EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

4-Methylbenzylidene Camphor

COLIPA N° S60

Adopted by the SCCP
during the 9th plenary meeting of 10 October 2006

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1. BACKGROUND

Based on current scientific knowledge the Scientific Committee on Cosmetics and Non-Food Products (SCCNFP) adopted on 12 June 2001 an 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.

In 2001 the European Commission received a request from the Danish authorities in which they asked for a safety evaluation of the UV filters 4-Methylbenzylidene camphor, Octyl methoxycinnamate and Oxybenzone when used in sunscreen products and other cosmetic products for children. The Danish authorities expressed their concern on the calculation of the margin of safety for the above-mentioned UV filters when used by children and asked for a special risk assessment to be made for the general use of the above-mentioned UV-filters in sunscreen products for children. In particular, they asked to specify the influence of factors like critical endpoints e.g. for 4-MBC to take into account the effects on the thyroid gland.

In January and February 2004, Merck KGaA, Germany provided information detailing the comprehensive toxicological study program for 4-MBC and submitting a study and a statement regarding thyroid gland effects.

Based on the information then available, the SCCNFP adopted on 25 May 2004 opinion SCCNFP/0779/04, where it stated that *because of the very low MOS (Margin of Safety) which can be derived from currently available information, it is requested that the … data should be provided as a matter of urgency*.
Following data has been requested: complete physico-chemical data; data on dermal penetration 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.

In May 2004 the SCCNFP received submissions from Merck KGaA on estrogenic potential of this UV-filter, a reproduction toxicity study in rats and data on toxicokinetics.

2. TERMS OF REFERENCE

1. *On the basis of provided data the SCCP is asked to assess the risk to consumers when 4-MBC is used in sunscreen products?*

2. *Does the SCCP recommend any further restrictions for the use of 4-MBC in cosmetic products based on the provided information?*

3. OPINION

3.1 HISTORICAL BACKGROUND

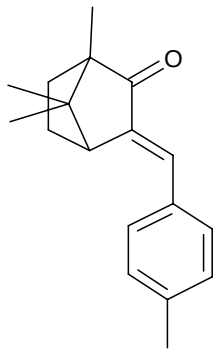
As stated under Section 1, a number of opinions related to the cosmetic use of 4-MBC [3-(4-Methylbenzylidene)camphor], have been issued in the past. The relevant toxicological data and conclusions thereof are summarized below:

3.1.1 Taken from opinion n° XXIV/1377/96, adopted on 21 January 1998

Chemical and physical specifications

Primary name:	4-Methylbenzylidene Camphor (INCI) Enzacamene (USAN)
Chemical name:	3-(4-Methylbenzylidene)-camphor 3-(4-Methylbenzylidene) bornane-2-one
Trade name and abbreviations:	Eusolex® 6300 COLIPA S 60
CAS number:	36861-47-9
EINECS number:	253-242-6

Structural formula:



Empirical formula:	$C_{18}H_{22}O$
Molecular weight:	254.37 g/mol
Purity, composition, substances codes:	The powder used in the tests is stated to be greater than 99.5% pure. Other preparations provided by the manufacturer, are assumed by the investigators to be equally pure; in some cases analytical data are provided.
Impurities:	Camphor (GC): $\leq$ 0.02 % Methylbenzaldehyde (GC): $\leq$ 0.1 %
Appearance:	white crystalline
Odour:	weak camphorlike
Melting point:	66 – 69 °C
Boiling point:	no data
Density:	no data

Loss on drying (50 C):	≤ 0.20%
Vapour Press:	no data
Log Pow:	5.14 (Method EC A.8)
Spec. absorption:	930 – 990 (at 299 nm: 1%, 1 cm; methanol)
Solubility:	Poorly soluble in water (0.00013 g/100 ml; 20 °C) Slightly soluble in ethanol and vegetable oils Very slightly soluble in chloroform
Function and uses:	As an ultraviolet filter in sunscreen and other cosmetics at a maximum concentration of 4%. Maximum absorption at 300 nm

Comment
The source of the cited chemical and physical specifications is not stated.

Acute oral / dermal / intraperitoneal toxicity data

LD_{50}-oral-mouse	>	10,000 mg/kg
LD_{50}-oral-rat	>	10,000 mg/kg
LD_{50}-oral-dog	>	5000 mg/kg
LD_{50}-dermal-rat	>	10,000 mg/kg
LD_{50}-i.p.-rat	>	5000 mg/kg

Skin irritation

- No skin irritation observed in the rabbit Draize test.
- 3 tests were carried out on man:
  a) a 5% containing Duhring chamber was glued on the skin of 10 ♀ subjects on the same site 5 days a week for 2 weeks. Exposure time was not stated. No skin irritation was observed.
  b)2 groups of 10 subjects were exposed on the same way as above but here the skin was first scarified and exposure was for 24h. The test was repeated on the same area of the skin 3 times. No irritation was observed.
  c)2 groups of 6 subjects who had a stinging sensation after application of lactic acid to the naso-labial fold. The test substance was applied on the same way. No reports of discomfort were reported.

Mucous membrane irritation

No mucous membrane irritation observed in the rabbit Draize test.

Skin sensitisation

- Concentrations of up to 3% 4-MBC in arachis oil or 0.5% aqueous carboxymethylcellulose did not cause any sensitising effect in the guinea pig (Freund's Complete Adjuvans was not used).

- 5 men and 25 women were treated with 5% 4-MBC-containing w/o and o/w emulsions for 3 weeks at a rate of 3 applications/week. After 8-10 days, the applications were done again to a fresh area. No evidence of sensitisation was noted.

Subacute oral toxicity

- 17-day oral study with the Wistar rat, dosages of 0, 30 & 300 mg 4-MBC/kg bw/day.
  At 300 mg/kg bw/day:
  - TSH increase 1.7 times for ♂ and 7.5 times for ♀ at top dose,
  - increased thyroid gland weight at top dose,
  - endothelial hypertrophy of thyroid glands at top dose,
  - dose-related decrease in prostate weight in ♂.

  Proposed oral NOAEL by authors = 30 mg/kg bw/day.

- 28-day oral study with the Wistar rat at dosages of 0 and 1000 mg 4-MBC/kg bw/day.
  At 1000 mg/kg bw/day:
  - increased relative liver weight,
  - decreased absolute and relative prostate weight in ♂,
  - reduced absolute thymus weight,
  - increased absolute and relative thyroid gland weight,
  - increase in thyroxine (T_4) plasma levels,
  - significant fall in triiodothyronine (T_3) plasma levels,
  - marked pattern of stimulation of the thyroid gland, though different from the positive control propylthiouracyl, the latter inducing a fall in T_4 as well as in T_3.

