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

OPINION

CONCERNING

4-METHYLBENZYLIDENE CAMPHOR

COLIPA n° S60

Adopted by the SCCNFP during the 28th plenary meeting
of 25 May 2004

1. Terms of Reference

1.1 Context of the question

Based on actual scientific knowledge, the Scientific Committee on Cosmetic Products and Non-food Products intended for Consumers (SCCNFP) adopted during the 17th plenary meeting of 12 June 2001 an opinion that the organic UV-filters used in cosmetic sun-screen products, allowed in the EU market today, have no estrogenic effects that could potentially affect human health (doc. n° SCCNFP/0483/01).

Additional information on estrogenic activity has been announced by industry. These data will be evaluated in a future opinion.

Submission VII, however, provided a reassessment of the safety of 4-Methylbenzylidene camphor concerning possible effects on the thyroid gland. This reassessment states the effects observed with 4-MBC in rats on the thyroid hormone profile and the thyroid morphological analysis has no relevance for man.

The current opinion deals with the evaluation of the information provided in submission VII.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions:

* Does the SCCNFP consider that the provided information confirm that 4-MBC is safe for use in sunscreen products?

* Does the SCCNFP recommend any further measures for the use of 4-MBC in cosmetic products based on the provided information

1.3 Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.
The extent to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

2. Toxicological Evaluation and Characterisation

2.1. General

2.1.1. Primary name

4-Methylbenzylidene Camphor (INCI name)

2.1.2. Chemical names

Chemical name : 3-(4-Methylbenzylidene)-dl-Camphor
Synonyms : Bicyclo [2,2,1]Heptan-2-one,1,7,7-Trimethyl-3-[(4-Methylphenyl) Methylene]-

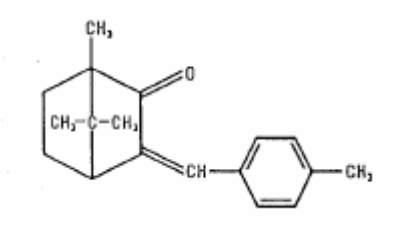
2.1.3. Trade names and abbreviations

Trade name : Eusolex 6300, Neo Heliopan
COLIPA n° : S 60

2.1.4. CAS / EINECS number

CAS : 38102-62-4, 36861-47-9
EINECS : 253-242-6

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{18}H_{22}O$
Mol weight : 254.37

2.1.7. Purity, composition and substance codes

Purity : 99.5 %

No data on impurities

2.1.8. Physical properties

Appearance : White powder with faint characteristic odour
Melting point : 66-68 °C

Boiling point : /
Flash point : /
Density : /
Rel. vap. density : /
Vapour Press. : /
Log P_{ow} : /

2.1.9. Solubility

Ethanol (96%) : approximately 25%
Isopropanol : approximately 25%
iso-propyl myristitate : approximately 25%
liquid paraffin : approximately 15%

Apparently not soluble in water.

2.1.10. Stability

/

2.2. Function and uses

Maximum authorised concentration: 4 % (as a UV filter).

General comments on analytical and physico-chemical characterisation

* Incomplete physico-chemical data

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Mouse
LD_{50}, greater than 10 g/kg bw.

Ref.: 1

Rat
On day 1, greater than 16 g/kg bw. Day 7, 10.6 g/kg bw.

Ref.: 2

Rat
i.p.: greater than 16 g/kg bw on day 1; 10.6 g/kg bw on day 7. In a second experiment, 8 g/kg bw on day 1 and 5.2 g/kg bw on days 7 & 14.

Ref.: 2, 3

Dog
A limit test was carried out on 2 beagles (1 male and 1 female). No abnormality was found. The LD_{50} was greater than 5 g/kg bw.

Ref.: 4

2.3.2. Acute dermal toxicity

Rat
Suspension in arachis oil: occlusion. The LD_{50} was greater than 10 g/kg bw. (2). A second experiment gave the same result.

Ref.: 2, 3

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

17-day oral study (pilot)

Species : rat (Wi-AF/Han)
Route : oral gavage
Test substance : Eusolex 6300 (TC630)
Batch : TT1076
Group size : 10/sex/dose
Dose : 0, 30, 300 mg/kg bw/day (vehicle: peanut oil)
Exposure : 17 days

Results

Dose (mg/kg bw/d)	0		30		300		dr
	m	f	m	f	m	f	
Mortality	-	-	-	-	-	-	dr
Clinical signs	-	-	After dosing: Restlessness, grooming, hypersalivation				
Body weight gain	-	-	-	-	-	-	
Food consumption	-	-	-	-	-	-	
Haematology	Not conducted						
Clinical chemistry	Not conducted						
Serum TSH levels	-	-	-	-	ic (1.9x)	ic (7.5x)	dr
Organ weights							
thyroids (r)	-	-	-	i	ic	ic	
prostate (r)	-	-	dc (82%)[1]		dc (75%)[1]		
thymus (r)	-	-	-	-	-	dc	
adrenals (r)	-	-	-	-	dc	-	
Pathology							
Macroscopy							
Enlarged thyroid gland	-	-	-	-	++	++	
Discoloration renal cortex	-	-	-	-	++	+	

Dose (mg/kg bw/d)	0		30		300		dr
	m	f	m	f	m	f	
Microscopy (thyroid only) Hypertrophy of follicular epithelium	- (4/20)		- (8/20)		ic (16/20)		

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
r relative organ weight (organ/bw ratio)
- not affected
+ present in one/a few animals
++ present in most/all animals
[1] Differences in comparison to control values (=100%)

Ref.: 5

4-week oral study (pilot)

Species : rat (Wi-AF/Han-EMD)
Route : oral gavage
Test substance : Eusolex 6300 (TC630)
Batch : TT1076
Group size : 10/sex/dose
Additional groups: paired fed group; positive control group (20 mg TPU/kg bw/d)
Dose : 0 and 1000 mg/kg bw/day (vehicle: peanut oil)
Exposure : 28 days

