Scientific Committee on Consumer Products

SCCP

OPINION ON

4-Methylbenzylidene camphor (4-MBC)

COLIPA n° S60

The SCCP adopted this opinion at its 16th plenary of 24 June 2008

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

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

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

SCCP

Questions concerning the safety of consumer products (non-food products intended for the consumer).
In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

Scientific Committee members

Claire Chambers, Gisela Degen, Ruta Dubakiene, Bozena Jazwiec-Kanyion, Vassilios Kapoulas, Jean Krutmann, Carola Lidén, Jean-Paul Marty, Thomas Platzek, Suresh Chandra Rastogi, Jean Revuz, Vera Rogiers, Tore Sanner, Günter Speit, Jacqueline Van Engelen, Ian R. White

Contact

European Commission
Health & Consumer Protection DG
Directorate C: Public Health and Risk Assessment
Unit C7 - Risk Assessment
Office: B232 B-1049 Brussels
Sanco-Sc6-Secretariat@ec.europa.eu

(ISSN)

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

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

Invited toxicokinetic expert:

Prof. Dr. Hermann M. Bolt

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TABLE OF CONTENTS

1. BACKGROUND

4-Methylbenzylidene Camphor ("4-MBC") is listed in the Cosmetics Directive 76/768/EEC as UV-filter which cosmetic products may contain (ref. no. 18 in Annex VII Cosmetics Directive 76/768/EEC).

Safety-concerns concerning 4-MBC have been voiced back in 2000. In response to that, the Scientific Committee for Consumer Products ("SCCP") issued an opinion on 12 June 2001 stating that "*based on the actual scientific knowledge, the SCCNFP is of the opinion that the organic UV-filters used in cosmetic sunscreen products, allowed in the EU market today, have no estrogenic effects that could potentially affect human health*" (SCCNFP/0483/01).

However, the SCCP, on 25 May 2004 issued an opinion (SCCNFP/0779/04) stating that: "*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.*"

In this opinion, the SCCP also stated that the additional data should be submitted "*as a matter of urgency*".

Industry submitted additional data in May 2004. However, as these data did not cover all questions posed by the SCCP, it was decided not to evaluate once more an incomplete dossier.

In February 2005, COLIPA informed the Commission that it intended to deliver the requested data in December 2005. In response to that, the Commission set a new deadline of 31 December 2005.

After assessing these data, the SCCP issued an opinion that presently the safe use of a maximum concentration of 4% 4-MBC in sunscreens cannot be established (SCCP/1042/06, 10 October 2006).

Following this opinion, some additional data was submitted by a company. Moreover, the Commission's attention was drawn to the fact that some data had not been forwarded to the SCCP and therefore not been considered in the previous safety evaluations.

2. TERMS OF REFERENCE

Does the SCCP, on the basis of the additional data submitted to it after its opinion of 10 October 2006 (SCCP/1042/06), maintain its view that the safe use of a maximum concentration of 4% 4-MBC in sunscreens cannot be established?

3. OPINION

3.1 Background

3.1.1. Previous reports related to 4-MBC

SCCNFP/0483/01: Opinion on the evaluation of potentially estrogenic effects of UV filters. Conclusion: "Organic UV filters used in cosmetic sunscreen products, allowed in the EU market today, have no estrogenic effects that could potentially affect human health."

SCCNFP/0779/04: Upon request of the Danish authorities the dossier for 4-MBC was re-opened and the subsequent data delivery by industry (Feb 2004) led to the calculation of a MoS of 110. The provided information was considered incomplete and the following was urgently required:

- 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.

SCCP/1042/06: In December 2005, industry provided some additional data without specifically answering the questions posed by the SCCNFP in 2004. Acute and subchronic toxicity of 4-MBC were assessed in the rat, while the kinetics of the compound and its two major metabolites were analyzed in human volunteers after single dermal application of a sunscreen and in the rat after single oral and repeated dermal application of 4-MBC containing sunscreens. The results of all those studies led to a proposed "Toxicokinetic-based MoS for the use of 4-MBC in sunscreen formulations". The SCCP pointed out that the questions posed in 2004 had not been answered. The newly calculated MoS was commented, though not accepted.

3.1.2 General toxicological profile of 4-MBC (taken from SCCNFP/0779/04 and SCCP/1042/06)

Acute toxicity
4-MBC displays low acute toxicity, with oral and dermal LD_{50} values of more than 2000 mg/kg measured in several species.

Skin and eye irritation
No irritative effects were reported after skin or eye contact with 4-MBC.

Skin sensitisation
Neither in guinea pigs, nor in human subjects, sensitisation effects were noted when 4-MBC was applied at concentrations of 3% and 5%, respectively.

Repeated dose toxicity
In oral 28 day and 90 day studies, 4 -MBC was administered daily to rats at dosage levels ranging from 25 to 312 mg/kg bw/day. The effects noted were mainly situated at the level of the thyroid axis, with deviations of normal thyroxine (T_4), triiodothyronine (T_3) and/or thyroid-stimulating hormone (TSH) levels, thyroid gland weight, etc.
The oral NOAEL (90d - rat) based upon thyroid effects showed to be 25 mg/kg bw/day.

When dermally applied to the rat skin for 90 days at reported dosage levels of 0, 100, 400 and 2000 mg/kg bw/day, some slight thyroid effects were observed at 400 mg/kg bw/day, while the animals of the high dosage group had to be sacrificed due to the severity of the local effects (epidermal lesions, wounds, necrosis, ...) they experienced.
The authors considered 400 mg/kg bw/day as the dermal NOAEL of 4-MBC and 100 mg/kg bw/day as its dermal NOEL.

Mutagenicity
The bacterial mutation (Ames) test and the *in vitro* chromosomal aberration test were both negative.

Photo-induced toxicity
The phototoxicity of 4-MBC was assessed in mice and humans and showed to be negative, while studies in guinea pig and human volunteers revealed the compound to be non-photosensitising at 4%.
In vitro photomutagenicity studies (Ames test and chromosomal aberration test) with 4-MBC were negative.

Reproductive toxicity
A teratogenicity study revealed a NOAEL value for developmental effects of 10 mg/kg bw/day, based upon the observation of some retardation of ossification at 30 mg/kg bw/day. There was no evidence of teratogenesis.
When tested in a one-generation reproduction toxicity study, 4-MBC displayed some minor thyroid effects at the highest dosage levels tested (25 and 50 mg/kg bw/day), though not at the lowest one (12.5 mg/kg bw/day). The study authors did not consider any of the observed effects relevant.

Dermal absorption
When applied at 5% in an oil-in-water emulsion on the forearm of 6 volunteers, 4-MBC displayed a dermal absorption value of 1.9%. However, due to the many shortcomings in the presented study, a final conclusion on the dermal absorption of 4-MBC could not be drawn.