Subchronic oral toxicity

- 90-day oral study with the Wistar rat, dosages of 0, 50, 125 & 312 mg 4-MBC/kg bw/day.

  At 312 mg/kg bw/day:
  - reduced body weights in ♀,
  - dose-related increase in packed cell volume (PCV) and haemoglobin (more marked in ♀), PCV still elevated in recovery animals after 17 weeks,
  - increased triglycerides in ♂ and increased cholesterol in ♀,
  - increased T_3 values for ♀ and ♂,
  - increased T_4 values for ♀ and ♂, though for the latter only in the recovery group,
  - elevated TSH values for ♀ and ♂, though for the latter only in the recovery group,
  - hypertrophied epithelium of the thyroid gland with increased mitotic activity in the secretory cells in ♂ and ♀.

  At 125 mg/kg bw/day:
  - reduced body weights in ♀,
  - dose-related increase in PCV and haemoglobin (more marked in ♀),
  - increased triglycerides in ♂ and increased cholesterol in ♀,
  - increased T_3 values for ♀ and ♂, though for the latter only in the recovery group,
  - increased T_4 values for ♀,
  - elevated TSH values for ♀,
  - hypertrophied epithelium of the thyroid gland with increased mitotic activity in the secretory cells in ♂ and ♀

  At 50 mg/kg bw/day:
  - reduced body weights in ♀,
  - dose-related increase in PCV and haemoglobin (more marked in ♀),

- increased T_3 values for ♀ and ♂, though for the latter only in the recovery group,
- elevated TSH values for ♀,
- hypertrophied epithelium of the thyroid gland with increased mitotic activity in the secretory cells in ♂ and ♀

× 90-day oral study with the Wistar rat, dosages of 0 & 25 mg 4-MBC/kg bw/day.
At 25 mg/kg bw/day:
- increased T_4 values for ♀,
- histological examination of the thyroid was normal.

Proposed oral NOAEL by authors = 25 mg/kg/day.

Phototoxicity and photosensitisation

A mice study with 5% 4-MBC and a human study with 4% 4-MBC did not reveal any phototoxic effect.
A guinea pig study with 5% 4-MBC and a human study with 4% 4-MBC did not reveal any photosensitising potential.

(Photo-)mutagenicity

The bacterial mutation (Ames) test and the *in vitro* chromosomal aberration test were both negative. Their photomutagenicity-counterparts also delivered negative results.

Dermal absorption

5% of ^{14}C-labelled 4-MBC in an oil in water emulsion was applied at a dose of 1g over a shaved area of 200 cm² on the forearm of 6 volunteers. The amount of radioactivity measured in urine and faeces indicated a dermal absorption of about 1.9%. However, viewing the shortcomings in this and some other presented studies, a final conclusion on dermal absorption could not be drawn.

Teratogenicity

In a first gavage study with pregnant rabbits, it was concluded that arachis oil was not a suitable vehicle for such a type of study.
A second gavage study with pregnant rats used dosages of 0, 10, 30 and 100 mg/kg bw/day in arachis oil. In the highest dosage group, the dams displayed reduced body weight gain. Some retardation of ossification in foetuses was noted in the high and intermediate dosage groups.
There was no evidence of teratogenesis.

Additional tests

A number of human tests for the effect on thyroid and pituitary hormones following cutaneous application were described.
In the largest (double-blind) study, 24 volunteers (12 ♂ and 12 ♀) were dermally exposed twice per day to 5 grams of an oil in water formulation containing 6% of 4-MBC, for 14 days. No significant change in thyroid-related hormones was noted.

The thyroid volume was found to be reduced by 1.7% in the treated group and increased by 3.11% in the placebo group (also consisting of 12 ♂ and 12 ♀ volunteers). Although these findings were found to be statistically significant, the authors attribute them to the inaccuracy of the method used rather than to any substance-related effect.

Opinion of the SCCNFP of 21/01/1998

The Margin of Safety of 4-MBC was calculated taking into account the NOAEL value of 25 mg/kg bw/day of the subchronic acute toxicity study and the dermal absorption value of 1.9%.

Thus a MoS of 110 was obtained and the use of 4-MBC up to 4% in sunscreens was accepted.

3.1.2 Taken from opinion n° SCCNFP/0483/01, adopted on 12 June 2001

As a result of a publication by Schlumpf et al. [2001], in which the potential estrogenic effects of 5 UV_B filters (including 4-MBC) were studied, the SCCNFP was requested to reconsider the safety evaluation of the organic UV-filters under investigation by M. Schlumpf.
In SCCNFP/0483/01, the results of Schlumpf et al. are summarized and discussed, together with data obtained from industry.

The Committee concluded that, *based on the actual scientific knowledge, organic UV-filters used in cosmetic sunscreen products, allowed on the EU market today, have no estrogenic effects that could potentially affect human health.*

3.1.3 Taken from opinion n° SCCNFP/0779/04, adopted on 25 May 2004

Based upon newly provided industry data, the SCCNFP was requested to re-evaluate the safety of 4-MBC when used in cosmetic products.
In SCCNFP/0779/04, the toxicological profile as described in 3.1.1 was combined with the results of the following newly introduced studies:

Repeated dose oral toxicity - Beagle dog

- 14-day oral study in Beagle dogs with 20 (day 1), 100 (day 2), 500 (day 3 + days 5-14)) and 2500 (day 4) mg 4-MBC/kg bw/day.
  The authors concluded that there were no treatment related effects, although the T_3 and T_4 levels obtained after exposure were consistently higher than those before exposure. There also appeared to be a gradual increase in time. Therefore the conclusion of the study was considered to be questionable.

- 21-day oral study in Beagle dogs with 0 (day 1), 20 (day 4), 100 (day 8) and 500 (days 11-21) mg 4-MBC/kg bw/day.
  Again the conclusions of the test were questionable and again, T_3 and T_4 levels appeared to be slightly higher after exposure.

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.
- **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.
- **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.

Toxicokinetics (incl. Percutaneous absorption) - human studies

- Six healthy male volunteers were tested. The a.i. was supplied with 14C labelling in an o/w formulation at a concentration of 5%. About 1 gm was applied over a shaved area of 200 cm² 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./cm²
  1.9% would be equivalent to an absorption of 4.75 µg a.i./cm²

- 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./cm².