Results

Dose (mg/kg bw/d)	0		1000		0 - Pair fed (Feed intake comparable to that in the treatment group)		20 TPU[1]	
Parameters	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	-	1/10	-	1/10	-	-	-	1/10
Clinical signs	-	-	After dosing: grooming, hypersalivation, disheveled fur		-	-	-	-
Body weight gain	-	-	dc	dc	dc	dc	dc	dc
Food consumption	-	-	dc	dc	dc	dc	dc	dc
Water consumption	-	-	ic	ic	-	-	dc	dc
Haematology	not conducted							
Clinical chemistry	not conducted							
Body temperature	-	-	-	-	not conducted		-	-
Cycle course (vaginal smears)		-		-	not conducted			-
Serum T3 (triiodothyronine)	-	-	ic (196%)[2]	ic (128%)[2]	not conducted		dc (95%)[2]	dc (63%)[2]
Serum T4	-	-	dc	dc	not conducted		dc	dc

Dose (mg/kg bw/d)	**0**		**1000**		**0 - Pair fed (Feed intake comparable to that in the treatment group)**		**20 TPU[1]**	
Parameters	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
(thyroxine)			(70%)[2]	(77%)[2]			(39%)[2]	(23%)[2]
Organ weights								
liver (r)	-	-	ic	ic	dc	-	dc	dc
kidneys (r)	-	-	-	ic	-	-	-	dc
thymus (r)	-	-	dc	dc	-	-	-	dc
prostate (r)	-		dc		-		-	
adrenals (r)	-	-	ic	ic	-	ic	-	dc
spleen (r)	-	-	-	-	-	-	dc	dc
thyroid (r)	-	-	ic	ic	-	-	ic	ic
Pathology								
macroscopy								
dilated stomach	-	-	++	++	-	-	-	-
reduced adrenal size	-	-	-	++	-	-	++	++
reduced thymus size	-	-	++	++	-	-	-	++
enlarged thyroid	-	-	++	++	-	-	++	++
microscopy								
Thyroid stimulation	-	-			-	-		
- mild-marked			++	++				
- excessive							++	++

dc/ic statistically significantly decreased/increased compared to the controls
r relative organ weight (organ/bw ratio)
- not affected
++ present in most/all animals
[1] An additional reference group of 10 rats/sex was included, receiving 20 mg/kg bw/day of the thyroid depressant propylthiouracil (TPU) in their drinking water. The rats in this group were also dosed with 5 ml peanut oil/kg bw/day.
[2] Differences in comparison to control values (=100%)

Ref.: 6

Conclusion
At 300 and 1000 mg/kg bw/day interference with thyroid function of rats was noted.
The relative weight of the prostate was decreased at all dose levels examined (30, 300 and 1000 mg/kg bw/day). The effects (at 1000 mg/kg bw/day) cannot be explained by the reduction in feed intake alone (as shown by the comparison with the pair fed group). The interference with thyroid function of the test substance (Eusolex) is not identical to that of TPU.

14-day oral tolerability study in beagle dogs

Species : Beagle dogs (approx. 9 months old)
Route : oral gavage before the 2-hour feeding period
Test substance : 3-(4-Methylbenzylidene)-camphor
Batch : 52000032
Group : One group comprising 1 male and 1 female
Exposure : 14 days
Treatment/ Dose : 20 mg/kg bw/day on day 1
100 mg/kg bw/day on day 2
500 mg/kg bw/day on day 3
2500 mg/kg bw/day on day 4

500 mg/kg bw/day on days 5-14
GLP : in compliance

Ref.: a

Results

Clinical signs	white particles in the faeces of both dogs on day 3-5. No mortality.
Body weight / Feed consumption	not affected
ECG (wk -1, day 1, 2, 3, 4 and 10; prior to and after treatment)	not affected
Blood pressure (wk -1, day 1, 2, 3, 4 and 10; prior to and after treatment):	not affected
Haematology (wk -1 and wk 2): **Clinical chemistry**	Trend for reduced red blood cell count, haemoglobin and PCV in the male dog in week 2. No very obvious changes
TT3, TT4 and TSH (RIA) (days -5, 1, 2, 3, 4 and 11; prior to and 2 h after treatment):	According to the authors, there were no treatment related effects. The data obtained after exposure were, however, consistently higher than those prior to exposure. Also there seemed to be a gradual increase in time (see data below). TSH was detected at the end of the study only. No reference ranges or data on circadian variation were given.

	Male			**Female**		
Day	TT3(ng/dl)	TT4(µg/dl)	TSH(ng/ml)	TT3(ng/dl)	TT4(µg/dl)	TSH(ng/ml)
-5	42.6	0.00	0.00	39.8	1.66	0.00
1 before	50.2	0.90	0.00	46.1	1.71	0.00
1 2h after	73.4	1.88	0.00	74.5	3.07	0.00
2 before	38.2	0.00	0.00	48.0	1.45	0.00
2 2h after	65.9	1.37	0.00	65.8	2.22	0.00
3 before	50.1	0.00	0.00	53.4	1.47	0.00
3 2h after	91.7	1.44	0.00	80.0	2.38	0.00
4 before	95.6	1.67	0.00	37.2	1.29	0.00
4 2h after	127.4	2.80	0.00	47.0	1.48	0.17
11 before	79.9	2.08	0.17	54.4	1.99	0.20

Pathology	the male dog showed minimal activation of the thyroid gland

3 week oral toxicity study in beagle dogs

Species : Beagle dogs (approx. 10 months old)
Route : oral gavage before the 2-hour feeding period
Test substance : 3-(4-Methylbenzylidene)-camphor
Batch : 52000032
Group : One group comprising 2 males and 2 females
Exposure : 14 days
Treatment/ Dose : 0 mg/kg bw/day on day 1 (wash-out phase)
20 mg/kg bw/day on day 4 (wash-out phase)
100 mg/kg bw/day on day 8 (wash-out phase)
500 mg/kg bw/day on days 11-21
GLP : in compliance