Toxicokinetics
As described in detail in SCCP/1042/06, a number of toxicokinetic and metabolism studies have been performed in rats and on human volunteers. The concentrations of 4-MBC and its two major metabolites (3-(4-carboxybenzylidene)-6-hydroxycamphor and 3-(4-carboxybenzylidene)-camphor) were measured in plasma and/or urine after dermal or oral administration of 4-MBC, either dissolved in corn oil or incorporated in a sunscreen formulation.
Since the toxicokinetics of 4-MBC form the major subject of this opinion, they will be further discussed in detail.

3.2 Overview of requested information and newly provided information

The following table summarizes the questions asked by the SCCNFP in 2004, together with the information provided by industry in December 2005 (discussed in SCCP/1042/06) and the most recently provided information (March 2007-July).

Information requested May 2004	Information received Dec 2005 New data provided March 2007
1) Complete physico-chemical data set	Physico-chemical data set
2) Dermal absorption study according to the Notes, including the study of the different factors affecting the quantitative outcome of the results	Summary report of 8 dermal absorption studies
3) Clear NOAEL obtained in relevant species	90d dermal study with 4-MBC in the rat

Information requested May 2004	Information received Dec 2005 New data provided March 2007
4) Exposure data on other uses (cosmetic and non-cosmetic) and on oral intake when used in e.g. lip products	Statement that 4-MBC is only used in cosmetics and not in lip care products
	Acute dermal study with the rat (4-MBC containing sunscreen)
	Kinetics in human volunteers (4-MBC containing sunscreen)
	Biotransformation and kinetics in the rat (4-MBC)
	Proposal for a toxicokinetic-based calculation of the MoS
	Argumentation in favour of previously proposed toxicokinetic-based calculation of MoS
	Additional study in human volunteers (4 day application of 4-MBC containing sunscreen), including full raw data package (sent separately)
	Additional literature on 4-MBC and its estrogenic effects.

The main topic of discussion currently is the applicability of a toxicokinetic-based calculation of the MoS, as proposed by industry. Therefore, every piece of newly introduced information will be discussed under its corresponding heading of potential paradigm for the calculation of the MoS, i.e. the "conservative approach" and the "toxicokinetic-based approach".

3.3 The "Conservative" MoS Calculation Approach

3.3.1 Request of the SCCNFP (SCCNFP/0779/04)

In 2004, the SCCNFP requested the following additional information for the calculation of the MoS of 4-MBC:

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

3.3.2 Newly introduced data (March 2007)

Statement on the exposure pattern of 4-MBC

The applicant states that there is no interest to keep 4-MBC for use in lip care products.
Like almost all UV Filters, 4-MBC is also used as UV filter for product protection. Typical use concentrations in that case are reported be 0.5 % or lower.

In addition, 4-MBC may be used to provide UV protection in daily skincare products (skin cream/lotions, anti aging...) which use UV protection as product feature (claimed or not claimed). The applicant mentions that concentrations for this use range from very low to the allowed maximum of 4%. He adds that it needs to be considered that the risk assessment for sunscreens already is based on a 18g daily exposure (not limited to seasonal time spans). Therefore the applicant argues that this approach covers the possibility of daycare products (approx 17 grams per day) containing 4-MBC. He points out that sunscreens and daycare products are in general not used concomitantly but usually in an either/ or fashion, wherefore aggregate exposure would not be reflective of real life.

Dermal absorption study

Guideline:	COLIPA Guideline for Percutaneous Absorption/Penetration (1995) Draft OECD TG 428: Percutaneous Absorption: *in vitro* Method (1994)
Date of test:	May 2004
Test system:	Pig skin
N° of samples:	24 (8 experiments, each in triplicate)
Test substance:	Cosmetic sunscreen formulation (composition stated), containing 4% 4-Methylbenzylidene Camphor
Batch:	Not stated (summary of eight experiments)
Purity:	Not stated (summary of eight experiments)
Applied amount:	Not mentioned (calculated to be ± 4.5 mg formulation/cm²)
Exposure time:	24 hours
GLP/QAU:	No signed documents available

Data from eight experiments (performed 1997-98) are summarized in this report. A typical cosmetic sunscreen formulation, containing 4% 4-MBC, was used.
The experiments are carried out with especially prepared, unboiled back skin of selected female pigs. The experiments are carried out in penetration cells of glass kept at 32°C with a thermostat. Skin discs (gently dry-shaved surface, thickness about 3-4mm, diameter 5 cm) are used.

The receptor fluid, which is suitable to solute hydrophilic and lipophilic tests samples, is composed as follows: 0.9% sodium chloride, 1% bovine serum albumin and 0.02% gentamycine sulphate in water. The analytical samples are prepared a follows:

- skin surface: gentle scraping with a spatula and/or threefold wiping with cotton wool ⇒ non-absorbed sample
- horny layer: about 15-20 fold stripping with self-adhesive "tesa-film" tape
- epidermis: heating of the skin disc (epidermal side for 45 seconds on a 80°C hot plate (Ceram), separation epidermis ⇔ dermis with forceps)
- dermis: 3 punched samples or total dermis
- receptor fluid: 2 aliquots

Analytical determination of the test substance was carried out with HPLC-DAD with established lab procedures. The limit of detection was approximately 0.03 µg/ml. The limit of quantification was approx. 0.1µg/ml test sample.

Results

Internal test #	Applied substance	Surface	Horny layer	Epidermis	Dermis	Recovery
137	182 µg/cm²	125 µg/cm²	38.2 µg/cm²	2.0 µg/cm²	0.34 µg/cm²	90.9 %
141	200 µg/cm²	159 µg/cm²	26.2 µg/cm²	1.3 µg/cm²	0.50 µg/cm²	93.8 %
146	172 µg/cm²	132 µg/cm²	27.6 µg/cm²	2.4 µg/cm²	0.72 µg/cm²	94.7 %
150	175 µg/cm²	119 µg/cm²	31.0 µg/cm²	1.7 µg/cm²	0.90 µg/cm²	87.2 %
151	171 µg/cm²	138 µg/cm²	23.5 µg/cm²	0.7 µg/cm²	0.34 µg/cm²	95.0 %
152	167 µg/cm²	132 µg/cm²	16.2 µg/cm²	1.4 µg/cm²	0.88 µg/cm²	90.0 %
153	187 µg/cm²	144 µg/cm²	17.1 µg/cm²	0.4 µg/cm²	not det.	86.8 %
191	173 µg/cm²	140 µg/cm²	23.5 µg/cm²	2.2 µg/cm²	not det.	95.6 %
Mean	**178 µg/cm²**	**136 µg/cm²**	**25.4 µg/cm²**	**1.5 µg/cm²**	**0.46 µg/cm²**	**91.8 %**
St. dev.	11 µg/cm²	12.5 µg/cm²	7.2 µg/cm²	0.7 µg/cm²	0.36 µg/cm²	3.5 %

No 4-MBC was detected in the receptor fluid.

