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

CONCERNING

ISOPIMPINELLIN

adopted by the SCCNFP during the 26th plenary meeting of
of 9 December 2003

1. Terms of Reference

1.1 Context of the question

The adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following question :

- *Does the data provided justify an update of the "Initial List of fragrance" for No 6 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 furocumarin-like substances in the finished cosmetic product do not exceed 1 ppm.*

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

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 Flavor & Fragrance Association (EFFA) to DG Enterprise stating (letter of 17.07.02):

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 (See Opinion SCCNFP/0740/03, final adopted 20.10.03) *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.*

A second submission concerning the photomutagenicity of the furocoumarin isopimpinellin was submitted 26.09.03.

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

In Submission II from EFFA *Furocoumarin content of essential oils* (dated 21.08.03) it was stated "*In recent studies on two of the major furocoumarins in citrus oils; bergamottin showed no evidence of photomutagenicity* [Submission I], *and isopimpinellin was photomutagenic in one of the five* [Salmonella typhimurium] *strains tested.*

2.1. General

2.1.1. Primary name

Isopimpinellin

2.1.2. Chemical names

4,9-dimethoxy-7H-furo(3,2-g)(1)benzopyran-7-one,
5,8-Dimethoxypsoralen

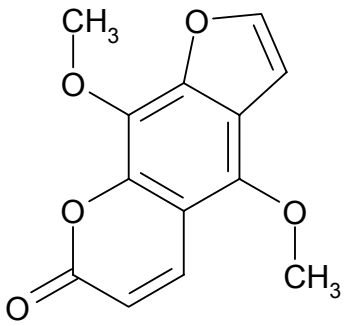
2.1.3. Trade names and abbreviations

Not available

2.1.4. CAS no. and EINECS no.

CAS no : 482-27-9
EINECS no : /

2.1.5. Structural formula



2.1.6. Empirical formula

$C_{13}H_{10}O_5$

2.1.7. Purity, composition and substance codes

Batch no. : 02093005
Purity : not stated.

2.1.8. Physical properties

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

2.1.9. Solubility

/

2.2 Function and Uses

Isopimpinellin is a natural chemical belonging to the group furocoumarins. It is a component of cold pressed lime oil (0.2% [2 000 ppm]) and rue oil (0.02% [200 ppm]).

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 isopimpinellin.

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)

Not evaluated

2.8. Genotoxicity

See section 2.10.5. Photomutagenicity.

2.9. Carcinogenicity

2.9.1. Animal studies

Mice

Oral administration of isopimpinellin has been found to inhibit ethoxyresorufin O-deethylase (EROD) and pentoxyresorufin O-dealkylase (PROD) activity in epidermis as well as in lung and forestomach of SENCAR mice. Oral treatment with isopimpinellin also increased liver cytosolic glutation S-transferase (GST). Oral administration of isopimpinellin reduced DNA adduct levels in liver and lung due to BaP and mammary epithelial cells due to DMBA. Moreover, isopimpinellin reduced DNA adduct formation in skin and tumour initiation by polycyclic aromatic hydrocarbon. Overall, it was found that isopimpinellin has a chemoprotective effect when administered orally on skin tumour initiation by BaP and DMBA in SENCAR mice.

Ref. : 1, 2, 3

2.9.2. Human studies

No data

2.10. Special investigations

2.10.1. Photochemical properties

Isopimpinellin has been reported to be active in the light-dependent induction of crosslinks in viral DNA.

Ref. : 4

The same authors that performed the experiment above (ref. 4) has later tested possible phototoxicity toward three target viruses, viz: bacteriophage T4; and the animal viruses murine cytomegalovirus (MCMV) and Sindbis virus (SV). All three of these viruses were readily inactivated in UVA by 8-MOP. The compounds were also tested against 3T3-L1 cells. While 8-MOP gave the predictable effects, isopimpinellin showed no significant phototoxicity to any of the organisms.

Ref. : 5

Later, it was reported that isopimpinellin of very high purity was not active in a photoassay that utilized a DNA repair mutant of *Escherichia coli*. These investigators concluded that pure isopimpinellin is photobiologically inactive and that reports of its photoactivity could most likely be attributed to the presence of active psoralens (such as bergapten) present as impurities in the isopimpinellin samples used in the assays.

Ref. : 6

Two studies have been performed in relation to the photoactivity of isopimpinellin on chick skin. In the first study, isopimpinellin was found to be phototoxic. The substance used in the study was obtained from the plant *Ammi majus,* which also contained a number of other highly phototoxic psoralens. In the second study with synthetic isopimpinellin, no phototoxicity was demonstrated, and it was claimed that the results from the first study, which was also performed in the same laboratory, was due to the present of impurities (2.4% xanthoxin and 0.013% bergapten). It is stated that the synthetic isopimpinellin contained at most only trace level of bergapten [detection limit 0.007%] and xanthoxin [detection limit 0.01%]).