Ref. : **a**

Clinical signs	not affected; no mortality.
Body weight / Feed consumption	not affected
ECG (wk -1, day 1, 4, 8, 11 and 16; prior to and after treatment):	not affected
Blood pressure (wk -1, days 1, 4, 8, 11 and 16; prior to and after treatment):	not affected
Haematology /Clinical chemistry (wk -1 and wk 3):	no very obvious changes
TT3, TT4 and TSH (RIA) (d-5, 1, 2, 3, 4 and 11; prior to and 2, 4, 6 and 24 h after treatment)	according to the authors there were no treatment related effects. The data were incompletely summarized. Figures obtained on TT3 and TT4 after exposure were consistently somewhat higher than those prior to exposure or 24 hours thereafter, but the circadian variation after dosing of 0 mg/kg bw seemed comparable to that after treatment with levels up to 500 mg/kg bw.
Pathology	no treatment related effects

2.3.5. Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Sub-chronic oral toxicity

13 week oral study in the rat

Part 1

Species : rat (Wi-AF/Han)
Route : oral feeding
Test substance : Eusolex 6300
Batch : TT1722
Group size : 20/sex/dose (half of the animals in each group were allowed a 1 month recovery period after 13 weeks of treatment)
Additional group : paired fed group; positive control group (20 mg TPU/kg bw/d)
Dose : 0, 50, 125, 312 mg/kg bw/day (vehicle : feed)
Exposure : 13 weeks
GLP : in compliance

Results

Dose (mg/kg bw)	**0**		**50**		**125**		**312**		**dr**
Parameters [5]	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality (not related to treatment)	-	-	-	1/20	3/20	3/20	-	-	
Clinical signs	-	-	-	-	-	-	-	-	
Body weight gain	-	-	-	-	-	dc	-	dc	
Food consumption	-	-	-	-	-	-	-	dc	
Water consumption	-	-	-	-	-	-	ic	-	
Ophthalmoscopy	-	-	-	-	-	-	-	-	
Haematology									
PCV	-	-	ic[2]	dc[2,4]	ic[2,4]	dc[2,4]	ic[2,4]	dc[2,4]	-
haemoglobin	-	-	-	dc[3]	dc[2]	dc[3]	dc[3,4]	dc[3]	dr
red blood cells	-	-	-	dc[3]	dc[1]	dc[3]	dc[1]	dc[3]	-
reticulocytes	-	-	ic[4]	-	ic[4]	-	ic[3,4]	-	-
Clinical chemistry									
creatinine	-	-	-	-	-	-	ic[3]	-	-
cholesterol	-	-	-	ic[2]	-	ic[2]	ic [3]	ic [3]	dr
triglycerides	-	-	-	-	dc[2]	-	dc[2]	-	-
GPT (=ALAT) activity	-	-	-	-	-	ic[3]	ic[3]	ic[2]	-
albumin	-	-	-	-	-	dc[1]	-	dc[2,4]	dr
total protein	-	-	-	-	-	-	ic[3]	-	-
urea	-	-	-	-	-	-	ic[2]	-	-
Serum T3 (triiodothyronine)	-	-	ic[4]	ic[2]	ic[4]	ic[2]	ic [2,4]	ic[2]	dr
Serum T4 (thyroxine)	-	-	-	(ic[3]) (+21%)	-	-	(ic[4]) (+46%)	(ic[1]) (+30%)	-
Serum TSH	-	-	i[4] (+43%)	ic[2] (+99%)	i[4] (+70%)	ic[2] (+106%)	ic[4] (+74%)	ic[3] (+350%)	dr
Organ weights									
liver (r)	-	-	- (0%)	i (+8%)	- (+4%)	ic[2] (+16%)	ic[2] (+14%)	ic[2] (+24%)	dr
thymus (r)	-	-	-	-	-	-	-	ic[2]	
prostate (a)	-		-		-		dc[2]		
adrenals (r)	-	-	-	-	-	-	dc[2]	-	
spleen (r)	-	-	-	-	-	-	ic[2]	ic[2]	
pituitary (a)	-	-	-	-	-	-	-	dc[2]	dr
thyroid (r)	-	-	-	-	ic[2]	ic[2]	ic[2,4]	ic[2,4]	
Pathology									
macroscopy	-	-	-	-	-	-	-	-	
microscopy Thyroid stimulation (hypertrophy and hyperplasia of the epithelium)	-	-	++[2,4]	++[2,4]	++[2,4]	++[2,4]	++[2,4]	++[2,4]	dr

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
r relative organ weight (organ/bw ratio)

a only absolute weight was affected
++ present in most/all animals
(ic) Not considered relevant by the authors
[1] In week 6/ 7 of the treatment period
[2] In week 13/ 14 of the treatment period
[3] Both in week 6/ 7 and week 13/ 14 of the treatment period
[4] Present in the treatment free period
[5] Generally only parameters showing effects were listed

Part 2

Species : rat (Wi-AF/Han)
Route : oral feeding
Test substance : Eusolex 6300
Batch : TT1722
Group size : 20/sex/dose (half of the animals in each group were allowed a 1 month recovery period after 13 weeks of treatment)
Additional group : paired fed group; positive control group (20 mg TPU/kg bw/d)
Dose : 0 and 25 mg/kg bw/day (vehicle: feed)
Exposure : 13 weeks
GLP : in compliance

Results

Dose (mg/kg bw)	**0**		**25**	
Parameters [5]	**m**	**f**	**m**	**f**
Mortality	1/20	1/20	-	-
Clinical signs	-	-	-	-
Body weight gain	-	-	-	-
Food consumption	-	-	-	-
Ophthalmoscopy	-	-	-	-
Haematology				
PCV	-	-	-	(dc[2])
haemoglobin	-	-	-	(dc[2])
red blood cells	-	-	-	(dc[2])
Clinical chemistry				
GPT	-	-	-	(ic[2])
Serum T3 (triiodothyronine)	-	-	-	-
Serum T4 (thyroxine)	-	-	(ic[2]) (+10%)	(ic [2,4]) (+22%)
Serum TSH	-	-	-	-
Organ weights				
liver I	-	-	-	(ic[2]) (+8%)
Pathology				
macroscopy	-	-	-	-