Ref.: Diembeck et al. 2004

Comments on the dermal absorption study

- Since only a summary of eight studies is provided, the test descriptions are insufficient.
- The solubility of the test substance in the receptor fluid is declared to be sufficient, though no data have been provided to support this.
- For the lower values, the standard deviations are very high.
- Taking into account the values obtained in eight separate experiments, the mean dermal absorption value of 1.96 µg/cm² will be used for further calculations.

3.3.3 Conservative calculation of the MoS and discussion

Before anything else, it must be pointed out that in section 3.1.2 under ' *Reproductive toxicity*', a developmental toxicity study is reported which results in a NOAEL of 10 mg/kg bw/day. Study of the full developmental toxicity test report (1988), however, reveals that the effects on which the above-mentioned NOAEL is based, are not clearly related to the test substance and the data obtained are not statistically significant. A 1-generation reproduction toxicity study with the rat (also discussed in earlier SCC(NF)P opinions) points towards a reproduction toxicity NOAEL value of 50 mg/kg/day.

Therefore, it is the opinion of the SCCP that the NOAEL value of 25 mg/kg/day of the 90-day oral toxicity study in the rat is the appropriate value to be used in the calculation of the MoS.

With regard to the dermal absorption value used in the calculation, the sum of the amounts measured in dermis and epidermis was used (1.50 µg/cm² + 0.46 µg/cm²).

Calculation of the MoS

Dermal absorption:		1.96 µg/cm²
Skin surface area (whole body, 1.8 m²):		18,000 cm²
Typical human body weight:		60 kg
No observed effect level NOAEL (90d-oral-rat):		25 mg/kg bw/day
Systemic exposure dose (SED)	((1.96.10^{-3} x 18,000) / 60)	0.588 mg/kg bw/day
MoS	**NOAEL / SED**	**42.5**

Discussion

- The MoS is below 100, indicating that 4-MBC cannot be declared safe as a UV filter in cosmetic sunscreen products at 4%.

- Seidlová-Wuttke et al. (2006a) argue that the majority of sunscreens are designed to reduce the dermal absorption of the UV filters. If that really is the case for modern sun products, this needs to be supported by appropriate dermal absorption studies, as already requested by the SCCNFP in 2004 ("*In vitro* dermal absorption study..., *including the study of the different factors affecting the quantitative outcome of the results*").

3.4 The Toxicokinetic-based MoS

3.4.1 The approach and studies presented by industry in 2005 and 2007

In the previous submission of December 2005, industry put forward a toxicokinetic-based calculation of the MoS. Herein the interspecies factor was proposed to be reduced from 10 (subdivided in 2.5 for toxicodynamics and 4 for toxicokinetics) to 1, since 4-MBC's metabolism and toxicokinetics were considered to be very similar between rats and humans. Moreover, it was stated to be well established that the rat is more susceptible to thyroid perturbations than humans.
The following dermal studies were presented to substantiate that approach:

Human volunteer study, single dermal application

Application: 2mg/cm² of "standard sunscreen formulation" (4% 4-MBC) applied on 90% of the body surface.
Dosage level(s): ± 22 mg 4-MBC/kg bw

Time (hrs)	Mean plasma levels (pmol/ml plasma)					
	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
0.5	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
12	87	152	47	70	129	211
24	12	51	47	83	65	111
36	0	21	15	15	38	46
48	0	0	9	17	26	35
60	0	0	4	5	19	32
72	0	0	3	12	15	25
84	0	0	3	9	12	20
96	0	0	5	11	10	20

Note: MET 1 = 3-(4-carboxybenzylidene)-6-hydroxycamphor
MET 2 = 3-(4-carboxybenzylidene)-camphor

Rat study, single dermal application

Application: 10g of "SW-06-02 or SW-06-01 sunscreen formulation" (4% or 20% 4-MBC) per kg body weight (area of 36 cm² treated, thus ± 70 mg sunscreen/cm² applied).
Dosage level(s): 400, 2000 mg 4-MBC/kg bw

Time (hrs)	Mean plasma levels (pmol/ml plasma)											
	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

Note: MET 1 = 3-(4-carboxybenzylidene)-6-hydroxycamphor
MET 2 = 3-(4-carboxybenzylidene)-camphor

Rat study, repeated dermal application for 90 days

Application: 10g of "SW-06-04, SW-06-02 or SW-06-01 sunscreen formulation" (1%, 4% or 20% 4-MBC) per kg body weight (area of 25 cm² treated, thus ± 100 mg formulation/cm² applied).

Dosage level(s): 100, 400, 2000 mg 4-MBC/kg bw/day

Time (hrs)	Mean plasma levels (pmol/ml plasma)											
	100 mg/kg bw/day, day 1						**100 mg/kg bw/day, 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

Time (hrs)	Mean plasma levels (pmol/ml plasma)											
	400 mg/kg bw/day, day 1						**400 mg/kg bw/day, 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

Time (hrs)	Mean plasma levels (pmol/ml plasma)											
	2000 mg/kg bw/day, day 1						**2000 mg/kg bw/day, 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					

Time (hrs)	Mean plasma levels (pmol/ml plasma)											
	2000 mg/kg bw/day, day 1						**2000 mg/kg bw/day, 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
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						

Note: MET 1 = 3-(4-carboxybenzylidene)-6-hydroxycamphor
MET 2 = 3-(4-carboxybenzylidene)-camphor

Human volunteer study, repeated dermal application for 4 days

Application: 2mg/cm² of "basic cream" + 10% Ethylhexylmethoxycinnamate, 10% Benzophenone-3 and 10% 4-MBC, applied on whole body surface.
Dosage level(s): ± 60 mg 4-MBC/kg bw

Measurements of:

- serum inhibin B, follicle stimulating hormone (FSH), luteinizing hormone (LH), sex hormone binding globulin (SHBG), testosterone and estradiol;
- serum free thyroxine (FT_4), free triiodothyronine (FT_3), thyroxine (T_4), triiodothyronine (T_3), thyroid-stimulating hormone (TSH) and thyroid-binding globulin (TBG);
- 4-MBC plasma concentrations.

Results
The study authors conclude that, with regard to serum hormone levels, no to minor biologically significant effects were observed.
The C_{max} for 4-MBC is reported to be reached in the plasma after 3-4h and amounts to 20 ng/ml (78.6 pmol 4-MBC/ml plasma). The study authors mention that in both sexes the 4-MBC plasma concentrations were significantly higher after 96h than after 24h, indicating accumulation after repeated daily applications.

