acid and trimethylcylohexanol are similar to the MoS calculated for homosalate itself.

4. CONCLUSION

Based on the information provided, the SCCP is of the opinion that the use of homosalate at a maximum concentration of 10% w/w in cosmetic sun screen product does not pose a risk to the health of the consumer.

Uses of homosalate in other types of cosmetic products at concentrations up to 10.0% also does not pose a risk to the health of the consumer.

Only the dermal application of homosalate was considered, not its use in 'spray'-applications.

5. MINORITY OPINION

Not applicable.

6. REFERNCES

1. Bayer (2002a) Neo Heliopan ® HMS, Uterotrophic assay in immature rats, Pharmaceutical Business Group, Report No. PH 31927, Bayer, Wuppertal, Germany, unpublished data, 08 April 2002
2. Bayer (2002b) Neo Heliopan ® HMS, In vitro studies on its androgen receptor binding properties, Research Toxicology, Report No. PH 32215, Bayer, Wuppertal, Germany, unpublished data, 23 July 2002
3. Bayer (2002c) Neo Heliopan ® HMS, In vitro studies on its estrogen receptor binding properties, Research Toxicology, Report No. PH 32216, Bayer, Wuppertal, Germany, unpublished data, 23 July 2002
4. Bonin A. M., Arlauskas A. P., Angus D. S., Baker R. S. U., Gallagher C. R., Greenoak G., Lane Brown M. M., Meher-Homji K. M. and Reeve V. (1982) UV-absorbing and

other sunprotecting substances: genotoxicity of 2-ethylhexyl P-methoxycinnamate, Mutation Res., 105, 303-308
5. Chatelain E., Gabard B and Surber C. (2003) Skin penetration and sun protection factor of five UV filters: Effect of the vehicle, Skin Pharmacol. Appl. Skin Physiol., 16, 28-35
6. Clinical Research Laboratories (CRL, 2003) Repeated insult patch test, MT#2101420 (10% Homosalate), Clinical Study No. CRL67703-8, Clinical Research Laboratories Inc., Piscataway, NJ, USA, unpublished data, 29 July 2003
7. Concordia Research Laboratories (2001) Human repeated insult patch test, Five formulations (Lotion 3 = 12% Homosalate sunscreen), 200 Subjects, Study No. PLO-041 A, Concordia Research Laboratories Inc., Cedar Knolls, NJ, USA unpublished data, 04 May 2001
8. Consumer Product Testing Co. (2003a) Photoallergy, Test material U03127.03 – 283285/1, Experiment Reference No. S03-0479.03, Consumer Product Testing Co., Fairfield, NJ, USA, unpublished data, 09 October 2003
9. Consumer Product Testing Co. (2003b) Phototoxicity, Test material U03127.03 – 283285/1, Experiment Reference No. S03-0478.05, Consumer Product Testing Co., Fairfield, NJ, USA, unpublished data, 10, June 2003
10. Cosmetic Toiletry and Fragrance Association (CTFA, 2005a) –Chromosome aberration test in vitro: Photomutagenicity in Chinese hamster V79 cells with Homosalate, RCC, Cytotest Cell Research GmbH, Rossdorf, Germany, RCC-CCR Study No. 908502, unpublished data, Draft
11. Cosmetic Toiletry and Fragrance Association (CTFA, 2005b) –Chromosome aberration test in Chinese hamster V79 cells with Homosalate, RCC, Cytotest Cell Research GmbH, Rossdorf, Germany, RCC-CCR Study No. 887602, unpublished data, 09 November 2005
12. Cosmetic Toiletry and Fragrance Association (CTFA, 2005c) –Photomutagenicity in a Salmonella typhimurium reverse mutation assay with Homosalate, RCC, Cytotest Cell Research GmbH, Rossdorf, Germany, RCC-CCR Study No. 908501, unpublished data, 08 December 2005
13. Cosmetic Toiletry and Fragrance Association (CTFA, 2005d) In vitro percutaneous absorption of [^{14}C]-homosalate through viable rat and human skin membranes using flow-though diffusion cells, TNO Report No. V6433, TNO Quality of Life, Physiological Sciences, Zeist, Netherland, unpublished data, 5 September 2005
14. Cosmetic Toiletry and Fragrance Association (CTFA, 2005e) Neutral red uptake phototoxicity assay in Balb/c 3T3 mouse fibroblasts, HVS Study No. 05AF05.140053, Institute for in vitro Sciences Inc., Gaithersburg, MD, USA, unpublished data, Draft
15. Cosmetic Toiletry and Fragrance Association (CTFA, 2005f) Salmonella thyphimurium reverse mutation assay with Homosalate, RCC-CCR Study No. 887601, RCC-Cytotest Cell Research GmbH, Rossdorf, Germany, unpublished data, 14 September 2005
16. DCSP (1996) Diffusion en Gel D'Agarose, Reference No. 96/01/006, unpublished data, 10 May 1996
17. Enslein K, Gombar VK, Blake BW, Maibach HI, Hostynek JJ, Sigman CC, Bagheri D (1997) A quantitative structure-toxicity relationship model for the dermal sensitization guinea pig maximization assay, Food Chem. Toxicol., 35, 1091-1098
18. Evic-Bio (1996) HET-CAM Test, Study No. (No. D'Etude) BC 056/96-0986, Evic-Bio, Division Biotechnique, France, unpublished data, 04 April 1996
19. Federal Register (1978), Proposed Rules, 43, 25 August 1978, 38232-38234
20. Fischer T, Bergström (1991) Evaluation of customers' complaints about sunscreen cosmetics sold by the Swedish pharmaceutical company, Contact Dermatitis, 25, 319-322
21. Formula 283221
22. Formula 283267
23. Formula 283273
24. Formula 283285-1

