Scientific Committee on Consumer Safety

SCCS

OPINION ON

3-Benzylidene camphor

COLIPA n° S61

References are cited in a different way in the opinion compared to the submissions

The SCCS adopted this opinion at its 2nd plenary meeting

of 18 June 2013

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 Safety (SCCS), 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 Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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1. BACKGROUND

Submission I on the UV-filter 3-benzylidene camphor with the chemical name 3-benzylidenebornan-2-one was submitted by COLIPA[1] in 1988.

The Scientific Committee on Cosmetic Products and Non-Food products intended for consumers (SCCNFP) adopted an opinion (1374/1998 on 3-benzylidene camphor at its plenary of 21 January 1998.

The substance is currently regulated in the Cosmetics Directive in Annex VII, part 1 n.19 ("List of permitted UV filters which cosmetic products may contain") in a concentration up to maximum 2%.

In October 2011, the French authorities notified the Commission that on 24 August 2011 the Agence française de sécurité sanitaire des produits de santé (AFSSAPS[2]), adopted a Decision, which was published in the Official Journal of the French Republic on 17 September 2011. The Decision adopted prohibits, as a safeguard measure in accordance with the provisions of Article 12(2) of the Directive 76/768/EEC, the manufacture, import, export, wholesale distribution, placing on the market free of charge or against payment, holding with a view to sale or distribution free of charge and use of cosmetic products containing 3-benzylidene camphor (CAS: 15087-24-8).

The AFSSAPS report states that the hazard characterisation for this substance is considered incomplete. In addition, the no observed adverse effect level (NOAEL) and the cutaneous absorption rate used by the AFSSAPS in connection with the risk assessment results in insufficient margin of safety to ensure consumer safety in accordance with the SCCS's notes of guidance[3]. Finally, as endocrine disruption effects were observed in the studies published in the scientific literature, in the current state of knowledge, the French authorities consider that it is not possible to conclude that there is no risk to humans.

2. TERMS OF REFERENCE

1. *Does the SCCS consider 3-benzylidene-camphor safe for use as a UV-filter in cosmetic products in a concentration up 2.0% taken into account the scientific data provided?*

2. *Does the SCCS have any further scientific concerns with regard to the use of 3-benzylidene-camphor as a UV-filter in cosmetic products taking into account the concern about its potential endocrine disruptor properties?*

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association "Cosmetics Europe"

[2] Now ANSM : Agence nationale de sécurité des médicaments et des produits de santé

[3] Scientific Committee on Consumer Safety (SCCS/1416/11) 2011. The SCCS's notes of guidance for the testing of cosmetic ingredients and their safety evaluation.

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

3-Benzylidene camphor

3.1.1.2. Chemical names

3-benzylidene-bornan-2-one
3-benzylidene-L-camphor.
1,7,7-trimethyl-3-benzylidene-2,2,1-bicyclo-2-heptanone
1,7,7-trimethyl-3-(phenylmethylene)bicyclo[2.2.1]heptan-2-one

3.1.1.3. Trade names and abbreviations

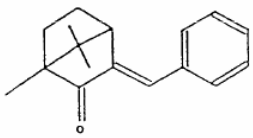
Mexoryl SD
UNISOL S-22

COLIPA No. S61

3.1.1.4. CAS / EC number

CAS: 15087-24-8
EC: 239-139-9

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{17}H_{20}O$

3.1.2. Physical form

White crystalline material

3.1.3. Molecular weight

Molecular weight: 240.4 g/mol

3.1.4. Purity, composition and substance codes

According to Submission II of 1991:

Batch No. 32778.6
Chemical characterisation of 3-benzylidine camphor was performed by IR, NMR and GC_MS
Purity: 99.1% (% GC Peak area, without using reference material)

3.1.5. Impurities / accompanying contaminants

According to Submission II of 1991:

Batch No. 32778.6
Camphor: < 500 ppm
Benzaldehyde: <500 ppm
Benzyl alcohol: < 500 ppm
Heavy metals: < 10 ppm
2-Propanol: 150 ppm
Water content: 0.232%
Ash: 0.16%

3.1.6. Solubility

Soluble in absolute alcohol and isopropanol
Insoluble in water.

Comment
Quantitative data on solubility was not provided

3.1.7. Partition coefficient (Log Pow)

Log Pow 5.37 (Soeborg et al., 2006)

3.1.8. Additional physical and chemical specifications

Melting point:	-/77.4°C
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
Absorption:	λmax:289 nm

3.1.9. Homogeneity and Stability

Test for photo-stability
A thin film (2um thick) of a.i. in a non-ionic emulsion was exposed to UV produced by selecting the UVA and UVB wavelengths from simulated solar radiation (SSR) by a dichroic mirror; the radiation was obtained from a xenon arc and suitable filters. The intensities were, respectively, 15 and 0.42 mW/cm²; in southern France and North Africa, the corresponding values for natural insolation, measured by the authors' equipment, were 5 and 0.14 mW/cm². Measurement of photo degradation in the film was by spectrophotometry and HPLC. The experimental methods were elaborated, and seem to have been carefully carried out. Corrections were made for differences between solar and SSR intensities and the values that would have been found in a 10 µm film were calculated. The a.i. was found to attain a photostable isomerisation very rapidly, following which there was a very slow irreversible degradation (no further details given).

Ref.: 14

SCCS General Comments to physico-chemical characterisation

-homogeneity and stability of test solutions used in various studies was not documented

3.2. Function and uses

3-Benzylidene camphor is proposed for use as a sunscreen agent at levels up to 6%; in a more recent submission, the concentration proposed by industry is 4%, because of formulation difficulties arising from the low solubility of the agent; in the most recent submission the proposed maximum concentration is 2%.

3.3. Toxicological Evaluation

The toxicological evaluation is based on the previous SCCNFP opinion from 1998 (1374/96) and on the dossiers I, II, III and IV on the UV-filter 3-benzylidene camphor with the chemical name 3-benzylidenebornan-2-one submitted by COLIPA respectively in 1988, 1991, 1992 and 1994. The submissions files are only summarizing the experimental studies and original data were not made available to the scientific committee. Recently published articles on 3-benzylidene camphor retrieved from the scientific literature are mentioned and discussed in the opinion. It concerned mainly endocrine properties of 3-BC and were also used to answer question 2 of the mandate.

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Rat

Five male and five female Sprague-Dawley rats were used. The study was performed following the OECD n°401 guideline. The a.i. was given by gavage suspended in 1% propylene glycol in a dose of 5 g/kg bw. Observation was for 14 days. No abnormality of any kind was seen. The LD50 was greater than 5 g/kg bw.

Ref.: 1

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Rabbit

A 6% solution of the compound in isopropyl palmitate was applied to abraded and non-abraded skin in 6 New Zealand rabbits and kept under semi-occlusion for 24 hrs. The compound was judged to be a mild irritant under these conditions.