- 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./cm².

Reproduction toxicity - rat

The study was conducted with dosages of 0, 12.5, 25 and 50 mg 4-MBC/kg bw/day.

F_0 (parents, ♀) group:	-	T_3, TSH and prolactin levels slightly elevated at 50 mg/kg bw/day,
	-	prolactin levels slightly elevated at 25 mg/kg bw/day.
F_1 (offspring):	-	slightly reduced levels of T_3 at 50 mg/kg bw/day,
	-	lower FSH-levels at 25 and 50 mg/kg bw/day.

None of these findings were considered being relevant, either because of their statistical (in)significance (T_3, TSH and prolactin levels) or by their alleged relation to the onset of puberty instead of substance administration (FSH levels in offspring).

Opinion of the SCCNFP, 25/05/2004

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 T_3 or T_4, 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.

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:

1) *complete physico-chemical data;*
2) *a dermal penetration study according to current guidelines, including the study of the different factors affecting the quantitative outcome of the results;*
3) *a clear NOAEL obtained in a relevant species;*
4) *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.

3.2 NEWLY INTRODUCED DATA (DECEMBER 2005)

The current submission does not mention nor addresses the questions posed by the SCCP.

It consists of the following studies:

1) Acute dermal toxicity study in the Wistar rat with a 4-MBC containing sunscreen (400 & 2000 mg 4-MBC/kg bw, 3♂ & 3♀/dosage group).
2) Kinetics of 4-MBC in human volunteers after single dermal application of a 4-MBC containing sunscreen (22 mg 4-MBC/kg bw, 3♂ & 3♀).
3) Biotransformation and kinetics of 4-MBC in the Sprague Dawley rat after single oral application (25 & 250 mg 4-MBC/kg bw, 3♂ & 3♀/dosage group).
4) 90-Day dermal toxicity study in the Wistar rat (0, 100, 400 & 2000 mg 4-MBC/kg bw, 20♂ & 20♀/dosage group).
5) Overall risk assessment: "Toxicokinetic-based Margin of Safety for the use of 4-MBC in sunscreen formulations".

3.2.1 Acute toxicity in the rat after single dermal application of a 4-MBC containing sunscreen

Date of study:	April 2005
Guideline:	OECD 402, EC B.3 (92/69/EEC)
GLP statement:	no signed document available
QAU statement:	no signed document available
Species/strain:	Wistar rat
Group size:	3 ♂ and 3 ♀ in the dosage groups 1 ♂ and 1 ♀ in the control group
Observation period:	4 days
Test substance:	4-MBC (Eusolex® 6300)
Test formulation:	SW-06-03, SW-06-02 and SW-06-01, containing respectively 0%, 4% and 20% of 4-MBC (for full composition, see below)
Batch nr. Eusolex® 6300:	5200032
Purity of test substance:	99.9%
Dosage levels:	0, 400 and 2000 mg 4-MBC/kg bw (10 g formulation/kg bw)

10 g/kg bw of SW-06-02 or SW-06-01 (containing respectively 4% and 20% of 4-MBC) was applied on an area of 36 cm² of the shaved skin of 3 female and 3 male Wistar rats, thus generating dosages of 400 and 2000 mg 4-MBC/kg bw, respectively. 1 female and 1 male rat were treated under the same conditions with SW-06-03 (control). The formulations were kept in contact with the skin for 24h under occlusive patch. After removal of the patch, any remaining test material was carefully wiped off.
Blood samples from the dosed and control animals were taken 0.5, 1, 3, 6, 24, 48, 72 and 96h after the start of the exposure.

	SW-06-3	**SW-06-02**	**SW-06-01**
4-MBC	0.00%	4.00%	20.00%

C12-15 Alkyl Benzoate	28.00%	29.00%	26.00%
Caprylic/capric Triglyceride	28.00%	29.00%	26.00%
Microwax	16.00%	16.00%	15.00%
Dibutyl Adipate	16.00%	16.00%	11.00%
Butyrospermum Parkii (Shea Butter)	12.00%	6.00%	2.00%

Results

There were no deaths during the course of the study.

No signs of toxicity were detected in the rats after treatment with the vehicle or the test material.

No local irritations were observed.

In the plasma samples of the treatment groups, the parent compound as well as two major metabolites, i.e. 3-(4-carboxybenzylidene)-6-hydroxycamphor and 3-(4-carboxybenzylidene)-camphor, were identified. Their mean values and areas under the curve are displayed in Tables 1 and 2. In the control animals, neither 4-MBC nor its metabolites were detected.

Table 1: Mean plasma levels (n=3) in pmol/ml plasma
MET 1 = 3-(4-carboxybenzylidene)-6-hydroxycamphor
MET 2 = 3-(4-carboxybenzylidene)-camphor

Time (hrs)	400 mg/kg bw						2000 mg/kg bw					
	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M
0.5	15	34	0	0	73	45	60	101	0	0	139	137
1	43	68	0	0	185	156	170	230	2	29	467	589
3	125	133	0	33	766	715	502	595	13	126	3060	2764
6	213	219	7	166	1683	1852	1032	932	72	734	7820	6067
24	211	233	44	1111	8162	5197	1181	1050	322	5590	35070	21514
48	82	96	153	3540	18838	10880	504	1035	792	17900	51761	36444
72	32	35	53	740	11489	3535	217	366	699	16600	42641	37500
96	0	0	16	423	5345	1844	51	83	697	4013	54542	13901

Table 2: Area under the curve (n=3) in pmol/ml*h.
MET 1 = 3-(4-carboxybenzylidene)-6-hydroxycamphor
MET 2 = 3-(4-carboxybenzylidene)-camphor

Dosage	Area Under Curve (pmol/ml*h)					
	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M
400 mg/kg bw/day	10809	11961	5890	119540	2578975	493975
2000 mg/kg bw/day	55182	70625	50940	1046213	3813694	983860

Conclusion

LD_{50}-dermal-rat > 2000 mg/kg.

Based upon plasma measurements after dermal exposure to 4-MBC in the rat, it was concluded that the systemic exposures to the parent compound and its metabolites were dose-dependent.