Dose (mg/kg bw)	0		25	
Parameters [5]	m	f	m	f
microscopy	-	-	-	-

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/I decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight
\+ present in one/a few animals
++ present in most/all animals
(dc/ic) Not considered relevant by the authors
[1] In week 6/ 7 of the treatment period
[2] In week 13/ 14 of the treatment period
[3] Both in week 6/ 7 and week 13/ 14 of the treatment period
[4] Present in the treatment free period
[5] Generally only parameters showing effects were listed

Conclusions
In part 1, interference with thyroid function of rats was noted at all dose levels (50, 125 and 312 mg/kg bw/day). In addition, changes in haematology, clinical chemistry and liver weights were noted at 50 mg/kg bw/day and above. Spleen and prostate weights were affected in the high-dose group. In part 2 (animals dosed at 0 and 25 mg/kg bw/day) slight increases in T_4 and in liver weight and slight decreases in red blood cell parameters in dosed females were thought by the authors not to be biologically significant. The authors propose a NOAEL of 25 mg/kg bw/day.

Ref.: 7

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

No data

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Rabbit
Six NZW male rabbits were used. In 3 animals the site was scarified. A suspension of 0.5 g of a.i. in arachis oil was applied under occlusion for 24 hours. No irritation was found. An identical test using water instead of arachis oil gave the same result.

Ref.: 2, 3

Human
Three tests were carried out:

(a) Ten female subjects were tested. A Duhring chamber containing 5% a.i. in mineral oil was glued to the skin on the same site 5 days a week for 2 weeks. Duration of exposure was not stated. There was no evidence of irritation.

(b) Two groups of 10 subjects were exposed in the same way as above, except that the skin was first scarified, and exposure was for 24 hours. The experiment was repeated on the same area of skin 3 times. There was no evidence of irritation.

(c) Two groups of 6 subjects were recruited who felt a stinging sensation on application of lactic acid to the naso-labial fold when they were perspiring profusely.
The test substance was similarly applied; there were no reports of discomfort.

Ref.: 20

2.4.2. Irritation (mucous membranes)

Rabbit
Six animals were used, 3 for testing and 3 as vehicle controls. A suspension of 0.5 gm a.i. in arachis oil was instilled into the eye of the test animals. There was no evidence of any irritant effect. In a similar test, a.i. by itself was used. The test was negative.

Ref.: 2, 3

A test was carried out on the chorio-allantoic membrane of the chick, using 1% and 5% of a.i. in olive oil. Twelve membranes were tested, and 12 were used as vehicle controls. There was no evidence of irritation.

Ref.: 25

2.5. Sensitisation

Guinea pig
A complex protocol is described. Ten animals formed a test group, and 10 formed a positive control group (DNCB). A further 10 animals formed a vehicle control group, and a group of 15 animals formed a second vehicle control group, of which some received a vehicle challenge and some received no challenge (to exclude the possibility that oil treatment of itself might cause hypersensitivity).
The induction was by the application of arachis oil, or of a.i. in arachis oil, to the clipped skin of the flank, 5 days a week for 2 weeks; in the case of the positive control, the application was of 2% DCNB in ether daily for 1 week. After a rest period, the challenge was made as a single application to the opposite flank. The applications were of 0.3% a.i. in arachis oil, 0.2% DCNB in ether, or vehicle only, depending on the group. Some animals received no challenge.
The positive control animals showed well marked erythema after the challenge; there was no skin reaction in animals of the other groups. The mean weight gain of the test animals was somewhat less than that of animals of the other groups over the period of the experiment; there was no obvious reason for this. It was concluded that sensitisation had not occurred.

Ref.: 2

In a similar experiment, the a.i. was used as a 3% suspension in 0.5% aqueous carboxymethylcellulose. The test was again negative.

Ref.: 3

Human

Tile experiment was carried out in 30 healthy subjects, 5 men and 25 women. The test applications were: 5% a.i. in 15/85 isopropyl myristitate/liquid paraffin; 5% a.i. in o/w emulsion; and 5% a.i. in w/o emulsion. The vehicles, without a.i., were also tested.
The applications were made initially to the skin of the back for 24 hours with occlusion. The skin was inspected after removal; there was no evidence of primary irritation.
The same applications were then made 3 times a week for 3 weeks. After a rest period of 8-10 days, the applications were made again to a fresh area. There was no evidence of sensitisation.

Ref.: 10

Test for capacity to induce photo-sensitisation

Guinea pig

Three groups of 5 animals (sex not stated) were used. (1) The test group was treated with a.i. in the chosen vehicle, followed by irradiation. (2) The vehicle control group was treated with the appropriate vehicle, and irradiated. (3) The radiation control group was treated with the application containing a.i., but not irradiated.
The experiment was carried out in 3 stages. In the first stage, an area on the neck was prepared. A volume of 0.05 ml of a 5% solution of a.i. in alcohol was painted on the skin of animals of group 1. Animals of group 2 had an application of vehicle. Both these groups were irradiated as in the photo-toxicity experiment. Animals of group 3 had the a.i. applied, but were not irradiated. In the second stage, after a 9 day rest period, the same procedure was carried out on 2 successive days, except that the vehicle was arachis oil. In the third stage, the skin of either flank was prepared: animals had an application of 2 ml of a 5% solution of a.i. in an 8% soap solution on one flank; the opposite flank was treated with vehicle. Gauze was applied for 1 hour. Animals of groups 1 and 2 were then irradiated, and those of group 3 kept in the dark. This procedure was carried out on 3 consecutive days, with observations made every 24 hours.
Animals which had been irradiated showed similar scores, whether or not a.i. was used; their mean score was 1.8, while the animals which had not been irradiated showed no change. The test was considered negative.