Ref.: 5

Rabbit, repeated application

A 6% solution in isopropyl palmitate was used. The skin was carefully clipped on both flanks and each animal had 2 ml of the test material rubbed into the flank on one side. A similar procedure, without the solution, was carried out on the other flank. Animals received applications on 5 days per week for 6 weeks. The experiment was then continued for a further week without treatment, to study recovery. The weekly mean index of irritation (maximum, 8) was: 1.83; 1.83; 1.21; 2.23; 1.13; 2.07 and (recovery) 1.0. Under these conditions the substance was judged to be a mild irritant.

Ref.: 6

3.3.2.2. Mucous membrane irritation

Rabbit

A 6% solution of a.i. in isopropyl palmitate was applied to one eye. The untreated eye served as control. The indices of ocular irritation were as follows: 5.33/110 directly after administration; 2.67/10 on day 1; 0.67/10 on day 2; thereafter negative. The authors judged the compound under the conditions of the test to be "very slightly irritant."

Ref.: 4

3.3.3. Skin sensitisation

Guinea pig

Twenty albino animals of the Hartley strain were used. For induction, 0.5 g of the compound, as a powder, was applied under occlusion for 48hrs, 3 days a week, for 10 applications. On days 1 and 10, an intradermal injection of 0.1 ml of Freund's complete adjuvant, 50% in saline, was given.

After a 12 day rest, a challenge application, the same as the induction application, was made to a new site. No sign of sensitisation was observed.

Ref.:7

3.3.4. Dermal / percutaneous absorption

Taken from previous opinion

Man

Four volunteers were treated. Areas of 100 cm^2 were delineated on the upper arm. The 14C-labelled compound was made up in a concentration of 5.02% in an o/w emulsion.

About 0.5 g of ointment was applied to the delineated areas (exact amount calculated by difference). Contact was for 6 hours, without occlusion. At the end of the experiment, the skin was swabbed clean and also stripped. Urine and faeces were collected for 5 days.

The mean amounts in the urine and faeces over 5 days, as a percentage of the amount applied, equalled 3.54% +/- 1.77%.

Ref.: 13

In submission IV (1994), the applicant considered that this study did not have adequate recovery. Therefore a new study has been performed.

Man

In another similar investigation, an o/w emulsion containing 5% a.i. (0.5% labelled with 14C was applied over 100 cm^2 of the forearm in 6 healthy male volunteers). About 300 mg of formulation was applied (e.g. 15 mg of active ingredient); the exact amount was calculated by difference. Occlusion was not used, and the application was allowed to remain for 6 hours. At the end of that time, the amount remaining on the skin was removed with a spatula, and the skin swabbed 5 times with ether. All the removed samples were counted. In addition, 10 cm^2 of the area of application was stripped 15 times, and radioactivity counted in the strips in batches of 5 strips. Urine was collected from 0 to 6 hours, 6 to 24 hours, and every 24 hours for a total of 120 hours. Faeces were collected every 24 hours for 120 hours.
In urine, the total amount of radioactivity found amounted to 0.53% +/- 0.25 of the net amount applied. For faeces, the corresponding amount was 1.37% +/- 0.66. In all, 1.89 +/- 0.70% of the 14-C activity applied was recovered in the urine and faeces within 5 days. The total recovery was 91.95% +/- 2.83. If one makes the assumption that the percentages in the urine and faeces represent the amount absorbed, the mean absorption found is approximately 0.12 mg/kg bw.

Ref.: 21

SCCS Comments
Both studies were performed with a low number of male volunteers which does not allow conclusions to be drawn on the interindividual variability in humans. The original data of these studies were not made available to the SCCS and no explanations were given to explain the higher absorption measured in the first study. Therefore the SCCS considered that, based on the results of the second study which has an adequate recovery rate, the amount + 2SD of skin penetration should be used for risk assessment: 1.89 + 2x0.70 = 3.29%.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral toxicity

Taken from previous opinion 1374/98

Rat

A 4 week oral study by gavage was carried out according the OECD guideline n° 407 and complies with GLP. Groups of 10 male and 10 female animals were given doses of 0, 250, 375 and 550 mg/kg bw/day. The a.i. was suspended in 1% carboxymethylcellulose. In addition to the usual clinical observations, weighing, etc., blood and urine samples were taken immediately before sacrifice. All animals were subjected to necropsy, and 10 organs were fixed and subjected to histological examination; numerous other tissues were also fixed for future examination if required. The stability of the a.i. in its vehicle was confirmed by analysis.

There were no deaths. Clinically the only abnormal finding was alopecia. It was observed in a few males from the group 3 (375 mg/kg bw/day), and in the female from the three treated groups.
Body weight gain showed a significant reduction in all dosed female groups.

Food consumption was not affected. There was a significant dose related increase in weight of the adrenal glands in female animals; the weight of the liver showed a significant, dose related increase in all dosed animals. No macroscopic or microscopic abnormalities were found in any group. In female animals, haematological investigations showed a significant, dose related fall in haemoglobin and packed cell volume; there were highly significant

reductions in MCH in all dosed groups, but they were not dose related. On the other hand, mean corpuscular haemoglobin concentration, red cell count and mean corpuscular volume were normal. In male animals there were no important changes in the blood picture. In both sexes, the prothrombin time showed a significant increase, which was not dose-related, in all dosed groups.

Biochemical investigation of the blood showed a very striking reduction of cholesterol levels in all dosed groups (cf. Results from sub-chronic toxicity, infra). In male animals, bilirubin was reduced in a dose related manner, and protein was somewhat increased. In female animals glucose, protein and alkaline phosphatase levels were all very significantly increased in all dosed groups, but the increases did not seem to be dose related. Lipids do not seem to have been measured. Examination of the urine revealed no abnormality.

Apparently, a NOAEL (which would be lower than 250 mg/kg bw/d) has not been established from that study.

Ref.: 2

Rat

Groups of 10 male and 10 female SD rats were given doses of a.i. of 0, 25 and 50 mg/kg bw/day by gavage for 6 weeks. The study was conducted according to GLP.

There were no deaths. The chief clinical finding was alopecia chiefly in females at the high dose.

Body weight and body weight gain were unaffected. There were some falls in food consumption in female animals in two of the weeks and rises in other weeks, probably not biologically significant.

Haematological investigations were carried out at the end of the experiment; there were falls in haemoglobin and haematocrit in female dosed animals, but these were small and within normal limits.

Biochemical investigations showed changes in female animals only, as follows. Cholesterol had reduced by about 55% in dosed animals. Triglycerides had increased by 63% and 88% respectively in dosed animals. Albumin had reduced by about 8% and globulins had increased by about 40% in dosed animals. Electrophoresis of the plasma in female animals showed that the increase in albumin was not dose related, and that there was a significant dose related increase in alpha1 globulins without any increase in alpha2, beta or gamma globulins.

The blood levels of thyroid hormones showed some changes in both male and female animals. In males, triiodothyronine (T3) showed a dose related increase, significant at the higher dose, but no change in thyroxine (T4). In female animals, T3 showed no change, but T4 showed a significant dose related increase in both dosed groups. In females, there was a dose related reduction in aspartate aminotransferase.

Urinalysis showed no abnormality in either sex.

At necropsy, no macroscopic abnormalities were found.