Ref.: Janjua 2004, Janjua 2007

Letter of consulted expert in favour of the toxicokinetic approach

In a letter addressed to the SCCP experts, a consulted expert declares his support for the toxicokinetic approach with the following arguments:

- The toxicokinetic approach is far more reliable than a risk assessment based upon oral toxicity and *in vitro* dermal absorption data, which also use a single and not a repeated administration schedule. In addition, using the toxicokinetic approach avoids the uncertainty of possible species differences in skin penetration rates.
- Although it is acknowledged that kinetic data from a repeated dose human study would be preferable, repeated dose kinetic data after topical application are highly unlikely to be fundamentally different from single dose data.
- The dosage applied in the above-mentioned human study (2 mg/cm²) by far exceeds the daily use level of UV filters and leads to a maximal concentration in the plasma of 20 ng/ml (78.6 pmol/ml).

Ref.: Dekant 2007

Additional literature on the estrogenic properties of 4-MBC

Several studies confirm the fact that 4-MBC displays estrogenic effects. In all cases, the UV filter's potency is much lower than that of positive control compounds such as 17 estradiol and estradiol benzoate. It is also shown that the mechanism of action differs between 4-MBC and the estrogenic positive control substances.

Ref.: Maerkel et al. 2007, Schmutzler et al. 2004, Seidlová-Wuttke et al. 2006a&b

3.4.2 SCCP discussion on the toxicokinetic approach

Reduction of the toxicokinetic factor of the MoS from 4 to 1

As indicated in SCCP/1042/06, a toxicokinetic-based approach for the calculation of the MoS requires the comparison of:

a) the shape and the area under the curve for plasma concentrations plotted as a function of time after repeated dermal administration of the substance at its NOAEL in a relevant species;

WITH

b) the shape and the area under the curve for plasma concentrations plotted as a function of time after repeated dermal administration of the substance at its in-use concentrations in human volunteers

In case the shapes of the curves are comparable, the factor 4.0 becomes 1 and a MoS of 25 might be considered.

In the case of 4-MBC, however, not all the required data (plasma levels) after repeated dermal exposure in human volunteers are available. In the only repeated human study (4 days) where plasma levels were measured, only 4-MBC, and not its major metabolites, were determined. In order to perform a correct calculation, the areas under the curve and the shapes of the curves of the plasma levels as a function of time, also need to be available for the metabolites of 4-MBC. In addition, the relevance of the hormone levels measured in this study is compromised by the presence of 10% 4-MBC instead of the requested 4% and the combination of three UV filters at a high concentration. By preference, the study should be performed by making use of a sunscreen formulation only containing 4-MBC (not a combination of several filters) at the in-use concentration of 4%.

Finally, all available data (rat and human studies) indicate accumulation of 4-MBC and/or its metabolites after single and repeated exposure (see tables under section 3.4.1), which makes the application of the toxicokinetic approach very difficult. It also renders the automatic extrapolation from data from a single application study to a repeated application situation, inappropriate.

Since the submitted studies were all performed under different conditions with diverging dosage levels and forms of 4-MBC, the SCCP did not accept the reduction of 4 to 1 of the toxicokinetic factor of the MoS.

Reduction of the toxicodynamic factor of the MoS from 2.5 to 1

A reduction of the toxicodynamic factor from 2.5 to 1 was not accepted either, although the SCCP acknowledged that rats are more susceptible to thyroid perturbation than man. Thyroid hormone-related measurements were not included in the human dermal study and robust data supporting the hypothesis that 4-MBC or one of its metabolites would be the

active compound with regard to human toxicity, were not available. More elaborated mechanistic data involving the use of pure metabolites were required to help clarifying the toxicodynamic issue.

In order to have more reassurance in the decision-making in this complex area, the SCCP invited an independent expert in toxicokinetics to express his opinion on the toxicokinetic approach proposed by the applicant.

Some additional remarks

- In the dermal studies with the rat, 70 and 100 mg sunscreen/cm² were applied and the surplus was washed off after 24 hours. Therefore, the mentioned dosage levels probably represent an overestimate.
- Physicochemical data are newly provided and correspond to the data already described in SCCNFP/0779/04. However, no (photo-)stability data are provided, neither are data on the solubility of 4-MBC in the receptor fluid of the *in vitro* dermal absorption study. Photostability appears quite interesting, since a newly introduced publication (Scalia et al. 2007) suggests that 4-MBC undergoes marked degradation under sunlight exposure.

3.4.3 Independent expert's opinion on the toxicokinetic approach

During the SCCP *ad hoc* meeting of 13 November 2007, an independent expert in pharmaco- and toxicokinetics and toxicology expressed his consent with the concerns expressed earlier by the SCCP.

Since no univocal conclusion could be drawn based upon the points mentioned under 3.4.2, it was decided to approach the interpretation of the toxicokinetic data from a different angle.

Rat plasma levels after repeated exposure to 4-MBC

As also pointed out by the SCCP, the individual 4-MBC and its metabolites plasma levels and their graphical representation from the 90 day dermal study in the rat can be extracted from the data set submitted. The following pages (*A. Plasma levels measured in a 90 day dermal study in the rat*) contain the individual plasma level graphs as used by the expert, accompanied by the relevant C_{max} values. The latter are preferred over the areas under the curve, as enzyme induction clearly takes place. In addition, preference is given to the plasma levels measured at the NOEL (100 mg/kg/day) instead of the NOAEL (400 mg/kg/day) in order to be on the safest side.

For the parent compound, the expression "**distribution phenomenon**" is used when the initial peaks in plasma concentration are not considered to reflect the actual C_{max}, but rather an elevated concentration of the compound under study due to an incomplete initial distribution phase. In those cases the second peak, and not these early 0.5-1 hour values, is taken as C_{max}.

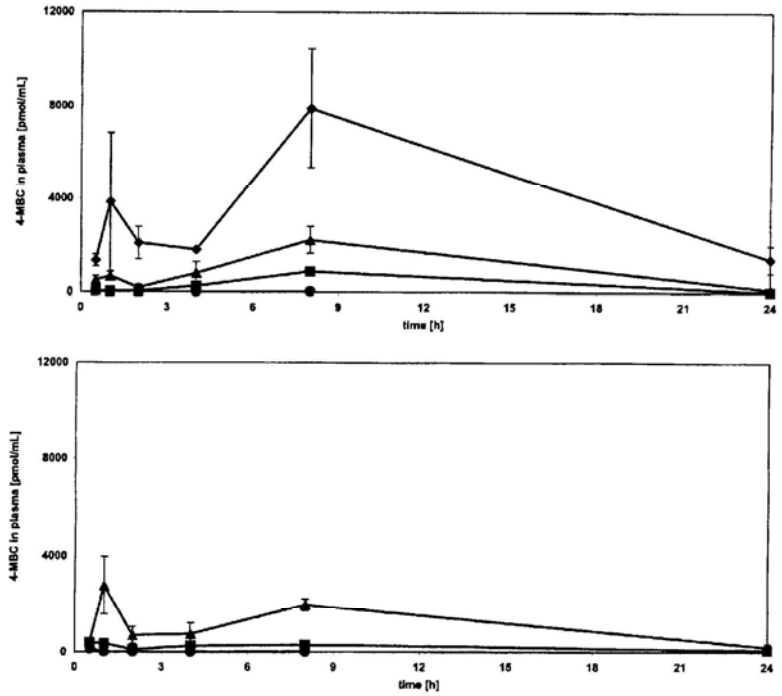
For the metabolites, "**delayed metabolism**" means that it takes more than 24 hours before C_{max} is reached, meaning that the metabolism of the parent compound into the metabolite under study is not complete after 24 hours. Therefore it is difficult to deduce the exact C_{max} values from the day 1 plasma level graphs. In the cases where this phenomenon occurs, the mentioned C_{max} values are the ones indicated by the performing laboratory.