Ref.: 9

Human

Five healthy subjects were used, 3 male and 2 female.
In the first stage, 0.05 ml of a 4% solution of a.i. in alcohol was applied to the forearm (area 1). This was then irradiated, in the manner described for the photo-toxicity experiment. At the same time a second area was treated with the a.i. but not irradiated (area 2).
Finally a third area was treated with vehicle only and irradiated (area 3). After a 10 day rest period, stage 2 was begun. The procedure of stage 1 was repeated except that the vehicle was liquid paraffin instead of ethanol.
After a further rest period of 10 days, stage 3 was begun. The a.i. was made up as a 4% solution in 8% soap solution. A volume of 1 ml was applied to area 1 and covered with gauze for 1 hour. The remaining 2 areas were treated with vehicle only, and also covered with gauze for 1 hour.

After removal of the gauze, all 3 areas were irradiated.
The only reactions were slight ones in the vehicle control areas. The test was considered negative.

Ref.: 12

Human
(a) Twenty-five healthy subjects (12 males and 13 females) were recruited. The MED for each subject was determined by exposing an area of the back of each subject to SSR from a xenon arc with appropriate filters. The dose of radiation was increased by 25% increments.
The a.i. was formulated in a lotion (no details of composition of the vehicle or of concentration of a.i. are given).
An area of the back 2x2 cm had 100 µl of the lotion applied, followed by occlusion for 24 hours. The patch was removed, and the area exposed to 3 MEDs. This procedure was repeated 24 and 48 hours later, and this sequence was repeated in the following 3 weeks, making 9 applications in all.
After a 10 day rest, 10 µl of the lotion was applied in duplicate to 2 previously untreated areas of the back, and occluded for 24 hours. After removal of the patches, one of the sites was irradiated with 4 J/cm^2 of UVA.
An adjoining untreated area of the skin was similarly irradiated. Reading was at 24 and 48 hours. There was no evidence of any reaction.

(b) An identical procedure was carried out using a preparation coded "Cream W". No reaction was produced.

(c) An identical procedure was carried out using a preparation coded "Cream U". No reaction was produced.

Ref.: 21

2.6. Reproductive function

2.6.1. Embryotoxicity/ Teratogenicity

Rabbit
Four groups each of 3 animals were used, and a.i. in arachis oil was given by gavage daily from days 6 to 10 of pregnancy, in doses of 0, 25, 50 and 100 mg/kg bw/day. All animals suffered from initial diarrhoea. The numbers of offspring were unusually low in all groups.
There was no evidence of teratogenicity or embryo-toxicity; it was also concluded that arachis oil was not a suitable vehicle for this sort of study.

Ref.: 23

Rat
Four groups of 25 Wistar rats were used; the study was carried out in conformity with GLP guidelines. The a.i. was suspended in arachis oil and given by gavage from days 6 to 16 of pregnancy, in doses of 0, 10, 30 and 100 mg/kg bw/day. Gross autopsy of the dams showed no abnormality; those of the top dose showed lower body weight gain than the others. Foetuses: In group 4, the foetuses were significantly lighter than in the other groups. The degree of ossification of the sternum was somewhat lower in the intermediate- and high-dose male and female foetuses, while ossification of the extremities was delayed in males of the high-dose group. A dose-dependent increase of rudimentary lumbar ribs in foetuses of both sexes of the

intermediate- and high-dose was reported. The authors concluded that 'the maternal animal was sufficiently stressed to express the developmental instability inherent in the species'.

There was some retardation of ossification in foetuses of the intermediate and high dose groups. There was no evidence of teratogenesis.
Because developmental effects were noted at 30 and 100 mg/kg bw/day, it is concluded that the NOAEL for developmental effects is 10 mg/kg bw/day.

Ref.: 26

Fertile hen's eggs
Groups of 20 eggs were treated with doses (mg/egg) of 0, 0.1, 0.5, 1.0, 5.0 and 10.0. Two series were carried out: in the first, the injections were made on the first day of incubation, and in the second, on the fifth day of incubation. There was no evidence of toxic or teratogenic effect in the surviving chicks; a no effect level of 0.1 mg/egg is suggested, whether the injection was made on the first or fifth day of incubation.

Ref.: 24

2.6.2. Reproduction study

Species	:	Wistar rat (HanBrl:WIST)
Route	:	oral gavage; once daily (only F_0-females)
Vehicle	:	0.25% hydroxypropyl methylcellulose
Test substance	:	3-(4-Methylbenzylidene)-camphor
Batch	:	52000032
Purity	:	99.9%
Groups (F_0)	:	Four groups comprising 10 females each.
Study design	:	- F_0-Females were treated during a 28-day premating period, and during mating, gestation and lactation. - F_0-males remained untreated. - About half of the F_1-offspring were reared until day 55 post partum; the remaining F_1-offspring were sacrificed at weaning and examined macroscopically. F_1-offspring were not (directly) treated with the test substance.
Dose	:	0, 12.5, 25 and 50 mg/kg body weight/day
GLP	:	in compliance

Ref.: b

Results

F_0 -Clinical signs	not affected.
F_0 -Body weight / Feed consumption	not affected
F_0 -Water intake	50 mg/kg bw: (ca. 30%) increased during premating period and during gestation 25 mg/kg bw: (ca 13%) increased during premating period only 12.5 mg/kg bw: (ca 14%) increased during premating period and gestation period
Reproductive data (estrous cycle, fertility indices, mating performance, duration of gestation, implantation rate, post implantation	not affected

loss, litter size, postnatal pup loss)	
F_0 - Organ weights (thyroids, uterus, ovaries, adrenals, thymus, pituitary)	not affected
F_0 Hormone analyses at day 21 of the premating period and at weaning (TT3, TT4, TSH, FSH, LH, estradiol, testosterone)	not affected; TT3 levels in the high dose group seemed slightly elevated without reaching statistical significance at day 21 premating. TSH levels in the high dose group seemed slightly elevated without reaching statistical significance at weaning Prolactin seemed slightly elevated in the mid- and high dose group without reaching statistical significance at day 21 premating. All changes were slight, within control range and ascribed to inter-animal variation.
F_1 offspring (sex ratio, anogenital distance, pup weights, developmenytal indices, nipple retention in male pups, learning (water maze test), necropsy)	not affected
F_1 offspring - Organ weights day 21 or day 55 post partum (including thyroid and reproductive organs)	not affected
F_1 offspring - Hormone analyses at day 55 post partum (TT3, TT4, TSH, FSH, LH, estradiol, testosterone)	Lower levels of TT3 and TSH in high-dose males were ascribed to inter-animal variation. Lower FSH levels (dose related and statistically significant) were noted in males of the mid- and high-dose group.
F_1 offspring - Histopathology at weaning (ovaries, uterus, prostate, testes)	not affected

Conclusion

The oral administration levels up to 50 mg 4-MBC/kg bw/day did not affect reproductive function of female rats or the development of the offspring.