There was a dose related increase in absolute liver weight in female animals, reaching significance at the higher dose; similarly with the adrenals, although this reached only the level of $p = 0.05$, whereas the former reached $p = 0.01$.

The relative weights of livers and spleens showed some increase, but although this was statistically significant, it was small and probably not biologically significant (no dose given). A similar observation applies to the adrenal glands.

Microscopic examinations indicated a follicular and epithelial thyroid hyperplasia and a hypertrophy of the fasciculated zone in adrenals from the treated female groups. Ophthalmological examinations at sacrifice showed no differences between control and treated animals.

Apparently, a NOAEL (which would be lower than 25 mg/kg bw/d) has not been established from that study.

Ref.: 15

Guinea pig

Female animals only, of the Dunkin Hartley strain, were used in this study. In a preliminary experiment, one animal was treated by gavage with 500 and one with 1000 mg/kg bw/day for 15 days. No abnormality was found, and the dose of 500 mg/kg bw/day was chosen for the main study.

Two experiments were conducted in parallel, in accordance with GLP. In one, groups of 5 female animals were used, one group receiving 0 and one 500 mg/kg bw/day by gavage, for 6 weeks. In the second experiment, groups of 10 animals were similarly treated for 8 weeks. The mode of administration of the a.i. is not clear. It was suspended in 2% polysorbate 80 and given by oral route.

The available study summary states, that there were 3 deaths, one in the control group and 2 in the dosed group. In each case the death was attributed to aspiration of feed. The only abnormal finding was alopecia in the nuchal region in one control animal and one treated animal: on the basis of the histological appearances, the former was attributed to rubbing off the food hopper, and the latter to underlying dermatitis.

Body weight gain was unaffected. At autopsy, there were no important macroscopic findings.

Histological examination was made of only a few tissues from 7 animals in all: 2 animals had examination of the areas of skin which had alopecia, and 2 other animals also had skin sectioned and examined microscopically; 3 other animals had lung and trachea examined microscopically, and one of these also had microscopic examination of the thyroid. There were no important findings in any of the sections.

Blood was taken from a number of the animals for estimation of blood levels of the a.i., but this was not carried out, and the blood was preserved for future analysis if required. Similarly, the thyroids were removed and kept for future histological examination if required.
This was a very limited protocol, apparently directed entirely towards the problem of the alopecia found in the rat experiments; in this regard it was negative.

Ref.: 16

SCCS comments
A no adverse effect level seems not to have been established in this experiment.

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

Taken from previous opinion 1374/98

Rat

A 13 week study was performed following the OECD n° 408 guideline. The compound was administered by gavage in doses of 0, 100, 250 and 500 mg/kg bw/day, 5 days a week, to groups of 10 male and 10 female Sprague-Dawley rats.
Treatment was stopped after 6 weeks in one half of the animals in each group, and these served as recovery groups.
One female rat from the low dose group died at day 26 but it was not considered related to the treatment. Reversible ruffled and yellowish aspect of the furs were observed in all groups as well as depilation in high and mild dose groups and occasionally in low dose group. Decreased body weight gains were observed in males from the high dose group and in females from the high and mild dose groups. At day 13, a decrease in red blood cell count and haemoglobin concentration was observed in the high dose group. An increase in plasma lipids at 13 weeks in female animals at all dose levels. In addition the plasma cholesterol levels were increased in males at the top dose, and in females at the intermediate and top doses. No abnormality of lipids or of cholesterol was found in any animal after 6 weeks without treatment. A decrease testes weight was observed at the highest dose tested at day 13.

In conclusion, the authors considered that since changes in the aspect of the fur and a slight increase in serum total lipids level were seen in rats from the 100 mg/kg bw/day group (females only), this dosage level may represent the LOAEL.

Ref.: 3

Rat

A further study using groups of 25 males and females Sprague-Dawley rats was carried out, using doses of 0 (5 males and 5 females), 20 (10 males and 10 females) and 40 (10 males and 10 females) mg/kg bw/day in a similar manner. An abnormally high value for plasma lipids was found in females given 40 mg/kg bw/day; the increase at the lower dose was not significant. The no adverse effect level is probably 20 mg/kg bw/day.

Ref.: 3 bis

SCCS comments

The results of these two studies are scarcely reported in submission I report. In the 6 weeks toxicity study described above, effects were observed at the doses of 25 and 50 mg/kg bw/day. In the second study, based on the submission file, no change of biological relevance was seen in animals treated and 40 mg/kg bw/day could be considered as the NOAEL.

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

Taken from previous opinion 1374/98

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

An Ames test was carried out according to GLP, using strains TA 1525, TA 1537, TA 98 and TA 100. Toxicity experiments were not carried out; in strain TA 100 there was a decrease in

revertants with increasing dosage, suggesting some toxicity. Testing was carried out up to 1000 µg/plate in each strain. There was no evidence of mutagenic activity.

Ref.:10

A culture of Chinese hamster ovary cells was used to test for the production of chromosomal aberrations *in vitro* following the OECD guideline n°473. Without activation, doses up to 80 µg/ml were used, and with activation, doses up to 25 µg/ml. Mytomycin C and cyclophosphamide were used as control. There was a highly significant increase in aberrations in the preparation with activation in a dose of 25 µg/ml at 24h.

Ref.: 11

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

Active ingredient dissolved in peanut oil in a constant volume of 10 ml/kg was administered intra-peritoneally at the dose of 700 or 1400 (males), 800 or 1600 (females) mg/kg in OF1 mice. Bone marrow was sampled 24, 48 and 72h after injection. The toxicity on the bone marrow was checked by counting the ratio of normo and polychromatic erythrocytes. No genotoxic effect related to the test compound was observed.

Ref.: 12

SCCS comments

Based on the poor quality of the available tests, a firm conclusion on mutagenicity cannot be drawn.

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Taken from previous opinion 1374/98

Rat

An oral study according to GLP was carried out using groups of 24 mated female Sprague-Dawley rats dosed with 0, 15, 50, 100 and 150 mg/kg bw/day of a.i. The numbers of pregnant animals in each group were, respectively, 22, 23, 24, 24 and 22. The a.i. was suspended in 1% carboxymethylcellulose and given by gavage from days 6 to 15 of gestation. Groups of 5 animals were also dosed in the same manner and these were termed "satellite groups". The chief purpose of this part of the experiment was to obtain blood at the 10^{th} and 15^{th} days of pregnancy for the estimation of blood levels of a.i.; the animals were sacrificed at 15 days and autopsy and examination of the foetuses was carried out in these animals also.

There was one death at the top dose. Autopsy revealed the cause of death to be uterine haemorrhage. Clinical examinations showed that animals receiving 50 mg/kg bw/day and above had a reddish brown vaginal discharge. This was attributed to the number of resorptions occurring in these groups. At 50 mg/kg bw/day there was a significant dose related increase of pale extremities. Body weight gain was reduced at doses of 50 mg/kg bw/day and above, partly due to resorptions, but also an effect of the a.i. In the last 5 days of treatment, there was a reduced intake of food in animals receiving 50 mg/kg bw/day and above.