Ref. : 7, 8

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 isopimpinellin, 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-Methoxy-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. : 9, 10

Bacterial Reverse Mutation Test

Guideline	:	/
Species/strain	:	*Salmonella typhimurium,* TA98, TA100, TA1535, TA1537, TA102
Replicates	:	Triplicate plates
Test substance	:	Isopimpinellin in DMSO solution
Batch no	:	02093005, purity not stated
Concentrations	:	6 concentrations covering two logarithmic decades from 3.16-1000 μg/plate without metabolic activation
GLP	:	Quality Assurance Statement included

Isopimpinellin 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 and 4 mJ/cm^2 for strain TA100, positive control sodium azide, 12 and 24 mJ/cm^2 for strain TA1535, positive control sodium azide, 8 and 16 mJ/cm^2 for strain TA1537, positive control 9-aminoacridine and DMBA, and 60 and 120 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). The concentration range of 15.8 - 5000 μg/plate was selected.

Conclusions
The authors concluded that isopimpinellin did not induce mutation in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537), when tested under the conditions employed for this study. These conditions included treatments of concentrations up to 1000 μg/plate (a precipitating dose level) alone and at two separate UV light exposure levels appropriate for each strain. In strain TA102 isopimpinellin did not induce mutation in the absence of UV light, but induced mutation in the presence of UV light. The photomutagenicity of isopimpinellin was confirmed in a second experiment.

Ref. : 11

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 isopimpenellin 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. Effects of concern

Photomutagenicity and photocarcinogenicity are the main effects of concern in relation to the use of furocoumarins in cosmetics. The data submitted by EFFA on isopimpinellin is not adequate for evaluation of the safety of the substance in relation to photomutagenicity and photocarcinogenicity. The submitted data demonstrated that isopimpinellin is photomutagenic in *Salmonella typhimurium* TA102.

2.12. Opinion

The submitted data demonstrated that isopimpinellin is photomutagenic in *Salmonella typhimurium* TA102.

SCCNFP is of the opinion that there is incomplete information on photomutagenicity and on photoclastogenicity of isopimpinellin to enable a safety evaluation in order to provide an update of the “Initial List of fragrance” for No 6 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 [doc. n° SCCNFP/0392, 25.09.01]).

2.13. References

1. 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)anthracene in SENCAR mice. Carcinogenesis 18: 1521-1527, 1997.
2. Kleiner HE, Vulimiri SV, Miller L, Johnson WH, Whitman CP, DiGiovanni J. Oral administration of naturally occurring coumarins leads to altered phase I and II enzyme

activities and reduced DNA adduct formation by polycyclic aromatic hydrocarbons in various tissues of SENCAR mice. Carcinogenesis 22: 73-82, 2001.

3. Kleiner HE, Vulimiri SV, Starost MF, Reed MJ, DiGiovanni J. Oral administration of the citrus coumarin, isopimpinellin, blocks DNA adducts formation and skin tumor initiation by 7,12-dimethylbenz(a)anthracene in SENCAR mice. Carcinogenesis 23: 1667-1675, 2002.
4. Altamirano-Dimas M, Hudson JB, Towers GHN. Induction of cross-links in viral DNA by naturally occurring photosensitizers. Photochem Photobiol 44: 187-192, 1986
5. Hudson JB, Miki T, Towers GH. Isopimpinellin is not phototoxic to viruses and cells. Planta Med 53: 306-307, 1987.
6. Ashwood-Smith MJ, Ceska O, Warrington PJ. Isopimpinellin is photobiologically inactive. Photochem Photobiol 56: 563-565, 1992.
7. Ivie GW. Linear furocoumarins (psoralens) from the seed of Texas *Ammi majus* L. (bishop's weed). J Agric Food Chem 26: 1394-1403, 1978.
8. Ivie GW, Beier RC. Isopimmpinellin is not phototoxic in a chick skin assay. Photochem Photobiol 63: 306-307, 1996.
9. IARC Furocoumarins. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans 40: 289-376, 1986.
10. IARC 5-Methoxypsoralen and 8-Methoxypsoralen plus ultraviolet irradiation. Monographs on the Evaluation of Carcinogenic Risks to Humans. Suppl 7: 242-245, 1987.
11. Ballantyne M. Isopimpinellin: Reverse mutation in five histidine-requiring strains of Salmonella typhimurium, in the presence of ultra violet light. Research Institute for Fragrance Materials, Inc., 2003.