

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

CONCERNING

FUROCOUMARINS IN SUN PROTECTION AND BRONZING PRODUCTS

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.

Commission Directive 95/34/DC of 10 July 1995 amended Annex II, reference number 358 as follows: “Furocoumarines (e.g. trioxysalan, 8-methoxypsoralen, 5-methoxypsoralen) except for normal content in natural essences used. In sun protection and in bronzing products, furocoumarines shall be below 1 mg/kg.” The technical adaptation was based on an opinion adopted by the Scientific Committee on Cosmetology (SCC) in 1990. Furocoumarines are recognized to photomutagenic and photocarcinogenic. The SCC had not been able to conclude from the available scientific, technical and epidemiological data at that time that the association of protective filters with furocoumarines would guarantee the safety of sun protection and bronzing products containing furocoumarines above a minimum level. Therefore, in order to protect public health, furocoumarines were limited to less than 1 mg/kg (1 ppm) in these products.

The European Commission received in July 2003 a letter from Jean-Jacques Goupil indicating that new documents on the safety and efficacy of sun protection and bronzing products with an efficient dose of 15 to 60 ppm 5-methoxypsoralen had been transmitted to Health and Consumer Protection DG. Dr. Patricia Martin-Lamanthe supported the request by another letter in July 2003.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following question :

- *Does the data provided justify a higher limit for furocoumarins in sun protection and bronzing products?*
- *If yes, which limit for furocoumarines is scientifically justified in sun protection and bronzing products in association with UV-filters?*

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

The present Opinion will primarily deal with questions concerning photomutagenicity and photocarcinogenicity of the furocoumarines 5-methoxypsoralen and 8-methoxypsoralen.

2.1. General

2.1.1. Primary name

5-Methoxypsoralen (5-MOP)

8-Methoxypsoralen (8-MOP)

2.1.2. Chemical names

5-MOP

Chem. Abstr. Name: 4-Methoxy-7H-furo[3,2-g][1]benzopyran-7-one

IUPAC Systematic Names: 6-Hydroxy-4-methoxy-5-benzofuranacrylic acid, δ -lactone; 4-methoxy-7H-furo[3,2-g][1]benzopyran-7-one

8-MOP

Chem. Abstr. Name: 7H-Furo [3,2g] [1] benzopyran-7-one, 9-methoxy-

IUPAC Systematic Name: 9-Methoxy-7H-furo[3,2g] benzopyran-7-one; 6-Hydroxy-7-methoxy-5-benzofuranacrylic acid δ -lactone

2.1.3. Trade names and abbreviations

5-MOP

Bergaptan; bergapten; bergaptene; heraclin; majudin; 5-methoxy-6,7-furanocoumarin

8-MOP

6,7-furocoumarin; 9-methoxypsoralen; metoxsalen; oxypsoralen

Ammoidin; Meladinin (VAN); Meladinine; Meladoxen; Meloxine; Methoxa-Dome; Mopsoralen; Oxsoralen; Soloxsalen; Trioxun; Xanthotoxin; Xanthotoxine

2.1.4. CAS no. and EINECS no.

5-MOP

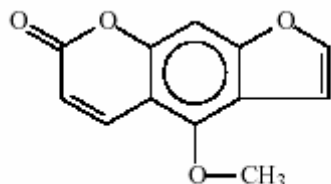
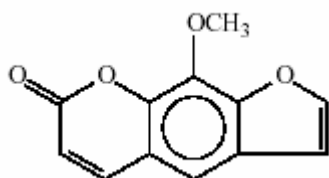
CAS no : 484-20-8

EINEC no : 207-604-5

8-MOP

CAS no : 298-81-7

EINEC no : 206-066-9

2.1.5. Structural formula**5-MOP****8-MOP****2.1.6. Empirical formula and molecular weight****5-MOP**C₁₂H₈O₄ Mol.wt: 216.19**8-MOP**C₁₂H₈O₄ Mol.wt: 216.19**2.1.7. Purity, composition and substance codes**

One preparation of 5-MOP contained 7.3% of a dimethoxypsoralen isomer
8-MOP is available in USA as a USP grade with 98.0-102.0% active substance

2.1.8. Physical properties**5-MOP**

Subst. Code : /
Appearance : Needles when crystallized from ethanol
Melting point : 188°C with sublimation
Boiling point : /
Density : /
Rel. vap. dens. : /
Vapour Press. : /
Log P_{ow} : /
Flash point : /

8-MOP

Subst. Code	:	/
Appearance	:	White to cream-coloured, odourless, fluffy, needle-like crystals
Melting point	:	143-148°C
Boiling point	:	/
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P _{ow}	:	/
Flash point	:	/

2.1.9. Solubility**5-MOP**

Practically insoluble in water, slightly soluble in glacial acetic acid, benzene and warm phenol, soluble in absolute ethanol.