All animals were subjected to autopsy, and about half the foetuses examined for visceral abnormalities, and the remainder for bone abnormalities.

In the dams, the main abnormal findings were: enlarged spleens; haemorrhagic uteri; haemorrhagic and necrotic placentae; pale liver and kidneys. These were found only in animals receiving 50 mg/kg bw/day and above. Their incidence was dose related up to 100 mg/kg bw/day; the number of abnormal findings slightly decreased at 150 mg/kg bw/day. If the numbers of animals showing abnormalities are added up for each of the groups, the values are: 0, 0, 6, 32 and 6, respectively. There was increased foetal loss at doses of 50 mg/kg bw/day and above; some of the highest dose animals produced no living foetuses at all. The mean foetal weights decreased at 50 mg/kg bw/day and above, but the differences were not statistically significant. Increased major abnormalities were found in the foetuses of animals receiving 100 mg/kg bw/day and above; the increases were significant at 100 mg/kg bw/day -but not at 150 mg/kg bw/day, (although the latter figure was higher than the laboratory's background incidence); a significant dose related increase in minor abnormalities and retardation of ossification were found in the offspring of animals receiving 50 mg/kg bw/day and above. In the satellite animals, 2 at the top dose had vaginal discharge, and 1 animal had pale extremities; there were reductions of body weight gain and food consumption, and an increase in foetal toxicity.

This appears to have been a well conducted study, and is fully reported; there is clear evidence of embryo-toxicity at 50 mg/kg bw/day and above. The observed major external/visceral abnormalities (at 100 and 150 mg/kg bw/day), most plausibly result from retarded development and *in utero* pressure (the finding of retarded ossification is in line with this hypothesis). The development of these effects may be associated with the maternal toxicity (note that maternal toxicity is already present at 50 mg/kg bw/day).
The NOAEL for maternal toxicity and embryo-toxicity in this study is 15 mg/kg bw/day. In an appendix a method for the estimation of the blood levels by HPLC is given, but no figures for these values are provided.

Ref.: 18

SCCS comments

As reported in the Afssaps report, this study does not follow an OECD guideline. However the protocol seems to be very similar of the OECD TG 414 except that the dams were treated only until GD 15th (instead of GD 18th). A NOAEL of 15 mg/kg bw/day can then be derived from this study, based on maternal and embryo toxicity. This NOAEL can be used for risk assessment.

Mouse

A similar test to the above was carried out in cr1 CD1 (ICR) BR mice, in conformity with GLP.
Groups of 25 to 26 animals were mated; the numbers pregnant in each group were 26, 25, 23, 24, 24. Dosing was by gavage at levels of 0, 15, 50, 100 and 250 mg/kg bw/day, from day 6 to day 15 of pregnancy.

The a.i. was suspended in 1% aqueous carboxymethylcellulose. There was one death in the high dose group from an intubation error. Clinical examination revealed no important signs attributable to the a.i. There was no effect on body weight gain or food consumption in the dams.
The numbers of implantations, post implantation losses, and of live foetuses showed no significant differences between the groups. Necropsy of the dams showed no abnormality attributable to the a.i.
Foetal weights were not significantly different between the groups. There was no evidence of an increase in major abnormalities. A minor abnormality (extra ossification centres in the sternebrae) was found to be significantly higher than control only in the groups given 15 and 250 mg/kg bw/day. This finding was thought to be fortuitous. There was no evidence of teratogenic activity, or of toxicity in the dams, and analysis of the dose forms showed that the target concentrations were very nearly reached. A no effect level of 250 mg/kg bw/day is proposed by the authors.

Ref.: 19

Rabbit

Four groups of Himalayan rabbits of the HM strain were used. The a.i., suspended in 2% polysorbate 80, was administered by gavage over days 6 to 18 of gestation in doses of 0, 50, 150 and 450 mg/kg bw/day. The numbers of pregnant females in each group were, respectively, 11, 12, 12 & 11.
There were no deaths. Regular clinical examination showed no abnormalities. There was decreased body weight gain from days 6 to 10 in the middle and high dose animals, but the ultimate body weights at sacrifice (on day 29) showed no significant differences between the groups. Consumption of food and water is not reported.
One animal in group 3 (150 mg/kg bw/day) suffered a necrotic accessory lobe of liver with haemorrhage and death of all foetuses. At necropsy, all other dams displayed no abnormalities, and there was no evidence of teratogenic activity.

Ref.: 20

General comments of the SCCS on reproductive toxicity

Teratogenicity of 3-BC has been investigated in 3 species: rat, cr1 CD1 (ICR) BR mice and Himalaya rabbits. Both studies on rats and mice seem to have been well conducted even if they do not completely comply with an OECD guideline. The protocol seems to be very similar of the OECD TG 414 except that the dams were treated only until GD 15th. Based on the results of these 3 studies it seems that rats are more sensible to the teratogenic and foeto-toxic effects of 3-BC than the other species investigated. Indeed the effects reported in rat dams and foetuses have not been reproduced in mice and rabbits. The teratogenic effects observed may be associated with the maternal toxicity.

Since the previous opinion from the SCCNFP (1998), new studies reporting some reprotoxic effects of 3-BC were published:

In 2009, Faass *et al.* have investigated effects of 3-BC but also of 4-MBC (4-methylbenzylidene camphor) administered in chow to F0 rats before mating, during pregnancy and lactation and also to the F1 offspring until adulthood. Female sexual behaviour was recorded on videotape in adult female F1 on proestrus evening at the beginning of the dark phase. 3-BC at the doses of 2.4 and 7 mg/kg bw/day reduced proceptive behavior (jump and ear wiggling) and receptive behaviour (lordosis quotient), and increased rejection behaviour toward the male. Estrous cycles were also modified as well as expression of target genes (ERα, ERβ, SRC-1 and PR *(progesterone receptor)*) (see 3.3.12 for detail). The protocol of this study is not described in detail in the publication and without the original data, it is difficult to estimate how the observed effects may impair the reproductive function. This study has also some limitations: the study was performed in

separate experiments due to infrastructural limitations which makes it difficult to compare results for the different doses. Only females of F1 were studied.

In 2012, in a review, Krause *et al*., reported effects of selected UV-filters in cosmetic products including 3-BC. They reported results from *in vivo* (Schlumpf *et al*., 2004; Hofkamp *et al*., 2008) and *in vitro* studies (Kunz and Fent, 2006; Schreurs *et al.,* 2002. The data concerning influence of 3-BC on hormonal activities are discussed in section 3.3.12.

3.3.9. Toxicokinetics

Taken from previous opinion 1374/98

Investigation of metabolites

Hairless rat

Two groups of 5 female animals were used. The a.i. was made up as a suspension in propylene glycol/Tween 80/water and given to animals of the test group in a dose of 30 mg/kg bw by gavage. Animals of the control group received vehicle only. After 30 minutes, animals were anaesthetised with gamma-butyrolactone and bled. Plasma from the 5 animals in a group was pooled for analysis. Analysis was by HPLC; the internal standard was 3-(4'-methylbenzylidene)-d,1-camphor (S60). The sensitivity of the method enabled 2.5 ng/ml of a.i. to be detected, and 5 ng/ml and above to be quantified.