25. Gerberick GF, Ryan CA (1989) Contact photoallergy testing of sunscreens in guinea pigs, Contact Dermatitis, 20, 251-259
26. Gerberick GF, Ryan CA (1990) A predictive mouse ear-swelling model for investigating topical photoallergy, Food. Chem. Toxicol., 28, 361-368
27. Gonzales E, Gonzales S (1996) Drug photosensitivity, idiopathic photodermatoses and sunscreens, J. American Academy Dermatology, 35, 871-885
28. Gottschalck TE, McEwen Jr GN (eds, 2004) International Cosmetic Ingredient Dictionary and Handbook, 10th Edition, Washington DC, USA
29. Harrison Research Laboratories (HRL, 2002) Repeated insult patch test (RIPT), Test material #6: U02195.05 –283221, HRL Panel #02-109, Harrison Research Laboratories Inc., Union, NJ, USA, unpublished data, 02 July 2002
30. HMS formula U23131, Symrise
31. Homosalate (2005) Two week oral gavage range-finding study in rats, in: Roberts MS (2005) Opinion on the systemic toxicity of homosalate given topically repeat doses, Therapeutics Research Unit, School of Medicine, University of Queensland, Australia, unpublished data, 01 December 2005
32. Johnson & Johnson (2005a), Cumulative irritation test (CIT), 16 test materials, Harrison Research Laboratories, Union, NJ, USA, unpublished data, 31 May 2005
33. Johnson & Johnson (2005b), Phototoxicity test (PT), SPF-30 sunscreen, formula #769-187, batch # 0015C-P, reference # 31135.06, Harrison Research Laboratories, Union, NJ, USA, unpublished data, 14 April 2005
34. Johnson & Johnson (2005c), Photoallergy test (PA), SPF-30 sunscreen, formula #769-187, batch # 0015C-P, reference # 31135.07, Harrison Research Laboratories, Union, NJ, USA, unpublished data, 24 May 2005
35. Johnson & Johnson (2005d), Repeated insult patch test (RIPT), SPF-45 sunscreen, formula #769-190, batch # 0015C-V, reference # 31136.03, Harrison Research Laboratories, Union, NJ, USA, unpublished data, 25 May 2005
36. Johnson & Johnson (2005e), Phototoxicity test (PT), SPF-45 sunscreen, formula #769-190, batch # 0015C-V, reference # 31136.06, Harrison Research Laboratories, Union, NJ, USA, unpublished data, 14 April 2005
37. Johnson & Johnson (2005f), Photoallergy test (PA), SPF-30 sunscreen, formula #769-190, batch # 0015C-V, reference # 31136.07, Harrison Research Laboratories, Union, NJ, USA, unpublished data, 24 May 2005
38. Johnson & Johnson (2005g), Repeated insult patch test (RIPT), SPF-30 sunscreen, formula #769-187 batch # 0015C-P, reference # 31135.03, Harrison Research Laboratories, Union, NJ, USA, unpublished data, 25 May 2005
39. Johnson & Johnson (2005h), Photoallergy test (PA), SPF-30 sunscreen, formula #769-193, batch # 0015C-M, reference # 31137.07, Harrison Research Laboratories, Union, NJ, USA, unpublished data, 24 May 2005
40. Johnson & Johnson (2005i), Phototoxicity test (PT), SPF-30 sunscreen, formula #769-193, batch # 0015C-M, reference # 31137.06, Harrison Research Laboratories, Union, NJ, USA, unpublished data, 14 April 2005
41. Johnson & Johnson (2005j), Repeated insult patch test (RIPT), SPF-30 sunscreen, formula #769-193 batch # 0015C-M, reference # 31137.03, Harrison Research Laboratories, Union, NJ, USA, unpublished data, 25 May 2005
42. Kligman AM (1977) Synopsis report –Objective: To determine the contact-sensitizing potential of Homomenthyl salicylate, unpublished data, 03 November 1997
43. Ma R, Cotton B, Lichtensteiger W, Schlumpf M (2003) UV filters with antagonistic action at androgen receptors in the MDA-kb2 cell transcriptional-activation assay, Toxicol. Sci., 74, 43-50
44. Menz J, Muller SA, Connolly SM (988) Photopatch testing: A six-year experience, J. American Academy Dermatology, 18, 1044-1047
45. National Toxicology Program (2005), Database Search Application, 3,3,5-trimethylcyclohexyl Salicylate, Salmonella Study Summary, 1-2 and Salmonella Study Details, 1-3, Study ID 216212, Year completed 1982, download 27 September 2005

