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

CONCERNING

BERGAMOTTIN

adopted by the SCCNFP during the 25th plenary meeting of
of 20 October 2003

1. Terms of Reference

1.1 Context of the question

The SCCNFP adopted during the 18th plenary meeting of 25 September 2001 an opinion concerning an Initial List of Perfumery Materials which must not form part of Cosmetic Products except subject to the restrictions and conditions laid down (doc. n° SCCNFP/0392/00).
The opinion is based on information submitted as 'monographs' (synopses) on behalf of industry. On the basis of the available information and assessment of the cutaneous toxicity of the substances tabulated in the opinion, it is the recommendation of the SCCNFP that these substances may be used as ingredients in cosmetic products only under the conditions and restrictions specified in the table attached to the opinion.

The European Commission received in July 2002 a letter from the European Flavour and Fragrance Association (EFFA) announcing that a report on the photo-mutagenic properties of furocoumarins in essential oils would be sent to the Commission services by September 2002. The letter also included an updated IFRA Standard on Methyl-N-methylanthranile.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* *Does the data provided justify an update of the "Initial List of fragrance" for n° 6 and n° 21 of the table attached to this opinion (An Initial List of Perfumery Materials which must not form part of Cosmetic Products except subject to the restrictions and conditions laid down [SCCNFP/0392, Adopted 25.09.01]) and how should the restrictions and conditions laid down be changed accordingly?*

The restriction in No 6 reads: *May be used in cosmetic products, provided that the total concentration of furocoumarin-like substances in the finished cosmetic product do not exceed 1 ppm.*

No 21 concerns *Methyl N-methylanthranilate* and will not be addressed in the present Opinion.

The present Opinion will primarily deal with questions concerning photomutagenicity of bergamottin.

1.3 Statement on the toxicological evaluation

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

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation

with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.

The extent to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

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

2. Toxicological Evaluation and Characterisation

Introduction

This opinion is prepared in the response of a request from European Flavour & Fragrance Association (EFFA) to DG Enterprise stating :

> *The Fragrance Industry would like to inform you that in the last few days new data has become available on furocoumarins (see table 1 number 6 of the opinion).*
>
> *The SCCNFP treated all furocoumarins as one family and this cannot be accepted by our industry because they are clearly not equal. Only those known to be photomutagenic should be mentioned on the list.*
>
> *The Fragrance industry recently tested Bergamottin on photomutagenicity and the result was negative. That explains that not all furocoumarins are photomutagenic and should therefore not be treated in the same way.*
>
> *Bergamottin is a furocoumarin which is found in several citrus oils in larger quantities than 5-methoxypsoralen which is the major photomutagenic furocoumarin in these oils. Restricting all furocoumarins as proposed would therefore impose an unnecessary and unjustified restriction on these important oils.*

In order to exclude bergamottin from the group of furocoumarins in the *"Initial List of fragrance" for No 6.* it will be necessary to demonstrated that it is not likely to be photomutagenic.

In Submission I from EFFA *Furocoumarin content of essential oils* (dated 05.11.02) it was stated "*2.4.1 The photomutagenicity of bergamottin was investigated in Salmonella typhimurium tester strains TA98, TA100, TA1535 and TA1537 and Escherichia coli strain WP2."* No data concerning Escherichia coli have, however, been submitted.

2.1. General

2.1.1. Primary name

Bergamottin

2.1.2. Chemical names

7H-Furo(3,2-g)(1)benzopyran-7-one, 4-((3,7-dimethyl-2,6-octadienyl)oxy)-, (E)-
5-Geranoxypsoralen
Bergamotine

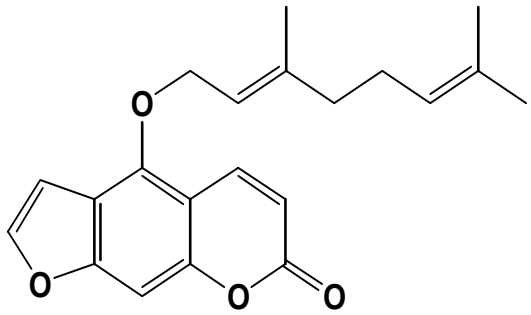
2.1.3. Trade names and abbreviations

Not available

2.1.4. CAS no. and EINECS no.

CAS no. : 7380-40-7
EINEC no : 200-782-5

2.1.5. Structural formula



2.1.6. Empirical formula

$C_{21}H_{22}O_4$

2.1.7. Purity, composition and substance codes

Batch no. 02060607. Purity stated as >99%.