The decrease in FSH in male offspring of the mid- and high-dose group was ascribed to a certain variation in the onset of puberty rather than to an estrogenic effect (suppression of the hypothalamus/pituitary axis). It was argued that LH was not affected and that developmental parameters and histopathology of testes and prostate did not reveal any treatment-related effects.

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Human

Six healthy male volunteers were tested. The a.i. was supplied with ^{14}C labelling in an o/w formulation at a concentration of 5%. About 1 gm was applied over a shaved area of 200 cm^2 on the forearm. Occlusion was not used, but the application was allowed to remain on the skin for 6 hours. At the end of this period, the skin was washed with soap and 1 litre of water, and then rinsed with 1.5 litres of water. The amount of radioactivity in the urine (over 3 days) and faeces (over 4 days) was followed. Overall recovery of radioactivity was poor, which the author attributes to the fact that the a.i. is very insoluble in water, so that the rinsing procedure did not remove all the a.i. Although the author concludes that percutaneous absorption amounted to 0.9% of the amount applied, careful reading of the experimental data suggests that the true figure is nearer to 1.9%, and in the interests of safety, this figure is used in the estimation of the margin of safety.

0.9% would be equivalent to an absorption of 2.25 μg a.i./cm^2

1.9% would be equivalent to an absorption of 4.75 µg a.i./cm^2

Ref.: 14

Human
Four healthy subjects were tested. A technique similar to that of the preceding experiment was used, except that xylene/ether was used for rinsing; this improved the recovery of radioactivity to about 90%. The amounts in urine and faeces over 3 days were calculated by the author to be 0.53% ± 0.26 (range 0.29 to 0.74%). Some of the tables are difficult to interpret. In addition, the skin was stripped 15 times in the area of application; in the first strippings, the percentage of net applied radioactivity found was 62.5% and 27.6%; later strippings yielded much smaller amounts. 0.53% would be equivalent to an absorption of 1.3 µg a.i./cm^2

Ref.: 15

Human
In a further report, the author combines the urinary and faecal findings of the previous 2 reports, and calculates that about 0.75% ± 0.21 of the amount of a.i. applied is absorbed. The interpretation of this experiment is difficult, as the figures given are not easily reconcilable with those given in the first two experiments. 0.75% would be equivalent to an absorption of 1.9 µg a.i./cm^2

Ref.: 16

These are old studies, and the protocols are thus not in accordance with modern guidelines.

2.8. Mutagenicity/Genotoxicity

2.8.1. Mutagenicity/Genotoxicity *in vitro*

A standard Ames test was carried out. There was no evidence of mutagenesis, with or without activation.

Ref.: 13

A test *in vitro* for chromosomal aberration was carried out using a Chinese hamster V79 cell line. The study was carried out according to OECD guidelines and GLP. Concentrations used (µg/ml) were : without S9, 10; with S9, 36 (7 hours exposure); without S9, 1, 5 and 11; with S9, 3, 15 and 36 (18 hours exposure); without S9, 11; with S9, 36 (28 hours exposure). Toxicity was found at 36 µg/ml in the cultures without S9. There was some increase in the percentage of aberrations after 7 and 28 hours exposure at the highest dose, when S9 mix was used. However, the test is evaluated as negative.

Ref.: 22

4-Methylbenzylidene camphor did not increase the number of revertants in the presence of UVA/UVB light. The compound was not able to induce photomutagenic effect on *S. typhimurium* TA 102 and TA 1537 and on *E. coli* under the conditions of this study. The positive control induced a clear photomutagenic effect in *S. typhimurium* TA 102 and in *E. coli* WP2. It was weakly photomutagenic in *S. typhimurium* TA 1537.

Ref.: 27

4-Methylbenzylidene camphor has been tested for photoclastogenicity potential on mammalian cells (CHO-K5) exposed to UVA/UVB light.

The CHO cells were treated with concentrations of 4-Methylbenzylidene camphor ranging from 1 to 6.6 µg/ml (3 doses) and concomitantly exposed to solar simulated irradiation at UV doses ranging from 200 to 2000 mJ/cm² of UVA and from 4 to 25 mJ/cm² for UVB.
Concomitant controls were made in the absence of UV light and with positive control exposed to UV light (chloropromazine).

Under the experimental conditions, there was no indication of photoclastogenicity induced by 4-Methylbenzylidene camphor.

Ref.: 30

2.8.2 Mutagenicity/Genotoxicity *in vivo*

No data

2.9. Carcinogenicity

No data

2.10. Special investigations

Test for capacity to produce photo-toxicity

Mouse
Groups of 5 male and 5 female animals were used.
A shaved area of the skin of the back was exposed to radiation from a quartz lamp (details not given). The a.i. was suspended in [probably] arachis oil at a concentration of 5%. The groups tested were: (a) treated with a.i. but not irradiated; (b) treated with vehicle, and irradiated ; (c) treated with vehicle and not irradiated: (d) treated with a.i. and irradiated. Reading was at 24, 48 and 72 hours. Scoring was on a scale of 4. The non-irradiated groups showed no abnormality.
The irradiated vehicle control group showed 0.4 ± 0.5 (m) and 0.6 ± 0.5 (f) at 48 hours, and l.0 ± 0.7 (m) and 0.8 ± 0.8 (f) at 72 hours.
The animals treated with a.i. and irradiated showed a reaction of l.0 ± 0.7 (m) and 1.4 ± 0.5 (f) at 72 hours.
The test was regarded as negative.