Human plasma levels after repeated exposure to 4-MBC

As the lack of these particular data was reported to be the major hurdle for the SCCP to accept the toxicokinetic-based reduction of the MoS of 100, the toxicokinetic expert proposes to make a realistic estimation of the 90 day dermal plasma levels in humans based upon the measured values after single exposure to human volunteers and the observed carry-over levels (see graphs under *B. Plasma levels measured in a single dose dermal study in human volunteers*).

As such, the above exercise allows comparison between rat and estimated human values and forms the basis for deciding on the potential reduction of the toxicokinetic factor of the MoS from 4 to 1.

A. Plasma levels measured in a 90 day dermal study in the rat

4-MBC	
 Fig. 2: Plasma concentrations of 4-MBC in female rats (n= 3 per gender, per dose) after dermal application of 0 (●), 100 (■), 400 (▲) and 2000 (◆) mg/kg bw 4-MBC on day 1 (upper panel) and day 90/91 (lower panel) of the study.	100 mg/kg bw/day (■): C_{max} (females)= 877 pmol/ml after 1 day 100 mg/kg bw/day (■): C_{max} (females) = 297 pmol/ml after 90 days NOTE: 385 pmol/ml at 0.5h is considered to be a distribution phenomenon. Therefore 8h C_{max} is used.

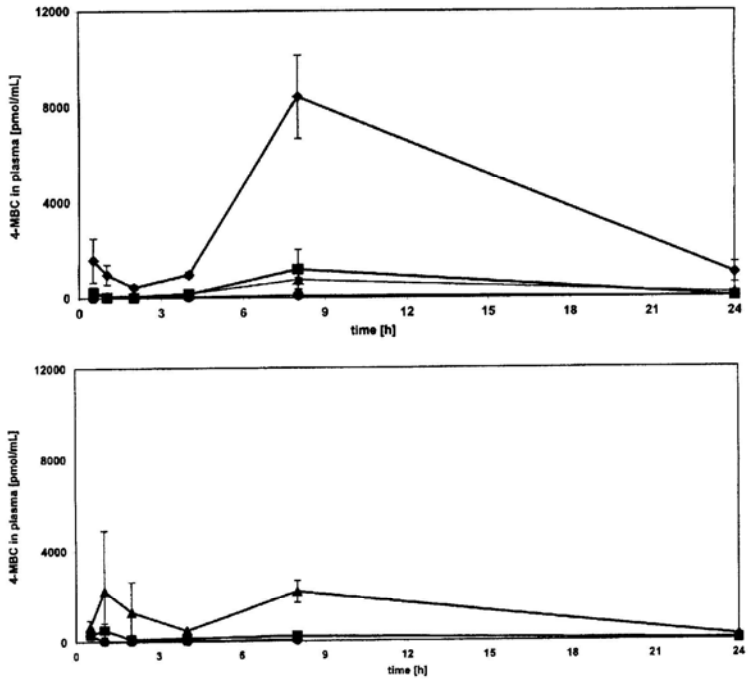


Fig. 1: Plasma concentrations of 4-MBC in male rats (n= 3 per gender, per dose) after dermal application of 0 (●), 100 (■), 400 (▲) and 2000 (◆) mg/kg bw 4-MBC on day 1 (upper panel) and day 90/91 (lower panel) of the study.

100 mg/kg bw/day (■):

C_{max} (males)= 1173 pmol/ml after 1 day

100 mg/kg bw/day (■):

C_{max} (males)= 295 pmol/ml after 90 days

NOTE:
503 pmol/ml at 1h is considered to be a distribution phenomenon. Therefore 8h C_{max} is used.

MET-2 [3-(4-carboxybenzylidene)-camphor]

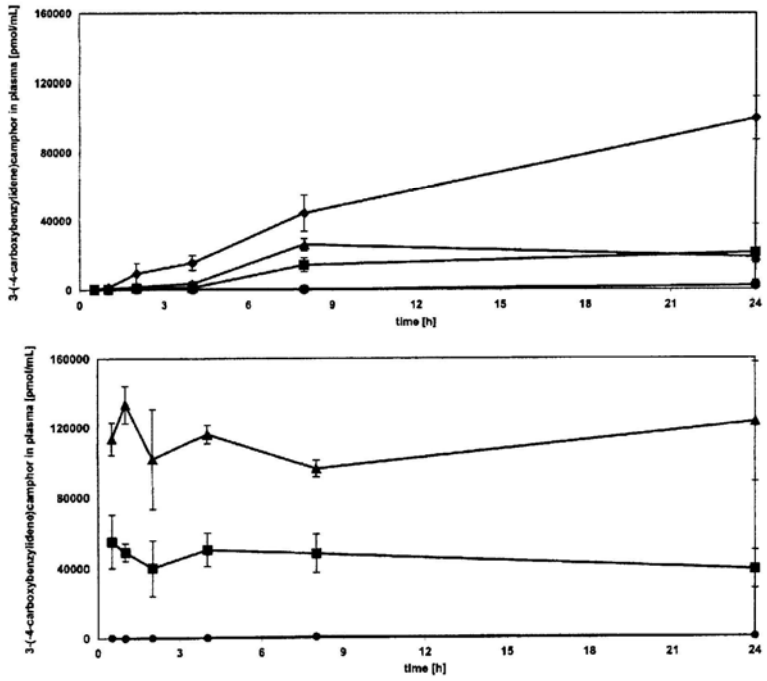


Fig. 6: Plasma concentrations of 3-(4-carboxybenzylidene)camphor in female rats (n= 3 per gender, per dose) after dermal application of 0 (●), 100 (■), 400 (▲) and 2 000 (◆) mg/kg bw 4-MBC on day 1 (upper panel) and day 90/91 (lower panel) of the study.