Preliminary experiments had suggested that the 4'-hydroxy derivative of the a.i. might be the main metabolite, and consequently 3-(4'-hydroxybenzylidene)-d,l-camphor was synthesised. Six peaks were found; the chief metabolite was, as predicted, 3-(4'-hydroxybenzylidene)-d,1-camphor. Some of the cis-isomer of the metabolite was also found. No values are given for the amounts found, or, at least, they could not be read on the microfiches supplied, which are not of the first quality.

Ref.: 22

Rat and human hepatocytes

Hepatocytes were obtained and cultured from (a) male SD rats (number of animals not stated) and (b) 4 human donors. Of the latter, the first was a woman of 55 with hepatic metastases from breast cancer; the second a man of 43 following an accident; the third a male of 53 with a myocardial infarct; and the fourth a female of 65 with hepatic metastases from colonic cancer. It is not clear whether the cells were obtained at operation or post mortem. The investigation started with previously frozen cells from donors 1 and 4; during the investigation fresh hepatocytes became available from donors 2 and 3. The a.i. was supplied as such and with a 14C label; the 4-hydroxy metabolite was also supplied. The a.i. was dissolved in DMS0 and suitably diluted.

It was found to penetrate rapidly into the hepatocytes, but it was also shown to adhere to the plastic of the tubes and wells used for the experiment, which led to some uncertainty about the percentages of metabolites produced. Some of the metabolites were the result of type II processes, and Helix pomatia extract was used as a source of sulfatase and glucuronidase to study these. The products of metabolism were studied by thin layer chromatography and by HPLC. The integrity of the cultured cells was studied morphologically and also by a study of a wide range of enzymatic activities.

The a.i. was toxic to human cells from about 5 x 10-5 M to 10-4 M. Rat cells were less susceptible to this effect.

Rat cells were found to metabolise the a.i. much more rapidly than human cells; an increase in metabolites was matched by a fall in the amount of a.i. There was a good deal of variability in enzymatic activity found in the cells from the 4 human donors. By thin layer chromatography some 10 metabolites were found, mostly in small quantities; by HPLC 4 major metabolites were found, of which one had the same retention time of the 4-hydroxy

metabolite. The report is somewhat difficult to follow in places, but it seems clear that numerous metabolites of the a.i. are formed in the systems studied, and are probably formed in man *in vivo* as well.

Ref.: 33

Pharmacokinetics

Hairless rat, intravenous

Fifteen female hairless rats were used for the test, and 10 for vehicle control. The a.i. was suspended in propylene glycol/water 75/25 and 3 mg/kg were injected into the tail vein under intraperitoneal gamma-butyrolactone anaesthesia. Sampling was carried out by anaesthecising 3 test animals and 2 control animals at each sampling time and taking blood from the abdominal aorta.

Sampling was at 0.25, 0.5, 1, 2 & 4 hours after administration. After extraction, plasma levels of a.i. and of its 4-hydroxy metabolite were estimated by HPLC. The internal standard was 3-(4'-methylbenzylidene)-d,l-camphor ("Fusolex 6300"). An additional procedure was to expose the 4-hydroxy metabolite (probably in methanol) to UV irradiation (wavelength not specified) before injecting onto the chromatogram. Under these conditions a further peak was obtained which was attributed to the cis-isomer of the metabolite.

The results show that the a.i. has a half-life of 8.35 hrs, and a terminal VD of 6.3 Litres. The 4-hydroxy metabolite appears within 15 minutes, and maintains a lower but parallel concentration throughout the experiment.

Ref.: 23

Hairless rat, oral

Female animals were used: 21 test and 14 control. The a. i. was dissolved in propylene glycol/water and 30 mg/kg were given by gavage under intraperitoneal gamma-butyrolactone anaesthesia. Blood samples were taken at (hours) 0.25. 0.5, 1, 2, 4, 6 and 24. Three test and 2 control animals were sacrificed under anaesthesia at each sampling time, and blood taken from the abdominal aorta.

The experimental procedure thereafter was identical with that of the preceding experiment

A feature of the values of a.i. in the test animals was a considerable variation between the animals at a given sampling time: e.g., at 0.5 hours, (ng/ml) 800, 14000, 7480; at 4 hrs, 16, 92, 1000. Variations in the concentrations of the metabolite were less marked.

Substantial amounts of the a.i. and its metabolite are found at 1 hour; the concentration of the metabolite is decidedly higher than that of the a.i. thereafter, and at 24 hours, when the level of the a.i. is below the limit of detection, the amount of metabolite present is substantial (mean value 23 ng/ml). This is attributed by the author to the presence of a saturable type II metabolic process in the disposition of the a.i.

Ref.:24

Mouse, oral

Swiss mice were used, 32 males and 32 females.

The a.i. was prepared as before (24) and given by gavage in a dose of 30 mg/kg bw. Sampling was at (hours) 0.25, 0.5. 1, 2, 4, 6 and 24; this was done under carbon dioxide anaesthesia and the animals sacrificed. In addition, a sample was taken from 4 male and 4 female animals which had not received any treatment. (Dosing and sampling were carried out by a different laboratory.

The findings show that the 4-hydroxy metabolite and the a.i. were present from 0.25 hours onwards, but the level of the metabolite was lower than that of the a.i., usually about one third to one half, with the exceptions of the 4 hour and 6 hour samplings, when the levels were about equal. Neither the a.i. nor the metabolite is detectable at 24 hours. There are differences between the findings in the male and female animals, but these do not seem to be systematic.

Ref.:25

Guinea pig, oral

The test was carried out in 32 Hartley animals, 16 males and 16 females. Dosing and blood sampling were carried out by CIT (28). Animals were given 30 mg/kg bw by gavage, except for 2 males and 2 females which had blood taken before any a.i. had been given. Of the remaining animals, 2 males and 2 females had blood removed from the abdominal aorta at the following times (hours): 0.25, 0.5, 1, 2, 4, 6 and 24.
Analysis was as described in the preceding experiment.

The results show that the levels of a.i. in the males reached a peak (of 36 ng/ml) at 1 hour; thereafter, the levels fell until there was none detectable at 24 hours. In the females, the levels were never detectable. The 4-hydroxy metabolite in the males showed a peak at 1 hour (98 ng/ml) and then fell progressively until none could be detected at 24 hours. In the females, the blood levels of the metabolite reached 3 ng/ml at 0.5 hours and 5 ng/ml at 1 hour; thereafter none could be detected. It seems that there is impaired absorption in female guinea pigs, compared with males, or that the metabolic handling of the drug is more rapid in females than in males, and perhaps different from the male in the metabolite produced or in its excretion.