2.1.8. Physical properties

Subst. Code	:	/
Appearance	:	Off white powder
Melting point	:	/
Boiling point	:	/
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{ow}	:	/
Flash point	:	/

2.1.9. Solubility

Bergamottin is poorly soluble in water, but readily soluble in alcohol (no specific numbers given).

Ref. : 1

2.2 Function and Uses

Bergamottin is a natural chemical belonging to the group furocoumarins. It is a major component of bergamot oil (2.2%) and cold pressed lime oil (2.5%).

Furocoumarins constitute a family of natural chemicals present in different plant extracts. These plant extracts are widely used as ingredients in fragrances.

Due to the phototoxic, photomutagenic and photocarcinogenic properties reported for certain furocoumarins, they are not permitted for use in cosmetic products as such, except for the normal content in natural essences if the total concentration of furocoumarin-like substances in the finished cosmetic product do not exceed 1 ppm.

TOXICOLOGICAL CHARACTERISATION

The present opinion will primarily deal with the photomutagenic properties of bergamottin.

2.3. Toxicity

Not evaluated

2.4. Irritation & corrosivity

Not evaluated

2.5. Sensitisation

Not evaluated

2.6. Reproductive toxicity

Not evaluated

2.7. Toxicokinetics (incl. Percutaneous Absorption)

In vitro studies have shown that bergamottin inhibits cytochrome P450 CYP3A and CYP1A1/2 enzymatic activities and induction of the corresponding proteins and mRNAs. This finding may explain why bergamottin inhibited metabolic activation of benzo(a)pyrene in cultured mouse keratinocytes and specifically inhibited the formation of DNA adducts derived from the anti diol-epoxide diastereomers from benzo(a)pyrene.

Ref. : 2, 3

2.8. Genotoxicity

See section 2.10.5 on Photomutagenicity

2.9. Carcinogenicity

2.9.1. Animal studies

Mouse

In initiation – promotion skin painting experiments with Sencar mice using benzo(a)pyrene as initiator and TPA as promoter, it was found that bergamottin inhibited skin papilloma formation when applied once to the skin 5 minutes prior to the initiator. The mechanism for reducing papilloma formation is probably due to the ability of bergamottin to inhibit cytochrome P450s involved in the metabolic activation of the initiator. See section 2.7. Toxicokinetics (incl. Percutaneous Absorption).

Ref. : 4

2.9.2. Human studies

No data

2.10. Special investigations

2.10.1. Photochemical properties

Bergamottin has been reported to undergo degradation with fairly high photoreactivity when irradiated with 361 nm UV light. Two photoproducts can be detected by HPLC. At low bergamottin concentration (30 μM) the photodegradation quantum yield is about 0.2. The oxygen effect on bergamottin photodegradation suggests involvement of the bergamottin triplet state in the photochemistry. It was concluded that bergamottin must be considered as a potential photosensitising compound in biological systems.

Ref. : 1

It has been reported that bergamottin did not cause phototoxicity when tested on guinea pig and rabbit skin.

Ref. : 5

2.10.2. Photosensitisation / Photoallergy

No data

2.10.3. Photomutagenicity

2.10.3.1. Photomutagenicity/Genotoxicity, *in vitro*

In order to evaluate the *in vitro* photogenotoxic data available for bergamottin, it is useful to consider information given in monographs by International Agency for Research on Cancer (IARC) concerning furocoumarins and UVA irradiation (see Table 1).