100 mg/kg bw/day (■):

C_{max} (females)= 21338 pmol/ml after 1 day (delayed metabolism)

100 mg/kg bw/day (■):

C_{max} (females)= 55493 pmol/ml after 90 days

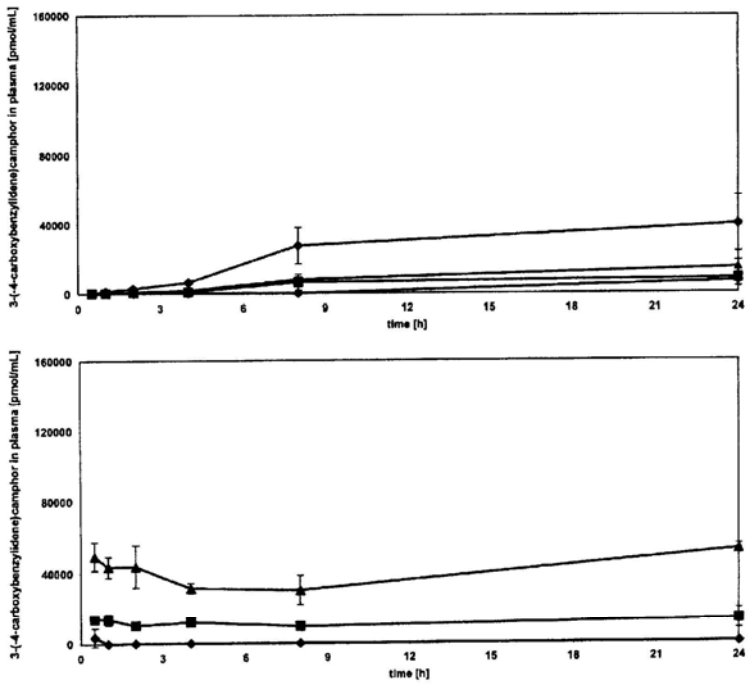


Fig. 5: Plasma concentrations of 3-(4-carboxybenzylidene)camphor in male rats (n= 3 per gender, per dose) after dermal application of 0 (●), 100 (■), 400 (▲) and 2 000 (◆) mg/kg bw 4-MBC on day 1 (upper panel) and day 90/91 (lower panel) of the study.

100 mg/kg bw/day (■):

C_{max} (males)=
8342 pmol/ml after 1 day
(delayed metabolism)

100 mg/kg bw/day (■):

C_{max} (males)=
14049 pmol/ml after
90 days

MET-1 [3-(4-carboxybenzylidene)-6-hydroxycamphor]

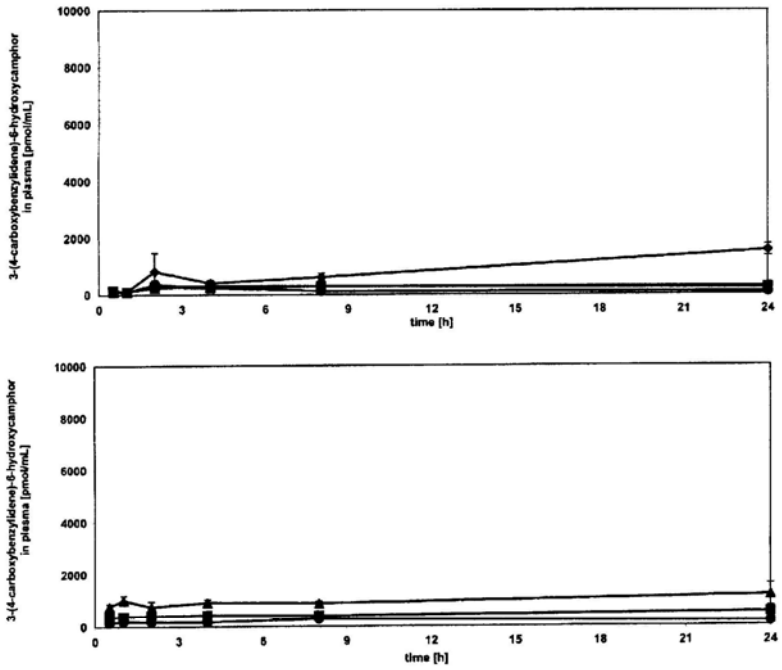


Fig. 4: Plasma concentrations of 3-(4-carboxybenzylidene)-6-hydroxycamphor in female rats (n= 3 per gender, per dose) after dermal application of 0 (●), 100 (■), 400 (▲) and 2 000 (◆) mg/kg bw 4-MBC on day 1 (upper panel) and day 90/91 (lower panel) of the study.

100 mg/kg bw/day (■):

C_{max} (females)=
292 pmol/ml after 1 day
(delayed metabolism)

100 mg/kg bw/day (■):

C_{max} (females)=
519 pmol/ml after 90 days

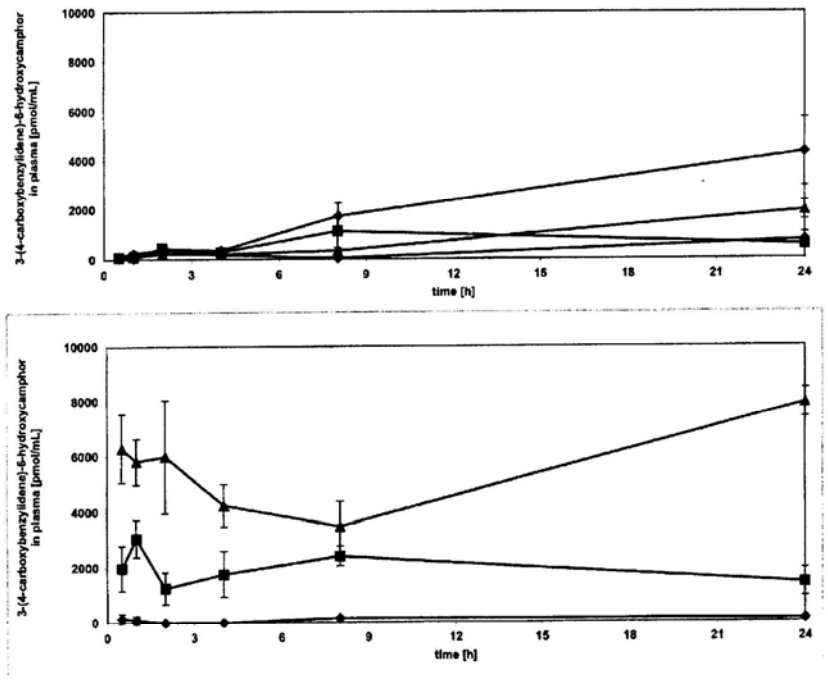


Fig. 3: Plasma concentrations of 3-(4-carboxybenzylidene)-6-hydroxycamphor in male rats (n= 3 per gender, per dose) after dermal application of 0 (●), 100 (■), 400 (▲) and 2000 (◆) mg/kg bw 4-MBC on day 1 (upper panel) and day 90/91 (lower panel) of the study.