Ref.: 26

Rabbit, oral

Groups of 2 NZW animals (1 male and 1 female) received 30 mg/kg bw by gavage. Sampling was carried out before the dose, and at 0.25, 0.5, 1, 2, 4 and 24 hours after it. (One male and 1 female animal was bled at each interval; after the second venepuncture, the animals were sacrificed, but no necropsy was carried out. Animal work was carried out by CIT (29).). The chemical investigation was as before. The plasma levels of a.i. were lower in females than in males: in males, the level peaked at 1 hour, at 87 ng/ml; in females at 0.25 hours at 34 ng/ml. It must be remembered, however, that each of these values represents a sample from one animal only. The levels of the 4-hydroxy metabolite were much lower, peaking at 10.5 ng/ml at 1 hour in the males, and 4.5 ng/ml in the females at 0.25 hours. The levels were so low that it was impossible to identify the metabolite definitely, but it was very probably the 4-hydroxy one as hitherto found.

Ref.: 27

Rat, oral

A similar procedure was carried out in groups of 4 SD rats (2 males and 2 females). In all, 8 such groups were used, being given 30 mg/kg bw of a.i. by gavage except for one group given vehicle only. Bleeding and sacrifice was carried out at (hours) 0, 0.25, 0.5, 1, 2, 4, 6, & 24. The animal work was carried out by CIT (32). The results show different patterns in the males and females.
In the males, the level of a.i. rises from 16 to 29 ng/ml from hours 0.25 to 4. It falls at 6 hours and is null at 24 hours. The plasma levels of the 4-hydroxymetabolite are similar but generally lower, though a little higher than the level of a.i. at 4 hours. In the females, the levels of a.i. are generally higher, peaking at 73 ng/ml at 0.25 hours, and thereafter falling. The levels of the 4-hydroxymetabolite are higher than those of the a.i. at all samplings, reaching 125 ng/ml at 1 hour, and remaining higher than the levels of the a.i. throughout the experiment.

Ref.: 31

SCCS Comments

These results show differences in the metabolism of 3-BC depending on the species and on sexes. Metabolism of 3-BC seems to be higher in rats compared to mice, guinea pigs and rabbits and in male rats compared to female rats. Based on *in vitro* study in rats and human hepatocytes, the applicant concludes that the metabolism profile is qualitatively similar in both species, but with important quantitative differences: the metabolism rate was 3 to 10 times higher in rat than in human hepatocytes. The applicant also considers that the higher level of the hydroxy metabolite in rats may explain the toxicity of 3-BC observed in rats in the toxicological studies by oral route. The SCCS considers that this hypothesis should be

confirmed by mechanistic explanation and especially a more comprehensive description of metabolism in human including human skin compared to rats.

Data published in the scientific literature and reported by Afssaps

- *Distribution of the UV filter 3-benzylidene camphor in rat following topical application* (Søeborg *et al.*, 2006)

This study concerns the development of an analytical method to assess the distribution of 3-BC in rats following topical application for 65 days. 3-BC (in 80/20 propylene glycol/isopropanol) was administered to 32 Sprague-Dawley rats (divided into 3 groups + control group) by topical application for 65 days at doses of 60, 180 and 540 mg/kg body weight/day. The animals were then euthanized by CO_2 gassing. The adipose tissue, brain, muscles and testicles were then removed to test for the presence of 3-BC. The concentration of 3-BC in the rat tissues are reported in the following table:

Table 1: Concentration of 3-BC found in different rat tissues in µg/g (according to Søeborg *et al.*, 2006)

Tissue	Control (n=3)	60 mg/kg body weight/day	180 mg/kg body weight/day.	540 mg/kg body weight/day
Adipose tissue	<LOQ	18.6+3.60	36.4+8.80	30.7+3.20
Brain	<LOQ	0.13+0.03	0.35+0.07	1.20+0.60
Liver	<LOQ	0.05+0.02	0.20+0.05	0.44+0.09
Muscle	<LOQ	0.18+0.06	1.00+0.60	1.30+0.70
Plasma (µg/l)	<LOQ	15.5+3.60	51.2+13.7	88.9+14.0
Testis	<LOQ	0.13+0.03	0.34+0.12	0.62+0.33

*n=8; LOQ = limit of quantification

All the tissues analysed show the presence of 3-BC after 65 days of exposure, with a higher accumulation in adipose tissue (see table 1). This can be explained by the $\log K_{ow}$ of 5.37 (lipophilic substance). Though, the highest concentrations are measured in the plasma.

Comments from the SCCS:
This study does not allow reaching a conclusion concerning the substance absorption rate. Although determining a rate of cutaneous penetration for 3-BC was not possible in this study, interesting information about the distribution of 3-BC into the body and target organs could be obtained.

3.3.10. Photo-induced toxicity

Taken from previous opinion1374/98

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

Rabbit, photo-toxicity
Six male and 6 female Albino Bouscat rabbits were used for the test and 6 animals were used as vehicle controls. One site about 5 X 5 cm in area was prepared on the left flank of each animal, and 2 on the right. The compound was made up as a 5% solution in 95% ethanol. One ml of this was applied to the left flank, and to one of the sites on the right flank, no application was made to the second site on the right flank. The sites on the right flank were irradiated with 1 med of ultraviolet light daily for 10 days. The irradiation was carried out by exposure to an "Osram Vitalux" lamp, but its spectral characteristics are not given. There was no evidence of phototoxicity.

Ref.: 8

Guinea pig, photo-sensitisation
Twelve male and 12 female albino Hartley animals were used in 3 groups.
Induction: Animals of group 1 (test) had injections of 0.2 ml of Freund's complete adjuvant in the foot on day 4 on days 1, 3, 5, 8 and 11, 0.5 ml of a 4% solution of the compound in olive oil was applied to a prepared site on the back of the neck, followed by exposure for 30 minutes to 2 lamps covering the range of 285 to 450 nm (UVA + UVB). Animals of group 2 (vehicle control) were similarly treated except that the applications were of olive oil only. Animals of group 3 had the Freund's adjuvant but no other treatment.
Challenge: On the 22nd day of the experiment, sites on either side of the lumbar vertebrae were prepared in all animals.
Applications of 10 µl of the following solutions were made to each side: olive oil; 2% of the compound in olive oil; 2% of the compound in ethanol. The sites were then exposed to irradiation by one of the lamps (320-450 nm: UVA) for 30 minutes. Readings were made at 24 and 48 hrs. There was no evidence of photo-sensitisation. There was no positive control, but the test was nevertheless judged by the authors to be satisfactory.

Ref.: 9

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

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3.3.11. Human data

No data submitted

3.3.12. Special investigations

Since the previous opinion, new studies have been published. Data published in the scientific literature reported by Afssaps and linked to endocrine effects are summarized below.

- Influence on estrogenic signalling
    - *Estrogenic activity and estrogen receptor beta binding of the UV filter 3-benzylidene camphor. Comparison with 4-methylbenzylidene camphor* (Schlumpf *et al.*, 2004a)

In this publication the oestrogenic potential of 3-BC was studied by two *in vitro* tests: a cell proliferation test on the oestrogen-dependent MCF-7 tumour cell line *(e-screen assay)* and a receptor (ERα or ERβ) ligand binding assay (3-BC), and an *in vivo* uterotrophic test on immature female rats.