Table 1. *Overall assessment of data from bacteria (or isolated DNA) and mammalian cells from short-term in vitro tests in the present of UVA (320 – 400 nm [max 355 nm]) irradiation and degree of evidence for animal carcinogenesis (A) and activity in short-term tests (G).*

Substance	**Organism**	**DNA damage**	**Muta-tion**	**Chromoso-mal effects**	**IARC[A] A G**
Angelicin	Bacteria (or isolated DNA) Mammalian cells	+ +	+	 +	L S
5-Methyl-angelicin	Bacteria (or isolated DNA) Mammalian cells	+ +[3]	+ +	 +	L S
4,4'-Dimethyl-angelicin	Bacteria (or isolated DNA) Mammalian cells	+	+		N L
4,5'-Dimethyl-angelicin	Bacteria (or isolated DNA) Mammalian cells	+ +	+ +		L S
4,4',6-Trimethyl-angelicin	Bacteria (or isolated DNA) Mammalian cells	+	+		N I
3-Carbethoxy-psoralen	Bacteria (or isolated DNA) Mammalian cells	+ +[1]	 +	 +	N S
5-Methox-psoralen	Bacteria (or isolated DNA) Mammalian cells	+ +	+ +	 +	S S
8-Methoxy-psoralen	Bacteria (or isolated DNA) Mammalian cells	+ +	+ +	 +	S S
Pyrido[3,4-c]-psoralen	Bacteria (or isolated DNA) Mammalian cells	+	 +[2]	 +	I S
7-Methylpyrido-[3,4-c]psoralen	Bacteria (or isolated DNA) Mammalian cells	+ +	 +[2]	 +	I S
4,5',8-Trimethyl-psoralen	Bacteria (or isolated DNA) Mammalian cells	+ +	+	 +	I S

Fungi/Green plant and Insects not included in the Table.

[A]Degree of evidence in evaluation by IARC. S = Sufficient evidence, L = Limited evidence, I = Inadequate evidence N = No data.

[1]Gunther EJ, Yeasky TM, Gasparro FP, Glazer PM. Mutagenesis by 8-methoxypsoralen and 5-methylangelicin photoadducts in mouse fibroblasts: Mutations at cross-linkable sites induced by monoadducts as well as cross-links. Cancer Res 55: 1283-1288, 1995.

[2]Moysan A, Vigny P, Dardalhon M, Averbeck D, Voituriez L, Cadet J. 3-Carbethoxypsoralen-DNA photolesions: Identification and quantitative detection in yeast and mammalian cells of the two *cis-syn* diastereoisomers formed with thymidine. Photochem Photobiol 47: 803-808, 1988.

[3]Papadopoulo D, Moustacchi E. Mutagenic effects photoinduced in normal human lymphoblasts by a monofunctional pyridopsoralen in comparison to 8-methoxypsoralen. Mutat Res 245: 259-266, 1990.

Ref. : 6, 7

Bacterial Reverse Mutation Test

Guideline	:	-----
Species/strain	:	*Salmonella typhimurium,* TA98, TA100, TA1535, TA1537, TA102
Replicates	:	Triplicate plates
Test substance	:	Bergamottin in DMSO solution
Batch no	:	02060607, purity stated as >99%
Concentrations	:	6 concentrations covering two logarithmic decades from 15.8-5000 μg/plate without metabolic activation
GLP	:	Quality Assurance Statement included

Bergamottin has been investigated for gene mutation in *Salmonella typhimurium,* using the direct plate incorporation method without S9 mix in the presence and absence of UVA (10 and 20 mJ/cm^2 for strain TA98, positive control 2-nitro-fluorene, 2.3 and 3.8 mJ/cm^2 for strain TA100, positive control sodium azide, 12.3 and 23.8 mJ/cm^2 for strain TA1535, positive control sodium azide, 7.7 and 16.1 mJ/cm^2 for strain TA1537, positive control 9-aminoacridine and DMBA, and 59.9 and 120.6 mJ/cm^2 for strain TA102, positive control glutaraldehyde and 8-methoxypsoralen. No controls were used in the UVA dose experiments except for TA1537 (DMBA) and TA100 (8-methoxypsoralen) in the high UVA dose experiments. Because of the lack of toxicity in the preliminary dose range finding assay, the concentration range of 15.8 - 5000 μg/plate was selected

Conclusions
The authors concluded that bergamottin did not induce mutation in five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA102), when tested under the conditions employed for this study. These conditions included treatments of concentrations up to 5000 μg/plate (a precipitating dose level) alone and at two separate UV light exposure levels appropriate for each strain.