3.2.2 Kinetics of 4-MBC in human volunteers after single dermal application of a 4-MBC containing sunscreen

Date of study:	Feb-Nov 2005
Guideline:	Internal protocol Institute of Toxicology
GLP statement:	no signed document available
QAU statement:	no signed document available
Species/strain:	healthy volunteers
Group size:	3 women and 3 men (20-34 years old)
Observation period:	4 days
Test substance:	4-MBC (Eusolex® 6300)
Test formulation:	"Standard sunscreen formulation", containing 4% of 4-MBC (full composition, see below
Batch nr. Eusolex® 6300:	5200032
Purity of test substance:	99.9%
Dosage levels:	20-25 mg 4-MBC/kg bw (2 mg formulation/cm² over 90% of the total body surface)

2 mg/cm² of a standard sunscreen formulation containing 4% 4-MBC (see below) was applied to 90% of the total body surface of 3 healthy female and 3 healthy male volunteers, thus resulting in a mean dermal dosage of 22 mg 4-MBC/kg bw. Subjects were permitted to take a shower 12h after application.

Blood and urine samples were collected in predetermined intervals:

- blood: 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 60, 72, 84, 96h after start of the treatment,
- urine: 0, 8, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88, 96h after start of the treatment.

4-MBC and its two major metabolites 3-(4-carboxybenzylidene)-6-hydroxycamphor and 3-(4-carboxybenzylidene)-camphor were measured at all these time points.
In the urine samples, the metabolites were determined with and without pre-incubation with glucuronidase in order to determine the extent to which they had been conjugated.

Standard Sunscreen Formulation

Aqua	73.25%
C12-15 Alkyl Benzoate	6.00%
Caprylic/capric Triglyceride	6.00%
Eusolex 6300 (**4-MBC**)	4.00%
Dibutyl Adipate	3.00%
Glyceryl Stearate & Ceteareth-15	2.00%
Stearyl Alcohol	2.00%
Microwax	1.00%
Butyrospermum Parkii (Shea Butter)	1.00%
Glycerin	1.00%
Dimethicone	0.50%
Xanthan Gum	0.25%

Results

Plasma measurements:

As displayed in Table 2, the plasma levels of 4-MBC reached a peak after 6 hours in all volunteers. The apparent half-life after the peak was stated to be 9h.

3-(4-carboxybenzylidene)-6-hydroxycamphor values reached a peak after 24h and displayed apparent half-lives after their peak of 20h for the women and 31h for the men. The highest 3-(4-carboxybenzylidene)-camphor concentrations were measured after 6 to 12 hours and the apparent half-lives after the peak were reported to be 23 to 26h.

Urine measurements:
4-MBC could not be detected in the urine of the treated volunteers. 3-(4-carboxybenzylidene)-6-hydroxycamphor was detected as such, while 3-(4-carboxybenzylidene)-camphor was present under the form of its glucuronide. Both metabolites peaked around 16h after the start of the treatment.

Table 3: Mean plasma and urine levels (n=3) in pmol/ml plasma and nmol/total urine volume, respectively.
MET 1 = 3-(4-carboxybenzylidene)-6-hydroxycamphor
MET 2 = 3-(4-carboxybenzylidene)-camphor

Time (hrs)	Mean **plasma** levels, n=3 (pmol/ml plasma)						Mean **urine** levels, n=3 (nmol/tot. urine volume)					
	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M
0	0	0	0	0	0	0			0	11	0	7
0.5	23	17	0	0	0	0						
1	14	60	0	0	15	5						
2	74	95	9	19	59	45						
4	98	161	30	13	121	109						
6	100	200	38	45	136	184						
8	97	186	37	58	124	206			1947	2197	523	687
12	87	152	47	70	129	211						
16									3352	5196	827	1395
24	12	51	47	83	65	111			2417	3387	733	1290
32									2271	4551	542	696
36	0	21	15	15	38	46						
40									1319	2115	242	259
48	0	0	9	17	26	35			1036	1096	292	334
56									946	1783	200	237
60	0	0	4	5	19	32						
64									675	1012	161	108
72	0	0	3	12	15	25			550	772	117	260
80									473	1100	51	114
84	0	0	3	9	12	20						
88									310	732	25	104
96	0	0	5	11	10	20			341	545	74	208

Table 4: Areas under the curve (n=3) of 4-MBC and metabolites in plasma and urine in pmol/ml*h and nmol*h, respectively.
MET 1 = 3-(4-carboxybenzylidene)-6-hydroxycamphor
MET 2 = 3-(4-carboxybenzylidene)-camphor

AUC **plasma**, n=3 (pmol/ml*h)	AUC **urine** n=3 (nmol*h)

4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M
1909	3884	1615	2657	3029	5782	-	-	123544	205203	25268	49479

Conclusion

3-(4-carboxybenzylidene)-6-hydroxycamphor, 3-(4-carboxybenzylidene)-camphor and their glucuronides were identified as the only significant metabolites of 4-MBC measured in blood and urine. Gender difference in peak levels of as well 4-MBC as its metabolites were noticed, but could not be explained by the performing laboratory, which only states that gender differences in blood levels have also been observed after dermal administration of other lipophilic compounds.

Considering the relatively low blood levels, the dermal absorption through human skin is considered to be very low (< 0.5% of dose).

The test report mentions that the molecular weights of the glucuronides of both 4-MBC metabolites are below 500 and thus below the threshold for biliary elimination in humans. Therefore the authors consider elimination of 4-MBC metabolites with the faeces as unlikely.