46. Orentreich Research Corporation (2001) Predictive patch test study, Body spray MT# 2047459, Orentreich Research Corporation, unpublished data, February 2001
47. Pernich P, Gallagher M (1950) Transmission spectra of representative sunscreen, J Society Cosmetic Ingredients, 92-99
48. Plough Inc. (1968) Homomenthyl Salicylate, Approximate acute oral LD50 in rats, Laboratory No. 90045, Food and Drug Research Laboratories, Maspeth, NY, USA, unpublished data, 05 August 1968
49. Pollock B, Wilkinson (2001) Photopatch test method: Influence of type of irradiation and value of 7-day reading, Contact Dermatitis, 44, 270-271
50. Pont AR, Charron AR, Brand RM (2004) Active ingredients in sunscreens act as topical penetration enhancers for the herbicide 2,4-dichlorophenoxyacetic acid, Toxicol. Appl. Pharmacology, 195, 348-354
51. Product Investigations (PI, 2003a) Determination of the irritating and sensitizing properties of 282267, U03036.01 on Human skin, Report: PII No. 16394, Product Investigations Inc., Conshohocken, PA, USA, unpublished data, 15 May 2003
52. Product Investigations (PI, 2003b) Determination of the irritating and sensitizing properties of 282273, U03036.03 on Human skin, Report: PII No. 16396, Product Investigations Inc., Conshohocken, PA, USA, unpublished data, 15 May 2003
53. Rietschel MRL, Lewis CCW (1978) Contact dermatitis to homomenthyl salicylate, Arch. Dermatol., 114, 442-443
54. RIFM (1978), Homomenthyl Salicylate, Acute oral toxicity in rats, dermal toxicity in rabbits, Project No. MB 77-2199, M B Research Laboratories, Spinnerstown, PA, USA, unpublished data, 01 February 1978
55. Roberts MS (2005) Opinion on the systemic toxicity of homosalate given topically repeat doses, Therapeutics Research Unit, School of Medicine, University of Queensland, Australia, unpublished data, 01 December 2005
56. Sarveiya V, Risk S, Benson HA. Liquid chromatographic assay for common sunscreen agents: application to in vivo assessment of skin penetration and systemic absorption in human volunteers. J Chromatogr B Analyt Technol Biomed Life Sci. 2004 Apr 25;803(2):225-31 in: Roberts MS (2005) Opinion on the systemic toxicity of homosalate given topically repeat doses, Therapeutics Research Unit, School of Medicine, University of Queensland, Australia, unpublished data, 01 December 2005
57. SCCNFP (2001), Opinion on the Evaluation of Potentially Estrogenic Effects of UV-filters adopted by the SCCNFP during the 17th Plenary meeting of 12 June 2001 (http://europa.eu.int/comm/food/fs/sc/sccp/out145_en.html)
58. Schlumpf M, Berger L, Cotton B, Conscience-Egli M, Durrer S, Fleischmann I, Haller V, Maerkel K, Lichtensteiger W (undated) Estrogen active UV screens
59. Schlumpf M, Cotton B, Conscience M, Haller V, Steinmann B, Lichtensteiger W (2001) In vitro and in vivo estrogenicity of UV screens, Environmental Health Perspectives, 109, 239- 244
60. Schreurs R, Lanser P, Seinen W (2002) Estrogenic activity of UV filters determined by an in vitro reporter gene assay and an in vivo transgenic zebrafish assay, Arch. Toxicol., 76, 257- 261
61. Shaath N. (2005) Sunscreens –Regulations and Commercial Development, Third Edition, edited by Shaath N, Taylor & Francis
62. Springborn Laboratories Inc. (SLI, 2001) A primary eye irritation study in rabbits with a 12% Homosalate sunscreen, SLI Study No. 3133 110, Springborn Laboratories Inc., Spencerville, Ohio, USA, unpublished data, 03 July 2003
63. Symrise (2002), Finished Product Specifications NO FP182, NeoHeliopan HMS (Homosalate USP), Product No. 182573, unpublished data, 13 March 2002
64. Symrise (2004) UV Protection Brochure
65. Symrise (2005a) Photostability of Homosalate in presence of a photo-labile UV-A absorbing research material, UV-Testlabor
66. Symrise (2005b) Homosalate UV spectrum, UV Testlabor
67. Symrise (2005c) Stability data
68. Symrise (2005d) Protocol for photostability test, Internal Memo

69. TKL Research (2003) Repeated insult patch study, 15% HMS sunscreen, TKL Study No. DS101803/102003-1, TKL Research Inc., Paramus, NJ, USA, unpublished data, 20 August 2003
70. Zeiger E., Anderson B., Haworth S., Lawlor T., Mortelmans K. and Speck W. (1987) Salmonella mutagenicity tests: III. Results from testing 255 chemicals, Environ. Mutagen, 9 (Suppl. 9), 1 –110,