Ref.: 8

Human
Five healthy subjects were tested using the method of Kligman & Breit. The a.i. was applied at 4% in alcohol. Applications of 0.05 ml were applied to each forearm and one area irradiated by a quartz lamp as in the preceding experiment. An area treated with alcohol alone was also irradiated. The test was negative.

Ref.: 11

Tests for effect on thyroid and pituitary hormones following cutaneous application

Human
In view of the effects on thyroid function found in animal experiments, a pilot study was carried out in man. Four healthy subjects were recruited, 2 men (subjects 1 & 2) and 2 women (subjects

3 & 4). The applications were 5% a.i, in w/o emulsion to subjects 1 & 4, and a 5% a.i. in o/w emulsion to subjects 2 & 3. The method of exposure was to apply 3 gm of preparation over 500 cm^2 of abdominal skin, and the same on the skin of the back, without occlusion, for 3 hours. The application was then repeated. At the end of the second 3 hour period, the subjects showered. Blood was taken at the beginning (0 hours) and at 3, 4, 6, 12, 24 and 48 hours. Estimation of plasma T_3, T_4 and TSH was carried out by radio-immunoassay.
TSH: This was below the level of detection throughout, in males. [Percentages indicate the values compared with the initial levels found]. In females, subject 3 (o/w) showed a fall to zero at 3 & 4 hours, followed by a steady rise to 156% at 24 hours, falling to 124% at 48 hours. Subject 4 (w/o) showed an increase to 270% at 3 hours, and then a steady climb to a maximum of 369% at 12 hours, falling to levels of 195% at 24 hours and 260% at 48 hours.
With regard to thyroid hormone levels, subject 3 had an increase in T_3 to a peak of 127% at 12 hours, followed by a fall to 87% at 24 hours. T_4 levels were little affected. Subject 4 showed little effect on T_3, but T_4 increased to 220% at 24 hours, falling to 81% at 48 hours.
Turning to the males (subjects 1 & 2), there was little effect on TSH, as stated; there was a rise in T_3 to 120% at 4 and 12 hours in subject 1, and subject 2 showed a rise in T_3 to 134% at 24 hours; there were no remarkable changes in T_4 levels.

Ref.: 19

Human
In view of the above findings, a further study was carried out in 11 healthy euthyroid subjects. Following a preliminary test with protirelin, it was found that 2 of the subjects gave anomalous responses, and the main test was therefore carried out with 9 subjects: 5 male and 4 female. Once a week, within a 3-wk period, the volunteers received topical applications of Eusolex (5%) ointment (1x; one week) or blank ointments (2x; the other two weeks) in a random order. In each week, 2 x 6 gram of Eusolex (5%) ointment or blank ointment was given in 2 topical applications on a single day (on a surface of 1200 cm^2 which remained uncovered). At 24 hours after the start of the test, protirelin was given. The results show that (a) the expected effect of protirelin (rise in TSH after 4 hours, fall below normal at 48 hours) was found; (b) there was statistically significant lowering of mean T_3 and T_4 values in the treatment group at 48 hours, but these changes were small and the experimenters believe they `were not biologically significant. This seems to have been a carefully carried out procedure, and the authors state that under the conditions of the test the a.i. does not affect thyroid function. In principle, however, this experiment was rather a test of pituitary function.

Ref.: 18

Human
A parallel double blind study in 24 male and 24 female subjects was carried out according to GCP guidelines.
Twelve male and 12 female subjects were exposed dermally to 5 gram of a w/o cream containing 6% of the active ingredient (Eusolex^R 6300) twice daily over a period of 14 days.
Once in the morning and once in the afternoon; 2.5 grams was applied to the abdomen and 2.5 grams to the back on each occasion. The total area covered was about 1200 cm^2. Daily dosage was thus 600 mg a.i. to an area of 1200 cm^2. Four hours after the second application, the subject took a shower. Occlusion was not used. The procedure for the control subjects was identical, except that vehicle only was applied.
Mean subject ages were 27.8 (male) and 27.7 (female). The 48 healthy volunteers gave informed consent to entering the trial, and the trial was approved by an independent ethical committee.

Volunteers who smoked were encouraged to continue their normal smoking patterns, and a small amount of alcoholic drink per day, in the pre-test phase, was allowed to those who wished it; however, alcoholic beverages were not allowed during the test proper. Two subjects failed to complete the test (both in the test group), one due to an attack of diarrhoea and one because of rashes. Eleven of the female subjects were taking oral contraceptive steroids; perhaps surprisingly, the menstrual status of the female subjects seems not to have been recorded.
Extensive testing was carried out before the experiment began. In addition to full clinical examination, medical history, and 12-lead ECG, the following physiological variables were tested:
Blood biochemistry: total. protein, total bilirubin, alanine transaminase, aspartate transaminase, gamma glutamyl transferase, glucose, sodium, potassium, bicarbonate, urea and creatinine.
(Females only) day before test and on test day 14: test for pregnancy.
Blood haematology: haemoglobin, red cell count, haematocrit, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume, total and differential white cell count ; platelet count.
Urine: protein, glucose, specific gravity, ketones, urobilinogen, bilirubin, blood and pH.
Drugs in urine: alcohol, cannabinoids, amphetamines, benzodiazepines.

The thyroid related variables tested for in the blood were: thyroid stimulating hormone, free thyroxine, free triiodothyronine. thyroxine, triiodothyronine, thyroid binding globulin.
Blood for the above tests was taken: pre- and post-study and at days 4, 7, 12, 14, 16 and 21.
Blood taken from female subjects was tested for pregnancy before the experiment and on day 14.
The volume of the thyroid gland was estimated by an ultra-sound method before and after the experiment. (The relevance of this measurement within 3 weeks may be doubtful).
The experiment proper lasted 14 days; it was argued that, since the thyroid related hormone with the longest half life in the serum, thyroxine, had a half life of 7 days, a 14 day study would pick up any variations in the levels of thyroid related hormones.