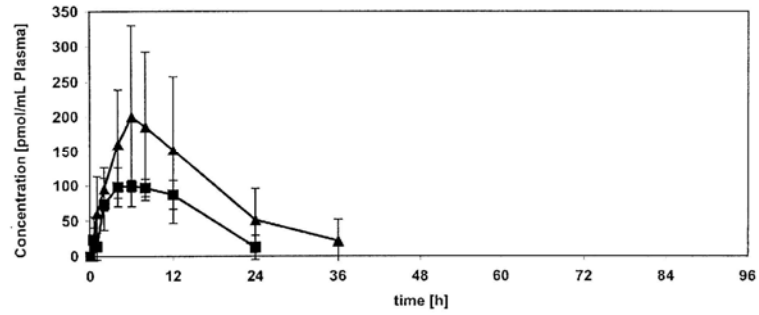
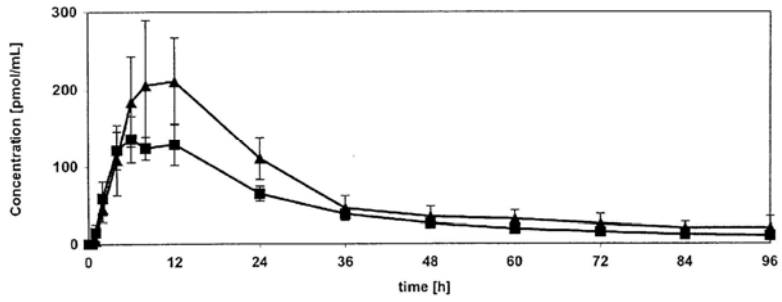
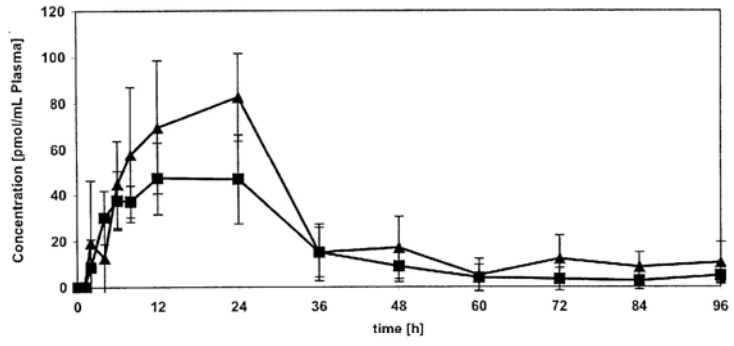
100 mg/kg bw/day (■):

C_{max} (males)=
1123 pmol/ml after 1 day
(delayed metabolism)

100 mg/kg bw/day (■):

C_{max} (males)=
3050 pmol/ml after 90 days

B. Plasma levels measured in a single dose dermal study in human volunteers

4-MBC  Fig. 2: Blood concentrations of 4-MBC in 3 male (▲, $t_{1/2}$ = 9 h) and 3 female (■) human subjects after dermal application of a typical sunscreen formulation containing 4% 4-MBC giving an applied dose of approx. 22 mg/kg bw. Concentrations were below the limit of detection after the time-points 24 h (female) and 36 h (male). (See annex I for details, metabolite structures are shown in scheme 1.)	6h C_{max} (females) = 100 pmol/ml 6h C_{max} (males) = 200 pmol/ml 24h C_{max} (females) = 20 pmol/ml 24h C_{max} (males) = 50 pmol/ml "Carry-over" is about 25% at 24h.
MET-2 [3-(4-carboxybenzylidene)-camphor]  Fig. 6: Blood concentrations of the metabolite 3-(4-carboxybenzylidene)camphor (mean ± SD) in 3 male (▲,$t_{1/2}$ = 26 h) and 3 female (■, $t_{1/2}$ = 23 h) human subjects after dermal application of a sun screen formulation containing 4% 4-MBC to give an applied dose of approx. 22 mg/kg bw. (See Annex III for details, metabolite structures are shown in scheme 1)	12h C_{max} (females) = 130 pmol/ml 12h C_{max} (males) = 200 pmol/ml 24h C_{max} (females) = 65 pmol/ml 24h C_{max} (males) = 110 pmol/ml "Carry-over" is about 50% at 24h.
MET-1 [3-(4-carboxybenzylidene)-6-hydroxycamphor]  Fig. 5: Blood concentrations of the metabolite 3-(4-carboxybenzylidene)-6-hydroxycamphor (mean ± SD) in 3 male (▲,$t_{1/2}$ = 31 h) and 3 female (■, $t_{1/2}$ = 20 h) human subjects after dermal application of a sun screen formulation containing 4% 4-MBC giving an applied dose of approx. 22 mg/kg bw. (See Annex II for details, metabolite structures are shown in scheme 1.)	24h C_{max} (females) = 50 pmol/ml 24h C_{max} (males) = 80 pmol/ml "Carry-over" is considered negligible. (Relatively low levels and rapid excretion)

The observation that the parent compound's C_{max} is lower in the rat after 90 days than after 1 day, strongly suggests that an enzyme induction phenomenon occurs in the case of repeated exposure. Therefore the C_{max} values of 4-MBC after 90 days are not used for comparison with human values. They are replaced by their corresponding day 1 values, which are higher and represent the worst case values.

As stated before, to estimate the plasma levels after repeated exposure in humans, the measured plasma concentrations of 4-MBC and its metabolites in the human single dose study are multiplied by their estimated "carry-over" ratio, deduced from the concentrations measured after 24h. For this purpose, initial carry-over levels after 1 day are mathematically extrapolated to 90 day total carry-over levels[1].