Ref. : 8

Comments
The Heraeus Suntest CPS solar simulator lamp used emits radiation across a spectrum similar to that of natural solar radiation. It is not clear how the UVA spectrum was obtained and the location of the maximum energy. It is not possible from the data submitted to assess if it had been appropriate to use higher UVA doses. 8-Methoxypsoralen has been demonstrated to be photomutagenic not only for TA102, but also for several other strains. Consequently, it should have been used as a positive control also in the case of the other strains where it is photomutagenic.

In order to study possible mutagenic effects of bergamottin in the dark, the substance should also have been tested in the presence of an exogenous metabolic system.

2.10.3.2. Mutagenicity/genotoxicity, *in vivo*

No data

2.11. Groups at extra risk

Not evaluated

2.11. Safety evaluation

2.11.1. Assessment of human exposure

Not evaluated

2.11.2. Conclusion

Photomutagenicity and photocarcinogenicity are the main effects of concern in relation to the use of furocoumarins in cosmetics. The data submitted by EFFA on bergamottin is not adequate for evaluation of the safety of the substance in relation to photomutagenicity and photocarcinogenicity.

2.12. References

1. Morlière P, Bazin M, Dubertret L, Santus R, Sa E Melo T, Hüppe G, Haigle J, Forlot P, Bernard A. Photoreactivity of 5-geranoxypsoralen and lack of photoreaction with DNA. Photochem Photobiol 53: 13-19, 1991.
2. Wen YH, Sahi J, Urda E, Kulkarni S, Rose K, Zheng X, Sinclair JF, Cai H, Strom SC, Kostrubsky VE. Effects of bergamottin on human and monkey drug-metabolizing enzymes in primary cultured hepatocytes. Drug Metabolism Disposition 30: 977-984, 2002.
3. Cai Y, Baer-Dubowska W, Ashwood-Smith M, DiGiovanni J. Inhibitory effects of naturally occurring coumarins on the metabolic activation of benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene in cultured mouse keratinocytes. Carcinogen 18: 215-222, 1997.
4. Cai Y, Kleiner H, Johnston D, Dubowski A, Bostic S, Ivie W, DiGiovanni J. Effect of naturally occurring coumarins on the formation of epidermal DNA adducts and skin tumors induced by benzo(a)pyrene and 7,12-dimethylbenz(a)anthrecene in SENCAR mice. Carcinogen 18: 1521-1527, 1997.
5. Naganuma M, Hirose S, Nakayama Y, Nakajima K, Someya T. A study of the phototoxicity of lemon oil. Arch Dermatol Res 278: 31-36, 1985.
6. IARC Furocoumarins. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans 40: 289-376, 1986.

7. IARC 5-Methoxypsoralen and 8-Methoxypsoralen plus ultraviolet irradiation. Monographs on the Evaluation of Carcinogenic Risks to Humans. Suppl 7: 242-245, 1987.
8. Ballantyne M. Bergamotin: Reverse mutation in five histidine-requiring strains of Salmonella typhimurium, in the presence of ultra violet light. Final Report. Part of Submission I from EFFA, 2002.

3. Opinion

SCCNFP concludes that it has insufficient information regarding the photo-toxic potential of bergamottin to perform an adequate safety evaluation. Consequently, the SCCNFP is unable to provide an update of entry n° 6 of Table 1 -List of perfumery materials which must not form part of cosmetic products except subject to the restrictions and conditions laid down – of the opinion concerning an Initial List of Perfumery Materials which must not form part of Cosmetic Products except subject to the restrictions and conditions laid down (doc. N° SCCNFP/0392/00 of 25.09.01).

Data on its mutagenic/genotoxic potential are needed.