8-MOP

Practically insoluble in cold water; sparingly soluble in boiling-water and diethyl ether; soluble in boiling ethanol, acetone, acetic acid, vegetable oils, propylene glycol, benzene and chloroform.

2.2 Function and Uses

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 does not exceed 1 ppm.

These agents are used as drug in combination with UVA to treat skin diseases.

TOXICOLOGICAL CHARACTERISATION

The present opinion will primarily deal with the photomutagenic and photocarcinogenic properties of 5-MOP and 8-MOP.

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

5-MOP tested in the absence of UVA, was reported to be weakly mutagenic to bacteria.

Ref. : 3

8-MOP tested in the absence of UVA, induced mutation in bacteria, but inconclusive results were obtained with respect to chromosomal aberrations and sister chromatid exchanges in human cells *in vitro*, gene mutation and DNA damage in rodent cells *in vitro* and mutation in yeast.

Ref. : 3

2.9. Carcinogenicity**2.9.1. Animal studies****5-MOP**

IARC has concluded that the studies available were inadequate to evaluate the local and systemic carcinogenicity of 5-MOP itself.

Ref. : 1, 3

8-MOP

IARC has concluded that when 8-MOP was tested alone in mice by intraperitoneal administration or by skin painting, it did not induce skin tumours. The studies were inadequate to evaluate the systemic carcinogenicity of 8-MOP.

Ref. : 2, 3

2.9.2. Human studies

One small survey showed no excess prevalence of skin tumors in workers in the bergamot oil (contain 0.23% 5-MOP and 2.2% bergamottin. 8-MOP not detected), production industry, but this study had methodological weaknesses. See also section 2.10.4.2.

Ref. : 1, 3

Evaluation and opinion on Furocoumarins in sun protection and bronzing products

2.10. Special investigations**2.10.1. Photochemical properties**

Not evaluated.

2.10.2. Photosensitisation / Photoallergy

Not evaluated.

2.10.3. Photomutagenicity/photocarcinogenicity**2.10.3.1. Photomutagenicity/Genotoxicity, *in vitro***

Table 1 summarises *in vitro* photogenotoxic data for some furocoumarines and the classification by IARC.

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

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

Substance	Organism	DNA damage	Mutation	Chromosomal 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

Evaluation and opinion on Furocoumarins in sun protection and bronzing products

^ADegree of evidence in evaluation by IARC. S = Sufficient evidence, L = Limited evidence, I = Inadequate evidence N = No data.

¹Gunther 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.

²Moysan A, Vigny P, Dardalhon M, Averbek 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.

³Papadopoulo 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. : 1, 3

5-MOP

Table 2 show results from *in vitro* photogenotoxic studies with 5-MOP + UVA radiation.

Table 2.