The *in vitro* cell proliferation test shows that 3-BC produces cell hyper-proliferation, with an EC_{50} of 6.84 x 10^{-7} M, *versus* 1.03 x 10^{-12} M for the reference compound (17β-oestradiol). This indicates that 3-BC is very weakly oestrogenic compared to 17β-oestradiol.

The *in vitro* ligand-receptor interaction test shows that 3-BC binds preferentially to ERβ receptors.

The *in vivo* uterotrophic test was carried out in accordance with the OECD guidelines, but some details are missing: GLP, physical and chemical characterisation of the product, the choice of dosage (9 doses ranging from 0.8 to 300 mg/kg body weight/day) and even the choice of animal strain. A dose ranging from 0.8 to 300 mg/kg body weight/day was administered to each group of immature female rats by oral gavage for 3 days; they were then sacrificed 24 hours after the last dose. The results clearly show that, in the experimental conditions described by the authors, 3-BC produces a significant increase in uterine weight of immature rats, by comparison with the control group, from a dose of 2 mg/kg body weight/day (ED_{50}=45 mg/kg). The dose *per os* which produces no observed effect is 0.8 mg/kg body weight/day.

- *The chemical UV-Filter 3-BC causes an oestrogenic effect in an in vivo Fish Assay (Holbech et al., 2002)*

In this study, 3-BC was investigated for its capability to cause vitellogenin induction, possibly *via* oestrogen receptor binding, in the *in vivo* fish assay: juvenile raibow trout, Oncorhynchus mykiss, vitellogenin ELISA. A clear relationship was reported by the authors between the dose of intraperitoneally injected 3-BC and the concentration of plasma vitellogenin level. Effective dose-values (ED-values) were determined. ED10, ED50 and ED90 of 3-BC after 6 days (2 injections) were 6.4, 16 and 26 mg/kg/injection, respectively. These values place 3-BC among the more potent xenooestrogens (10 times as potent as 4-MBC). The authors considered that even if results in fish and mammals should be compared with great caution, their results support the findings of Schlumpf *et al*. (2001).

- *Estrogenic activity of UV filters determined by an in vitro reporter gene assay and an in vivo transgenic zebrafish assay* (Schreurs et al., 2002)

Schreurs *et al.* (2002) also investigated antagonistic oestrogenic activity of 3-BC and other UV-filters but did not report any effects of the tested compounds.

- Influence on androgen activity

  - *UV filters with antagonistic action at androgen receptors in the MDA-kb2 cell transcriptional-activation assay* (Ma *et al*., 2003)

The authors evaluated the anti-androgenic potential of different UV filters using transactivation technique of an androgen-dependent reporter gene. When the cells (MDA-kb2 human tumour cell line) are exposed to the mixture containing the reference androgen substance and the studied product, the anti-androgenic effect is revealed by the reporter gene expression decrease engendered by the reference product alone. In the case of 3-BC, no antagonist effect was observed. Therefore 3-BC does not interfere with the expression of androgen-dependent genes.

- *Endocrine activity and developmental toxicity of cosmetic UV filters – an update* (Schlumpf *et al.*, 2004b)

This publication summarizes the results published previously concerning the oestrogenic effects of several UV filters. In addition, the authors published the results concerning the

effect of those UV filters (including 3-BC and 4-MBC) on pre- and post-natal development. 3-BC was administered to groups of first-generation (F0) Long Evans male and female rats *per os* for 10 weeks at several doses: 0.24, 0.7, 2.4 and 7 mg/kg body weight/day. After mating, 3-BC was then administered to the female rats during pregnancy and lactation. The F1 rats were then treated until reaching adulthood. The choice of doses was based on the study described previously by Schlumpf *et al.* (2004a). Weight gain of pregnant rats was reduced by 3-BC (and not by 4-MBC), early postnatal survival rate and thymus weight by both compounds at higher doses. 4-Methylbenzylidene camphor and 3-BC delayed male puberty, and dose-dependently affected reproductive organ weights of adult male and female F1 offspring, with partly different effect patterns. Thyroid weight was increased by higher 4-MBC doses. Tissue-specific changes in mRNA levels of estrogen-regulated genes in prostate, uterus and brain regions, determined by real-time PCR, and in their response to acute estradiol challenge in adult gonadectomized offspring were observed. Lowest effective doses were 0.24 mg/kg/day for 3-BC and 7 mg/kg/day for 4-MBC. These results were also included in a review on the toxicity of UV filters from Schlumpf *et al.* (2008).

- Multiple hormonal activities

    - *Multiple hormonal activities of UV-filters and comparison of in vivo and in vitro estrogenic activity of ethyl 4-aminobenzoate in fish. Aquat Toxicol 79, 305-324* (Kunz and Fent, 2006)

In this publication, the authors investigated a series of UV filters including 3-BC for multiple hormonal activities *in vitro* in human receptor systems and evaluate the predictive value of these findings for the activity in fish *in vitro* and *in vivo*. They systematically analysed the estrogenic, antiestrogenic, androgenic, and antiandrogenic activity of 18 UV filters including 3-BC and one metabolite *in vitro* at non-cytotoxic concentrations with recombinant yeast systems carrying either a human estrogen (hERalpha) or androgen receptor (hAR). All 19 compounds elicited hormonal activities, most of them multiple activities. They found 10 UV-filters including 3-BC having agonistic effects towards the hER alpha and also anti estrogenicity. 3-BC completely inhibited the activity of E2 at the highest concentration tested (10^{-2} M) and produced full dose-response curve. They also identified six UV filters including 3-BC and 4-MBC with antiandrogenic activities. 3-BC was not found having androgenic activity in the hAR assay.

    - *UV Interaction of polycyclic musks and UV filters with the estrogen receptor (ER), androgen receptor (AR), and progesterone receptor (PR) in reporter gene bioassays. (Schreurs et al. ,2002).*

In this publication the authors assessed the interaction of five polycyclic musk compounds and seven UV filters including 3-BC with the estrogen receptor (ER), androgen receptor (AR), and progesterone (PR) receptor, using sensitive and specific reporter gene cell lines. 3-BC was found to be antagonists toward the AR and PR. Most effects were observed at relatively high concentrations (above 10^{-6} M).

- *Region-specific growth effects in the developing rat prostate following fetal exposure to estrogenic ultraviolet filters* (Hofkamp *et al.*, 2008)

This study concerns the effect of 3-BC and 4-MBC on the neonatal development of the prostate in rats. No effect is observed when 3-BC is administered to the animals (0.07 and 0.24 mg/kg body weight/day; 4 rats given each dose).

- *Female sexual behavior, estrous cycle and gene expression in sexually dimorphic brain regions after pre- and postnatal exposure to endocrine active UV filters* (Faass *et al.*, 2009)

In this study, the effect of 3-BC on rats sexual behaviour, oestrous cycle and the expression of target genes in different regions of the brain following pre- and post-natal administration *per os* was evaluated. The study was also carried out on the F1 generation. The sexual behaviour of F1 females and their oestrus cycle are modified by 3-BC at doses of 2.4 and 7 mg/kg body weight/day. The expression of target genes (ERα, ERβ, SRC-1 and PR *(progesterone receptor)*) is disrupted (increased or reduced, depending on the anatomical brain area) in both males and females at all doses (0.24, 0.7, 2.4 and 7 mg/kg body weight/day).