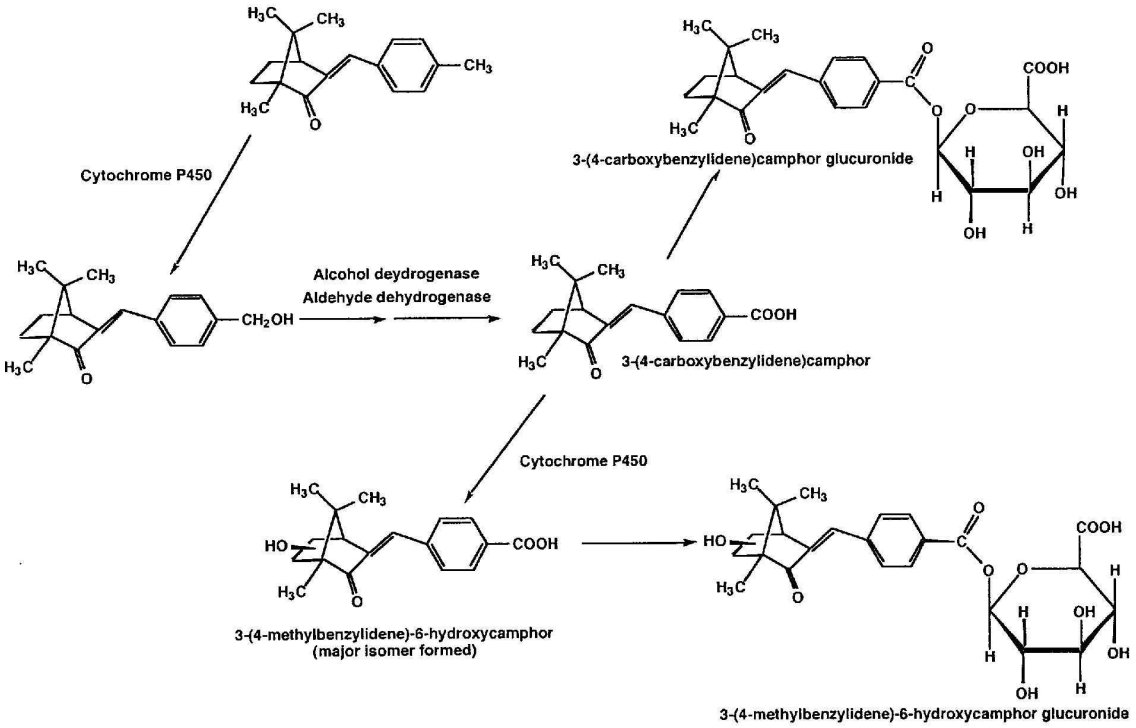


Fig. 1 Assumed metabolic pathway of 4-MBC *in vivo*

With regard to the metabolic pathway of 4-MBC, the study authors contend that the fact that 3-(4-carboxybenzylidene)-camphor is the major metabolic product found in blood, whereas its glucuronide and 3-(4-carboxybenzylidene)-6-hydroxycamphor are the major metabolites in urine, the following pathway can be assumed (see Figure 1):

- 3-(4-carboxybenzylidene)-camphor is initially formed from 4-MBC by a cytochrome P450 catalyzed oxidation of the aromatic methyl group, followed by further oxidation to the carboxylic acid.

- 3-(4-carboxybenzylidene)-camphor is subsequently either further oxidized by P450 to give 3-(4-carboxybenzylidene)-6-hydroxycamphor or conjugated with glucuronic acid.

3.2.3 Biotransformation and kinetics of 4-MBC after single oral administration to the rat

Date of study:	Feb-Nov 2005
Guideline:	Internal protocol Institute of Toxicology
GLP statement:	no signed document available
QAU statement:	no signed document available
Species/strain:	Sprague Dawley rat
Group size:	3 ♂ and 3 ♀ per dosage group
Observation period:	4 days
Test substance:	4-MBC
Test formulation:	4-MBC in corn oil
Batch nr. 4-MBC:	not stated
Purity of test substance:	> 95%
Dosage levels:	25 and 250 mg 4-MBC/kg bw

3 female and 3 male Sprague-Dawley rats were administered a single oral dosage of 25 or 250 mg/kg bw of 4-MBC in corn oil. Metabolites formed were characterized and the kinetics of the elimination from blood of 4-MBC and its metabolites and their excretion through urine, were determined.

Results

Analysis of the plasma and urine samples of the treatment groups confirmed the fact that 3-(4-carboxybenzylidene)-6-hydroxycamphor and 3-(4-carboxybenzylidene)-camphor were the two major metabolites after oral administration of 4-MBC to the rat. The results of LC-MS and NMR analysis support the proposal for the metabolic pathway of 4-MBC stated under 3.2.2 (sequential formation of 3-(4-carboxybenzylidene)-6-hydroxycamphor and 3-(4-carboxybenzylidene)-camphor glucuronide with 3-(4-carboxybenzylidene)-camphor as an intermediate).

4-MBC and its metabolites were measured in blood (mean values and areas under the curve in Tables 5 and 6) and in urine (mean values and areas under the curve in Tables 7 and 8) at regular time points.

Plasma measurements:

Table 5: Mean plasma levels (n=3) in pmol/ml plasma.
MET 1 = 3-(4-carboxybenzylidene)-6-hydroxycamphor
MET 2 = 3-(4-carboxybenzylidene)-camphor

Time (hrs)	25 mg/kg bw						250 mg/kg bw					
	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M
0	0	0	-	-	0	0	0	0	-	-	0	0
3	22	17	-	-	21597	13743	123	69	-	-	99296	80903
6	30	11	-	-	18764	12376	147	131	-	-	115873	88850
9	37	3	-	-	19278	10354	217	81	-	-	123418	86821
24	20	0	-	-	15727	0	173	108	-	-	73087	22140
48	8	0	-	-	4510	0	32	124	-	-	29862	0
96	8	0	-	-	0	0	4	26	-	-	1308	0

Table 6: Area under the curve (n=3) in pmol/ml*h.
MET 1 = 3-(4-carboxybenzylidene)-6-hydroxycamphor
MET 2 = 3-(4-carboxybenzylidene)-camphor

Dosage	Area Under Curve (pmol/ml*h)					
	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M
25 mg/kg bw/day	261	24	-	-	751360	207899
250 mg/kg bw/day	1886	1574	-	-	3518650	3519638

Blood concentrations of 4-MBC and 3-(4-carboxybenzylidene)-camphor showed to be dose-dependent and reached their peaks within 8 to 10h after administration. 3-(4-carboxybenzylidene)-camphor levels were much higher as compared to those of 4-MBC and declined following first-order kinetics with a half-life of about 15h after the peak.
The 3-(4-carboxybenzylidene)-6-hydroxycamphor levels failed to exceed the limit of detection in plasma.

Urine measurements:

Table 7: Mean urine levels (n=3) in nmol/total urine volume.
MET 1 = 3-(4-carboxybenzylidene)-6-hydroxycamphor
MET 2 = 3-(4-carboxybenzylidene)-camphor

Time (hrs)	25 mg/kg bw						250 mg/kg bw					
	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M
0			0	0	0	0			0	0	0	0
6									1035305	936345	128220	3455
14									398973	1398880	62727	34180
22									376867	892553	34970	16890
24			83493	87049	2964	0						
30									1111670	806153	33627	9000
38									694917	435790	142107	9247
46									335583	183947	54090	5393
48			18487	20880	2088	0						
54									206100	181440	19963	2985
62									225550	86120	31447	2000
70									56957	47407	26683	2910
72			11297	3475	213	0						
78									67123	34247	32167	2220
86									37863	23477	23450	0
94									22727	12697	17857	0
96			2103	1631	0	0						

In urine samples of the 4-MBC treated rats (both dosages), 3-(4-carboxybenzylidene)-6-hydroxycamphor showed to be the predominant excretory product. Peak concentrations were reached after 14h in male, whereupon the metabolite's level slowly declined. In female rats, the urinary elimination of 3-(4-carboxybenzylidene)-6-hydroxycamphor was slower and displayed a

plateau from 14 to approximately 38h after administration. The measured concentrations of 4-MBC-derived glucuronides in urine were low.