Results
There was no significant change of any kind in the thyroid related hormones. The thyroid volume was found to be reduced by 1.70% in the treated group and increased by 3.11% in the placebo group. These differences were not regarded as biologically significant by the author.
"These findings may be put into context by considering that the reproducibility of volumetry by ultrasound is estimated at about 10%, and the average inaccuracy of Brunn's formula with respect to the "true" [thyroid] volume is estimated at 15-20%, hence any changes smaller than this will not be reliably detected and within this limit of precision, should be disregarded.
The changes detected within this study fall well within these limits and may therefore be due to inaccuracy in the method used rather than to any actual physical change in the size of the thyroid."
There were some adverse effects, but none of any importance. The author concludes that there was no evidence of any effect on thyroid function following daily dermal application of 600 mg a.i. (in a 6% formulation) to an area of 1200 cm^2 during 2 weeks.

Ref.: 29

Test for capacity to induce liver enzymes.

Rat
Groups of 10 animals were used: one control and one given 312 mg/kg bw/day in the diet. The experiment lasted 4 weeks. After sacrifice, the livers were tested for protein content, DNA and

RNA content, cytochrome P_{450} content, and glucuronyl transferase activity. There was no evidence of induction of liver enzymes.

Ref.: 17

2.11. Safety evaluation

Acute toxicity was low.
Sub-acute and sub-chronic studies in rats suggest marked interference of 4-MBC in thyroid hormone metabolism as evidenced by changes in thyroid weight, levels of circulating thyroid hormones and histological evidence of thyroid stimulation. In addition, interference with thyroid function may have affected other parameters (e.g. red blood cell turnover).
Hypertrophy and hyperplasia of thyroid epithelium was observed at levels of 50 mg/kg bw/day and above. At the lowest level tested (25 mg/kg bw/day), no morphological thyroid effect was noted, but increases in serum T4 were still observed. In addition, effects on red blood cell parameters were still observed at the lowest level tested. Hence a clear no-effect level was not obtained and 25 mg/kg bw is a LOAEL rather than a NOAEL. No data were available on the long term consequences of prolonged thyroid stimulation.
Studies in dogs indicated that thyroid function is probably affected at higher levels of 4-MBC in dogs than in rats. The study duration was, however, limited (exposure to one dose lasted maximally 9 days, whereas rats were exposed for 13 weeks). In addition, the very limited number of dogs and the special dosing regimen (increasing dose, versus separate dose groups and a control group) did not allow a systematical investigation of other parameters that determined the NOAEL in rats (e.g. haematological effects). A NOAEL in dogs could therefore not be established on the basis of these studies.
A study of the effect of dermal applied active ingredient on thyroid function in man did not reveal compound-related changes in thyroid hormone levels. However, the exposure surface was only 1200 cm² and this study may not mimic the 'in use conditions'

A teratogenicity study revealed a NOAEL of 10 mg/kg bw/day with developmental effects at 30 and 100 mg/kg bw/day. In a reproduction study in rats, the oral administration of levels up to 50 mg 4-MBC/kg bw/day did not affect reproductive function of female rats or the development of the offspring.

Tests for skin irritation, sensitisation, photo-toxicity, photo-sensitisation and photo-contact allergy were negative. The animal tests for sensitisation, however, were unsatisfactory, in as much as Freund's complete adjuvant had not been used. It was noted, however, that the compound very rarely caused contact allergy in man.
The percutaneous absorption was probably between 1.3 – 4.75 µg a.i./cm^2 as judged from urinary and faecal excretion in man following cutaneous application of an o/w formulation containing 5% radioactive a.i. (250 µg a.i./cm^2) over a 6 hr period. The results of the percutaneous absorption studies are, however, difficult to interpret. Moreover, although the test substance is insoluble in water, no data were obtained with a w/o emulsion.

An Ames test and a chromosomal aberration test *in vitro* were negative. Tests for photo-mutagenicity were carried out using 2 strains *of S. typhimurium* and *E. coli* WP2. The results were negative. Tests for photo-clastogenicity were negative.

CALCULATION OF THE MARGIN OF SAFETY

Not applicable

2.12. Conclusions

Reassessment of old and newly provided data indicate that the current use of 4-Methylbenzylidene camphor in sunscreen products poses a reason for concern.
The changes in thyroid hormone profile and thyroid morphological analysis in rats are difficult to interpret with the data available. Increased TSH in combination with elevated T3 or T4, enlarged thyroids and thyroid proliferation suggests a major interference of 4-MBC in thyroid hormone metabolism. Despite some limits with respect to the extrapolation of rodent experimental results to human pathophysiology, the present findings in rats cannot be disregarded without a proper understanding of the mechanisms involved. As goitrogenesis is not a trivial process but is in general associated with increased possibility for thyroid autonomy or thyroid carcinoma, disturbances of the thyroid hormone axis should be considered with great caution. Risk assessment is further hampered by the lack of adequate data on dermal penetration and the fact that 25 mg/kg body weight/day is a LOAEL rather than a NOAEL in rats.

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3. Opinion of the SCCNFP

4-Methylbenzylidene Camphor is presently widely used in cosmetic sunscreen products and has been available to the consumer for many years. However, reassessment of the available data has raised issues of concern about its safe use in cosmetic sunscreen products.

For a better evaluation of these potential effects, the following additional information is required:

* complete physico-chemical data;
* a dermal penetration study according to current guidelines, including the study of the different factors affecting the quantitative outcome of the results;
* a clear NOAEL obtained in a relevant species;
* exposure data on other uses (cosmetic and non-cosmetic) and on oral intake when used in e.g. lip products.

Because of the very low MOS which can be derived from currently available information, it is requested that the above data should be provided as a matter of urgency.

4. Other considerations

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5. Minority opinions

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