The following table with toxicokinetic parameters can subsequently be generated:

Compound	Parameter	Males	Females
4-MBC	C_{max} (rat, value day 1)	1173 pmol/ml blood	877 pmol/ml blood
	C_{max} (human, single dose)	200 pmol/ml blood	100 pmol/ml blood
	Carry-over (day 1) Total carry-over (day 90)	25% 33%	20% 25%
	C_{max} (human, repeated dose) - estimated	266 pmol/ml blood	125 pmol/ml blood
	Ratio rat/human	**4.4**	**7.0**
MET-2 [3-(4-carboxy-benzylidene)-camphor]	C_{max} (rat, value day 90)	14,000 pmol/ml blood	55,500 pmol/ml blood
	C_{max} (human, single dose)	200 pmol/ml blood	130 pmol/ml blood
	Carry-over (day 1) Total carry-over (day 90)	50% 100%	50% 100%
	C_{max} (human, repeated dose) - estimated	400 pmol/ml blood	260 pmol/ml blood
	Ratio rat/human	**35**	**213**
MET-1 [3-(4-carboxy-benzylidene)-6-hydroxy-camphor]	C_{max} (rat, value day 90)	3,050 pmol/ml blood	519 pmol/ml blood
	C_{max} (human, single dose)	80 pmol/ml blood	50 pmol/ml blood
	Calculated carry-over	0%	0%
	C_{max} (human, repeated dose) - estimated	80 pmol/ml blood	50 pmol/ml blood
	Ratio rat/human	**38**	**10**

The ratio rat/human is > 1 in all cases (for the mother compound 4-MBC as well as for its metabolites, for females as well as for males), indicating that the plasma levels were higher in the rat at the NOEL-value of the study, compared to the plasma levels in human volunteers under worst case application conditions. These findings plead in favour of the reduction of the toxicokinetic factor from 4 to 1. Nevertheless, it must be emphasized that the values generated as presented above are based on two assumptions, namely:

1) 100 mg/kg bw/day is taken as the NOEL of the 90d dermal study with the rat. In that case analysis of the kinetic data supports the argumentation by the applicant to reduce the toxicokinetic factor of the MoS.
2) The conditions present in the toxicokinetic study with human volunteers represent a worst case scenario.

[1] **Starting from 50% carry-over**
Day 1: 50% carry over at end of day
Day 2: 50% of new dose + 50% of 50% carry-over from day 1: 75% total carry-over at end of day
Day 3: 50% of new dose + 50% of 50% carry-over from day 2: 82.5% total carry-over at end of day
After 14 days, a plateau of 100% total carry-over is reached
Starting from 25% carry-over
After 5 days, a plateau of 33% total carry-over is reached.

3.4.4 Overall discussion on the calculation of the MoS of 4-MBC

The conservative calculation of the MoS for 4-MBC takes into account:

- the lowest NOAEL-value of 25 mg/kg/day based on thyroid effects observed in the 90day oral toxicity study in the rat, and
- a dermal absorption value of 1.96 µg/cm² (measured *in vitro*).

The details are repeated below:

Dermal absorption value:	1.96 µg/cm²
Skin surface area (whole body, 1.8 m²):	18,000 cm²
Typical human body weight:	60kg
No observed effect level NOAEL (90d-oral-rat):	25 mg/kg bw/day
Systemic exposure dose (SED) ((1.96.10^{-3} x 18,000) / 60 kg)	0.588 mg/kg bw/day

MoS = NOAEL / SED = 42.5

As the MoS was below 100, the compound could not be declared safe for its intended use by the SCCP.

The applicant responded with a proposal for a toxicokinetic-based approach, leading to the conclusion that the MoS could be reduced from 100 to 10 for the following reasons:

1) In a MoS of 100, a factor of 4 accounts for the inter-species toxicokinetic variation between man and the experimental animal. According to the applicant, this factor could in the case of 4-MBC be reduced from 4 to 1 based on the results from dermal studies in rats and human volunteers in which plasma levels of 4-MBC and/or its metabolites were measured.
2) Also according to the applicant, the toxicodynamic factor of 2.5 could be reduced to 1, viewing the fact that the tested species (the rat) is known to be more susceptible to thyroid effects than man.

The SCCP did not accept this argumentation, since:

1) To reduce the toxicokinetic factor from 4 to 1, plasma levels of 4-MBC and its metabolites must be available in two settings for comparison:

   - after repeated dermal application to the rat at the corresponding NOAEL level
   - after repeated dermal application to human volunteers, by preference under worst case conditions

   As the human repeated dermal application plasma levels were not available, the SCCP did not accept the requested reduction of the MoS. Moreover, concern was expressed with regard to potential accumulation of 4-MBC and its metabolites in the human body.

2) Reduction of the toxicodynamic factor to 1 was not accepted either, since thyroid effects were noticed in human volunteers and the submission did not contain data identifying the exact compound (4-MBC and/or one of the metabolites) responsible for the thyroid effect in man. Neither was there an argumentation on the mechanistic background of the effects noticed.

Upon request of the SCCP, an external expert in toxicokinetics studied the dossier. Acknowledging the concerns of the Committee, the expert proposed to estimate the repeated dose plasma levels for 4-MBC and its metabolites, based upon the available single

dose plasma levels and the amounts still present after 24 hours, out of which the "carry-over" values (accumulation) could be calculated.

Comparing these levels with the plasma levels of 4-MBC and its metabolites in the rat at the NOEL instead of the NOAEL value (worst case), leads to the conclusion that the estimated human values are systematically lower than their rat counterparts, supporting approval of the requested reduction of the toxicokinetic factor from 4 to 1.

Nevertheless, this approach is based upon two assumptions, namely:

1) 100 mg/kg/day needs to be the actual NOEL-value for 4-MBC in the 90 day dermal study.
2) The presented human study needs to be considered as a worst case situation.

The SCCP is of the opinion that:

1) 100 mg/kg/day is the NOEL of the 90d dermal toxicity study, based upon thyroid effects occurring at higher levels.
2) the application of 2mg/cm² of a sunscreen formulation can be considered as a worst case scenario

As such, following the toxicokinetic expert's opinion and accepting that the toxicokinetic part of the MoS can be reduced from 4 to 1, a MoS of 25 needs to be achieved. As mentioned in point 3.3.3, the conservative calculation of the **MoS** results in a value of **42.5**, which is higher than the requested threshold of 25.

4. CONCLUSION

It is the opinion of the SCCP that, using the toxicokinetic data for 4-MBC (rat, human), the toxicokinetic factor present in the calculation of the MoS can be reduced from 4 to 1, bringing the requested MoS value for 4-MBC to 25.

As such, it is concluded that 4-MBC can be considered safe for use in finished cosmetic products (whole body application) at a concentration of up to 4%.

It must be emphasized that this opinion is restricted to the safety evaluation of 4-MBC after dermal application of a cosmetic product containing this UV filter. Exposure scenarios via the inhalation route (through aerosols, sprays, etc.) or the oral route (through e.g. lip care products) are not covered. In these cases, risk cannot be excluded.

5. NOTE ADDED AFTER ADOPTION OF THE OPINION

The SCCP is aware of a newly published review article related to the developmental toxicity of 4 MBC (Schlumpf et al. 2008). The raw data forming the basis of this article and of previous publications cited in the review have not been made available to the SCCP and their relevance could not be assessed. The publication does therefore not affect the opinion as it is currently formulated.

6. MINORITY OPINION

Not applicable

7. REFERENCES

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SCCP/1042/06 - SCCP opinion concerning 4-Methylbenzylidene Camphor, COLIPA n° S60, *adopted by the SCCP during the 9th plenary meeting of 10 October 2006*.

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