In the faeces of the 4-MBC treated rats, 4-MBC together with its two major metabolites were retrieved. However, due to some practical constraints, quantification of the individual levels was impossible. Urine and faeces recovery data are presented in Table 8.

Table 8: Mean (n=3) recovery data of 4 MBC and its metabolites in excreta after oral administration

	Female				**Male**			
	25 mg/kg bw		250 mg/kg bw		25 mg/kg bw		250 mg/kg bw	
	µmol	% of dose	µmol	% of dose	µmol	% of dose	µmol	% of dose
4-MBC applied	19	100	234	100	28	100	416	100
Urine	0.9	4.8	28	12	0.6	2.3	36	8.6
Faeces	n.d.	n.d.	103	44	n.d.	n.d.	216	52
Total			131	56			252	61

Conclusion
A dose-dependent increase in 4-MBC concentration in blood occurs in both male and female rats. 4-MBC appears to undergo extensive first-pass metabolism in rat liver resulting in very low blood levels of the parent compound.
The major pathway for the disposition of 3-(4-carboxybenzylidene)-6-hydroxycamphor and 3-(4-carboxybenzylidene)-camphor is contended to be represented by an efficient glucuronidation of both compounds. The corresponding glucuronides have molecular weights from 460 to 476 Dalton whereas the threshold for biliary elimination in rats is approximately 400 Da. Therefore they are assumed to be eliminated with bile and to undergo enterohepatic circulation. Enterohepatic circulation may explain the comparatively slow elimination of 4-MBC metabolites with urine.

3.2.4 90d dermal study in the rat

Date of study:	Jul - Nov 2005
Guideline:	OECD 411, Repeated dose toxicity under the Medicinal Products Directive
GLP statement:	no signed document available (draft report)
QAU statement:	no signed document available (draft report)
Species/strain:	Wistar rat
Group size:	19 ♀ and 19 ♂ rats in the intermediate dosage groups 24 ♀ and 24 ♂ rats in the control and high dosage group
Observation period:	17 weeks
Test substance:	4-MBC (Eusolex® 6300)
Test formulation:	SW-06-03, SW-06-04, SW-06-02 and SW-06-01, containing respectively 0%, 1%, 4% and 20% of 4-MBC (for full composition, see below)
Batch nr. Eusolex® 6300:	TT891085
Purity of test substance:	99.9%
Dosage levels:	0, 100, 400 and 2000 mg 4-MBC/kg bw/day (10 g sunscreen/kg bw/day)

10 g/kg bw of SW-06-03, SW-06-04, SW-06-02 or SW-06-01 (containing respectively 0%, 1%, 4% and 20% of 4-MBC, see below) was applied once daily on an area of 25 cm² of the shaved skin of 3 female and 3 male Wistar rats, thus generating dosages of 0, 100, 400 and 2000 mg 4-MBC/kg bw/day, respectively. The formulations were kept in contact with the skin for 6h under semi-occlusive patch. After removal of the patch, any remaining test material was carefully wiped off.

	SW-06-3	**SW-06-4**	**SW-06-02**	**SW-06-01**
4-MBC	0.00%	1.00%	4.00%	20.00%
C12-15 Alkyl Benzoate	28.00%	28.00%	29.00%	26.00%
Caprylic/capric Triglyceride	28.00%	28.00%	29.00%	26.00%
Microwax	16.00%	16.00%	16.00%	15.00%
Dibutyl Adipate	16.00%	15.00%	16.00%	11.00%
Butyrospermum Parkii (Shea Butter)	12.00%	12.00%	6.00%	2.00%

Clinical signs, irritation scores, food consumption, body weights, ophthalmology, haematology, clinical biochemistry, urinanalysis, hormone analysis, plasma analytics (toxicokinetics) and (histo)pathology were examined at pre-determined time points.

Results

Local toxicity signs:

Vehicle group:	9/48:	scabs
	17/48:	patchy erythema
	32/48:	general erythema
	28/48:	scaling
	8/24:	epidermal lesions (only in females)
100 mg/kg bw/day:	30/48:	very slight to well-defined general erythema
	23/38:	very slight to slight scaling
	11/38:	very slight patchy erythema
	10/38:	very slight scabs
	8/38:	very slight epidermal lesions
400 mg/kg bw/day:	36/37:	very slight to moderate/severe general erythema
	35/37:	very slight to slight scaling
	14/37:	very slight to well-defined patchy erythema
	11/37:	slight formation of scabs
	8/37:	epidermal lesions
	3/37:	well-defined general oedema
2000 mg/kg bw/day:	48/48:	very slight to moderate/severe general erythema
	43/48:	scaling
	38/48:	scabs
	41/48:	epidermal lesions
	17/48:	general oedema
	44/48:	wound formation
	2/48:	necrosis

Due to the severity of the local effects, the treatment at 2000 mg/kg bw/day was stopped prematurely after 15 days.

Systemic toxicity signs:

No clinical signs for general toxicity were observed in animals treated with the test item.

100 mg/kg bw/day:
- haematology: lower mean value for absolute basophile count, considered unrelated to the test substance;

- clinical biochemistry: higher creatinin and potassium levels in males and higher mean chloride values in females, all considered too small to be toxicologically relevant.

400 mg/kg bw/day:
- haematology: lower mean value for partial thromboplastin time in females, considered unrelated to the test substance;
- clinical biochemistry : higher creatinin and potassium levels and lower mean cholesterol values in males, lower mean values for glucose and bilirubin, higher ALAT activity and higher mean chloride values in females, all considered too small to be toxicologically relevant;
- hormone analysis : slightly increased total T_4 and TSH levels in males and slightly increased total T_3 levels in females, all considered to be incidental;
- pathology: slightly decreased heart to body weight ratio, slightly increased testes to brain weight ratio, the latter attributed to the slightly decreased mean brain weight of that group.