Setting aside the expression of the target genes, the first observable effects (sexual behaviour and modification of oestrus cycle) appear at the dose of 2.4 mg/kg body weight/day. Nevertheless, the authors do not indicate the number of animals in the parents (P) generation, while the four doses were administered not in parallel, but successively. Due to these shortcomings, the results of this study need to be confirmed and cannot be used to for calculation of the MOS but as supportive studies for risk assessment of 3-BC.

SCCS comment on studies on endocrine activity
Concerning the potential endocrine disruptor properties of 3-BC, multiple hormonal activities of 3-BC have been reported *in vitro*: estrogenic and anti-estrogenic effects as well antiandrogenic activities. *In vivo*, the expression of target genes (ERα, ERβ, SRC-1 and PR (progesterone receptor)) has been shown to be altered in both males and females rats at doses lower than the NOAEL used to calculate the MoS. Due to some shortcomings in the studies, the results need to be confirmed.

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Amount of cosmetic product applied daily	**F**	**=**	**18000 mg**
Concentration of ingredient in finished product	**C%**	**=**	**2%**
Total amount of active ingredient applied	**I=F x C/100**	**=**	**360 mg**
Typical body weight of human		**=**	**60 kg**
Absorption of active ingredient *	**A%**	**=**	**3.29%**
Total amount absorbed	**I x A/100**	**=**	**12.7 mg**
Systemic exposure dose (SED)	**9.8/60**	**=**	**0.21 mg/kg bw**
No Observed Adverse Effect Level (NOAEL) (teratogenicity study, maternal effects, oral, rat)		**=**	**15 mg/kg bw/d**
NOAEL corrected from bioavailability 50% (default)		**=**	**7.5 mg/kg bw/d**
MOS	**NOAEL/SED**	**=**	**36**

* This is the highest value of two experiments in man, which gave values of 3.29 and 1.9%

3.3.14. Discussion

3-Benzylidene camphor is proposed for use in sunscreen products at levels up 2%.

General Toxicity
Various batches used for toxicity testing have not been identified with respect to their purity and impurity. The homogeneity and stability of test solutions/suspensions have not been documented. Quantitative data on solubility in various solvents of 3-BC was not reported.

The toxicological evaluation is based on the previous SCCNFP opinion from 1998 (1374/98) and on the dossiers I, II, III and IV on the UV-filter 3-BC with the chemical name 3-benzylidenebornan-2-one submitted by COLIPA respectively in 1988, 1991, 1992 and 1994. The submissions files are only summarizing the experimental studies and original data were not made available to the scientific committee. Recently published articles on 3-BC retrieved from the scientific literature are also shortly described and discussed in the opinion. It concerned mainly endocrine properties of 3-BC and were also used to answer question 2 of the mandate.
Acute oral toxicity is low.

Irritation/Sensitisation
Tests for primary irritation of the skin and for irritation of the skin on repeated administration show only slight effects at 6%.
Tests for photo-toxicity and for photo-sensitisation were negative, although a positive control was not used for the latter.

Percutaneous absorption
Dermal absorption was investigated in male volunteers in two studies.
The SCCS considered that, the second study which has an adequate recovery rate could be used to estimate skin penetration. However, this study has a low number of male volunteers which does not allow to draw conclusions on the interindividual variability, the original data were not made available to the SCCS and no explanations were given to explain the higher absorption measured in the first study. The amount + 2SD of skin penetration should then be used for risk assessment: 1.89 + 2x0.70 = 3.29%.

Repeated dose toxicity
One 6 week oral study in the rat showed dose related increases in plasma triiodothyronine in males, significant at 50 mg/kg bw/day, and in plasma thyroxine in females, significant at 25 and 50 mg/kg bw/day.
In two 90 day oral toxicity studies in the rat, elevated plasma lipids were observed in female rats at doses as low as 20 mg/kg bw/day, although this was not statistically significant at this dose.
The results of the two 90 day studies are scarcely reported in submission I.
No NOAEL can be derived from these experiments.

Mutagenicity / Genotoxicity
A test for chromosomal aberration *in vitro* was positive, but the Ames test and an *in vivo* micronucleus test were negative. Based on the poor quality of the available tests, a firm conclusion on mutagenicity cannot be drawn.

Reproductive toxicity
In a teratogenicity study in rats, embryo-toxicity was observed at 50 mg/kg bw/day and above was observed. The observed major external/visceral abnormalities (at 100 and 150 mg/kg bw/day), most plausibly result from retarded development and *in utero* pressure (the finding of retarded ossification is in line with this hypothesis). The development of these effects may be associated with the maternal toxicity. The NOAEL for maternal toxicity and embryo-toxicity in this study is 15 mg/kg bw/day. This value was used for the MOS calculation.

Endocrine activity
In some recent studies, effect of 3-BC on rats sexual behaviour and oestrous cycle at low doses (2.4 and 7 mg/kg body weight/day) were reported. These effects may be due to endocrine activity of 3-BC. Multiple hormonal activities of 3-BC have indeed been reported *in vitro*: estrogenic and anti-estrogenic effects as well as anti-androgenic activities. 3-BC was not found having androgenic activity. *In vivo*, the expression of target genes (ERα, ERβ, SRC-1 and PR *(progesterone receptor)*) has been shown to be altered (increased or

reduced, depending on the anatomical brain area) in both males and females rats at all doses (0.24, 0.7, 2.4 and 7 mg/kg body weight/day).

Pharmacokinetics

Pharmacokinetic studies show differences in the metabolism of 3-BC depending on the species and on sexes. Metabolism of 3-BC seems to be higher in rats compared to mice, guinea pigs and rabbits and in male rats compared to female rats. The reasons for this discrepancy are not known. Based on *in vitro* study in rats and human hepatocytes, the applicant concludes that the metabolism profile is qualitatively similar in both species, but with important quantitative differences: the metabolism rate was 3 to 10 times higher in rat than in human hepatocytes. The applicant also considers that the higher level of the hydroxy metabolite in rats may explain the toxicity of 3-BC observed in rats in the toxicological studies by oral route. The SCCS considers that this hypothesis should be confirmed by mechanistic explanation and especially a more comprehensive description of metabolism in human including human skin compared to rats.

4. CONCLUSION

Due to MoS < 100, the SCCS considers that the use of 3-benzylidene-camphor as a UV-filter in cosmetic products in a concentration up 2.0% is **not** safe.

Concerning the potential endocrine disruptor properties of 3-BC, multiple hormonal activities of 3-BC have been reported *in vitro*: estrogenic and anti-estrogenic effects as well anti-androgenic activities. *In vivo*, the expression of target genes (ERα, ERβ, SRC-1 and PR (progesterone receptor)) has been shown to be altered in both males and females rats at doses lower than the NOAEL used to calculate the MoS. Due to some shortcomings in the studies, the results need to be confirmed.

5. MINORITY OPINION

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