2000 mg/kg bw/day:
- significant decrease in body weight on test day 8,
- early sacrifice of the animals (on day 15) due to severe skin reactions.

Toxicokinetics:

4-MBC and its major metabolites 3-(4-carboxybenzylidene)-6-hydroxycamphor and 3-(4-carboxybenzylidene)-camphor, were measured in the rats' plasma on day 1 and on day 90/91. The results are displayed in Tables 9-13.

Table 9: Mean plasma levels (n=9) in pmol/ml plasma, **control group**.
MET 1 = 3-(4-carboxybenzylidene)-6-hydroxycamphor
MET 2 = 3-(4-carboxybenzylidene)-camphor

Time (hrs)	0 mg/kg bw, **day 1**						0 mg/kg bw, **day 90/91**					
	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M
0,5	81	9	89	71	188	151	129	280	189	151	359	3662
1	0	0	88	48	92	129	0	0	194	100	8	6
2	1	48	355	382	409	552	0	0	186	0	217	77
4	23	50	238	173	350	231	0	0	177	0	202	80
8	44	95	123	33	201	35	13	37	288	147	676	2
24	0	9	81	749	2123	6746	0	0	160	104	342	2

Table 10: Mean plasma levels (n=9) in pmol/ml plasma, **100 mg/kg bw/day**.
MET 1 = 3-(4-carboxybenzylidene)-6-hydroxycamphor
MET 2 = 3-(4-carboxybenzylidene)-camphor

Time (hrs)	100 mg/kg bw, **day 1**						100 mg/kg bw, **day 90/91**					
	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M
0,5	55	245	159	89	240	257	385	290	376	1977	55493	14049
1	29	34	94	116	284	246	351	503	386	3050	49472	13827
2	45	0	292	458	870	767	97	87	419	1249	40282	10299
4	259	160	230	286	1237	899	235	125	427	1762	50775	12254
8	877	1173	292	1123	14236	6303	297	217	401	2400	48521	9585

24	17	13	233	556	21338	8342	65	31	519	1423	38873	13176

Table 11: Mean plasma levels (n=9) in pmol/ml plasma, **400 mg/kg bw/day**.
MET 1 = 3-(4-carboxybenzylidene)-6-hydroxycamphor
MET 2 = 3-(4-carboxybenzylidene)-camphor

Time (hrs)	400 mg/kg bw, **day 1**						400 mg/kg bw, **day 90/91**					
	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M
0,5	503	285	115	72	230	221	372	655	780	6295	113732	49595
1	676	81	124	92	653	396	2755	2210	1010	5813	133451	43521
2	185	89	243	218	1581	854	684	1288	761	5997	102113	43803
4	806	172	309	198	3507	1775	738	463	925	4230	116197	31532
8	2233	743	293	338	26092	7806	1962	2170	885	3467	96479	30211
24	114	153	273	1953	18627	14482	194	193	1175	7907	123063	52746

Table 12: Mean plasma levels (n=9) in pmol/ml plasma, **2000 mg/kg bw/day**.
MET 1 = 3-(4-carboxybenzylidene)-6-hydroxycamphor
MET 2 = 3-(4-carboxybenzylidene)-camphor

Time (hrs)	2000 mg/kg bw, **day 1**						2000 mg/kg bw, **day 90/91**					
	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M	4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M
0,5	1356	1581	122	110	350	335	NO DATA					
1	3853	975	121	233	1337	1316						
2	2099	444	823	383	9423	3011						
4	1816	965	404	359	15542	6303						
8	7894	8409	611	1737	44437	27324						
24	1413	989	1560	4333	99437	39648						

Table 13: Areas under the curve (n=3) in pmol/ml*h.
MET 1 = 3-(4-carboxybenzylidene)-6-hydroxycamphor
MET 2 = 3-(4-carboxybenzylidene)-camphor

Dosage	Time point	Area Under Curve (pmol/ml*h)					
		4-MBC F	4-MBC M	MET 1 F	MET 1 M	MET 2 F	MET 2 M
100 mg/kg bw/day	Day 1	10741	11615	6884	18232	339778	145792
	Day 90/91	4635	2645	10652	45882	1085003	270198
400 mg/kg bw/day	Day 1	34928	16666	7680	21233	502911	210936
	Day 90/91	29535	31458	23270	110104	2520350	1007126
2000 mg/kg bw/day	Day 1	124455	124099	21627	53533	2573155	671095
	Day 90/91	NO DATA					

Conclusion
The test laboratory concludes that daily dermal administration for 90 days of up to 2000 mg 4-MBC/kg bw/day did not cause any clinical signs of toxicological relevance.
In the highest dosage group, severe local effects on the skin (erythema, scabs, scaling and epidermal lesions, …) led to a premature sacrifice of all animals of the high dosage group.
The authors also state that gross necropsy, microscopy, clinical biochemistry and haematology (with special attention for thyroid hormone levels) did not reveal any test substance related abnormalities.
As far as toxicokinetics are concerned, the test item is reported to be absorbed in a dose-dependent manner and metabolized into its two major metabolites after dermal application.
Based upon the test results, the authors have established the following values:

- dermal NOAEL = 400 mg 4-MBC/kg bw/day
- dermal NOEL = 100 mg 4-MBC/kg bw/day

3.2.5 Toxicokinetic-based Margin of Safety for the use of 4-MBC in sunscreen formulations

In this document, the following argumentation is taken into account by the authors:

- After oral administration of 4-MBC to the rat, 3-(4-carboxybenzylidene)-camphor was identified as the major metabolite in the plasma. 4-MBC was found at very low level and the 3-(4-carboxybenzylidene)-6-hydroxycamphor concentration fell below its limit of detection. After dermal application of 4-MBC to the rat, the same metabolite (3-(4-carboxybenzylidene)-camphor) was predominantly retrieved in plasma, while 4-MBC was detected at a slightly higher level than after oral administration. However, the major difference was the plasma 3-(4-carboxybenzylidene)-6-hydroxycamphor level, which was significantly present after dermal administration compared to not being detectable after oral intake.
  It remained, however, at all times lower than the 3-(4-carboxybenzylidene)-camphor level. In humans, 4-MBC is predominantly present in the plasma after dermal administration,

followed by 3-(4-carboxybenzylidene)-camphor and 3-(4-carboxybenzylidene)-6-hydroxycamphor, respectively.
Out of these findings, the authors conclude that dermal administration to rats is a better model to mimic the human situation after dermal application than the oral administration.

- Based upon the available data, the authors consider the metabolic behaviour and the kinetics of 4-MBC after dermal administration similar between men and rats. They also state that rats are far more susceptible to thyroid perturbation than men, wherefore they find that it is justified to reduce the safety factor from 100 to 10.
- At a dermal dosage level of 100 mg/kg bw/day, 18-110 fold higher AUC values were seen for 4-MBC in rats in comparison with the 25 mg/kg bw/day oral dosage which was considered by the SCCP to represent a LOAEL value. Therefore, the company experts do not consider the parent compound to be responsible for the thyroidal effects; otherwise they would have been obvious at 100 mg/kg bw/day in the dermal study. The authors contend that the same goes for humans, i.e. that one of the metabolites and not the parent compound, is responsible for any possible thyroidal effect.

Based upon the above, the authors calculate the MoS for the use of 4-MBC in a sunscreen and declare it to be safe.

3.3 DISCUSSION

The majority of the questions raised by the experts in the SCC(NF)P opinion of May 2004, have not been addressed in this submission. Neither the *in vitro* dermal absorption study nor the exposure data on other uses (cosmetic and non-cosmetic) and on oral intake when used in e.g. lip products were provided.

The current submission consists of three rat studies and one human study, accompanied by an "Overall Risk Assessment" report.

In the submitted "Overall Risk Assessment" report, the authors propose a toxicokinetic based calculation of the MoS reducing the interspecies safety factor from 10 to 1 for the following reasons:

- as well 4-MBC's metabolism as its kinetic behaviour are considered very similar between rats and humans
- with regard to toxicodynamics, the authors find it well established that the rat is more susceptible to thyroid perturbations than humans.

The SCCP is of the opinion that the adaptation of the MoS, as proposed by the study authors, cannot be accepted for the following reasons:

- Out of the results of the three rat studies and the human study, it can be concluded that 4-MBC's biotransformation qualitatively appears to be quite similar between man and rat, the major metabolites being 3-(4-carboxybenzylidene)-6-hydroxycamphor and 3-(4-carboxybenzylidene)-camphor in both species. Quantitatively, however, there is a difference. The toxicokinetics of 4-MBC and its major metabolites have been shown to depend not only on the tested species, but also on the chosen exposure route and on the applied dosage.

- The submitted studies are all performed under different conditions with different dosage levels and forms (see below) of 4-MBC.

Species	Test formulation	Dose regime	Exposure route	Dosage level(s)
Wistar rat	Sunscreen "SW"	single dose	dermal	0, 400, 2000 mg/kg bw
Human volunteers	"Standard" sunscreen	single dose	dermal	22 mg/kg bw
Sprague Dawley rat	4-MBC in corn oil	single dose	oral	25, 250 mg/kg bw
Wistar rat	Sunscreen "SW"	repeated dose daily for 90 days	dermal	0, 100, 400, 2000 mg/kg bw/day

- As described by Renwick and Lazarus [1998], the interspecies factor of 10 can be subdivided in a factor 4.0 for toxicokinetics and a factor 2.5 for toxicodynamics.
  The toxicokinetic factor takes into account individual differences between the external and the internal dose and thus reflects the chronic blood concentration or the body burden of the substance under study. In order to set this factor to 1, plasma levels are measured in rats after repeated administration of the substance at its NOAEL and are compared with plasma levels measured in humans after repeated administration of the compound under in-use conditions.
  More specifically, these plasma concentrations are plotted as a function of time and in case the obtained areas under the curve show to be significantly lower in humans than in the rat, the factor 4.0 becomes 1 and a MoS of 25 might be considered.
  Unfortunately the current submission lacks the plasma levels for the repeated administration of 25 mg 4-MBC/kg/day (proposed NOAEL-value) in the rat.
  The presented 90 day dermal toxicity study with the rat results in a NOAEL value of 400 mg/kg/day. However, among the effects noted at that dosage level, perturbations of the thyroid system are included. Since the latter belong to the major concerns with regard to the toxicological profile of 4-MBC, it is preferable to use the NOEL value of 100 mg/kg/day for further calculations.
  Although the plasma concentrations for a NOEL of 100 mg 4-MBC/kg/day are available in the 90d dermal study in the rat, the corresponding plasma levels in man after repeated dermal administration are not available, which makes relevant comparison impossible. Human data were introduced earlier in the submission that was leading to opinion XXIV/1377/96 on 21 January 1998. But although 24 volunteers were exposed dermally for 14 days (twice per day, 5g formulations containing 6% of 4-MBC), no plasma levels were provided either.
  Moreover, in rat studies with higher daily dosages, 4-MBC and/or its metabolites were still significantly present after 24h, which makes the use of the areas under the curve quite difficult (for comparison of AUC's between rat and man, all levels should be near zero after 24h). This was also the case in the single dose dermal studies.
  The toxicodynamic factor cannot be reduced from 2.5 to 1 based upon the submitted data either, although it is acknowledged that rats are more susceptible to thyroid perturbation than man. Unfortunately, thyroid hormone-related measurements were not included in the human dermal study. They could have given relevant information.
  There are no robust data available supporting the hypothesis that 4-MBC or one of its metabolites would be the active compound with regard to human toxicity.
  Therefore more elaborated mechanistic data involving the use of pure metabolites would be required to help clarifying the toxicodynamic issue.

Finally, it should be noted that none of the currently presented toxicokinetic studies were specifically requested by the SCC(NF)P and have been introduced by industry on a voluntary basis. They clearly show that in man as well as in rat there is systemic exposure to 4-MBC after

dermal exposure and that 4-MBC and its metabolites are present in human plasma after single dermal application of 2 mg/cm² of a sunscreen formulation on 90% of the total body surface. Considering the fact that for a sunscreen multiple applications per day are not uncommon, the plasma concentrations might even be higher under real in-use conditions. Moreover, additional exposure from other sources, e.g. lipstick, has not been taken into consideration.
The kinetic studies on which this opinion is based, have recently been published [Schauer et al. 2006, Völkel et al. 2006]. Note that no new elements than those present in the submission of December 2005, are present.

4. CONCLUSION

The SCCP is of the opinion that presently the safe use of a maximum concentration of 4% 4-MBC in sunscreens cannot be established.

5. MINORITY OPINION

Not applicable

6. REFERENCES

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