5-Methoxypsoralen + ultra-violet A radiation

END POINT	TEST CODE	TEST SYSTEM	RESULT NO ACT	RESULT ACT	DOSE (HID/LED)	EMIC NUMBER	REFERENCE
D	PRB	PROPHAGE, INDUCT/SOS/STRAND BREAKS/X-LINKS	+	0	0.0050	54189	CONNOR, WHEELER & LOWE 1451, 1983
G	SA2	S. TYPHIMURIUM TA102, REVERSE MUTATION	(+)	0	1.0000	48672	LEVIN, HOLLSTEIN, CHRISTMAN 7445, 1982
G	SA5	S. TYPHIMURIUM (OTHER), REVERSE MUTATION	(+)	0	1.0000	48672	LEVIN, HOLLSTEIN, CHRISTMAN 7445, 1982
G	ECR	E. COLI (OTHER), REVERSE MUTATION	+	0	40.0000	34772	ASHWOOD-SMITH, POULTON, BARKER 407, 1980
G	EC2	E. COLI WP2, REVERSE MUTATION	+	0	40.0000	34772	ASHWOOD-SMITH, POULTON, BARKER 407, 1980
D	SSB	SACCHAROMYCES, STRAND BREAKS/X-LINKS	+	0	1.1000	66362	AVERBECK, PAPADOPOULO & QUINTO 127, 1984
R	SCG	S. CEREVISIAE, GENE CONVERSION	+	0	1.1000	60317	AVERBECK 217, 1985
G	SCF	S. CEREVISIAE, REVERSE MUTATION	+	0	11.0000	43805	AVERBECK, AVERBECK & DALL'ACQUA 492, 1981
G	SCF	S. CEREVISIAE, FORWARD MUTATION	+	0	1.1000	60317	AVERBECK 217, 1985
G	SCF	S. CEREVISIAE, FORWARD MUTATION	+	0	10.8000	66362	AVERBECK, PAPADOPOULO & QUINTO 127, 1984
G	SCR	S. CEREVISIAE, REVERSE MUTATION	+	0	1.1000	60317	AVERBECK 217, 1985
G	SCR	S. CEREVISIAE, REVERSE MUTATION	+	0	1.1000	66362	AVERBECK, PAPADOPOULO & QUINTO 127, 1984
D	DIA	STRAND BREAKS/X-LINKS, ANIMAL CELLS IN VITRO	+	0	1.1000	60324	PAPADOPOULO & AVERBECK 281, 1985
G	GCO	MUTATION, CHO CELLS IN VITRO	+	0	0.3200	66361	LOVEDAY & DONAHUE 149, 1984
G	G9H	MUTATION, CHO CELLS IN VITRO	+	0	1.1000	60324	PAPADOPOULO & AVERBECK 281, 1985
S	SIC	SCE, CHINESE HAMSTER CELLS IN VITRO	+	0	40.0000	41682	NATARAJAN, VERDEGAAL-IMMERZEEL 113, 1981
S	SIC	SCE, CHINESE HAMSTER CELLS IN VITRO	+	0	40.0000	34772	ASHWOOD-SMITH, POULTON, BARKER 407, 1980
S	SIC	SCE, CHINESE HAMSTER CELLS IN VITRO	+	0	0.6500	66361	LOVEDAY & DONAHUE 149, 1984
D	UHF	UDS, HUMAN FIBROBLASTS IN VITRO	+	0	80.0000	45472	WOTTAWA & VIERNSTEIN 279, 1982
S	SHF	SCE, HUMAN FIBROBLASTS IN VITRO	+	0	40.0000	41682	NATARAJAN, VERDEGAAL-IMMERZEEL 113, 1981
S	SIH	SCE, OTHER HUMAN CELLS IN VITRO	+	0	40.0000	41682	NATARAJAN, VERDEGAAL-IMMERZEEL 113, 1981
C	CHF	CHROM ABERR, HUMAN FIBROBLASTS IN VITRO	+	0	40.0000	41682	NATARAJAN, VERDEGAAL-IMMERZEEL 113, 1981
C	CIH	CHROM ABERR, OTHER HUMAN CELLS IN VITRO	+	0	40.0000	41682	NATARAJAN, VERDEGAAL-IMMERZEEL 113, 1981

Ref. : 4

The lowest doses giving a genotoxic response are given in ppm in column 6. It is apparent that for 12 of the 23 tests listed, the lowest dose giving a genotoxic response was of the order of 1 ppm or lower. The lowest doses giving response were found for mutation in CHO cells (0.3 ppm) and prophage induction (0.005 ppm).

In a later study using human lymphocytes increased chromosome aberrations were found with 0.001 ppm 5-MOP.

Ref. : 5

Table 3 show results from *in vitro* photogenotoxic studies with 8-MOP + UVA radiation.

Table 3.

Evaluation and opinion on Furocoumarins in sun protection and bronzing products

8-Methoxypsoralen + ultra-violet A radiation

END POINT	TEST CODE	TEST SYSTEM	RESULT NO	RESULT ACT	DOSE (HID/LED)	EMIC NUMBER	REFERENCE
D	PRB	PROPHAGE, INDUCT/SOS/STRAND BREAKS/X-LINKS	+	0	12.0000	29382	FUJITA, SANO & SUZUKI 35, 1978
D	PRB	PROPHAGE, INDUCT/SOS/STRAND BREAKS/X-LINKS	+	0	11.0000	52270	MOHN, KERKLAAN, VAN ZEELAND 153, 1984
D	BRD	BACTERIA (OTHER), DIFFERENTIAL TOX	+	0	20.0000	41177	BRIDGES & VON WRIGHT 229, 1981
G	SAS	S. TYPHIMURIUM (OTHER), REVERSE MUTATION	+	0	1.0000	48672	LEVIN, HOLLSTEIN, CHRISTMAN 7445, 1982
G	ECK	E. COLI K12, FORWARD OR REVERSE MUTATION	+	0	6.7000	37921	DE MOL, VAN HENEGOUWEN, MOHN 23, 1981
G	ECK	E. COLI K12, FORWARD OR REVERSE MUTATION	+	0	11.0000	52270	MOHN, KERKLAAN, VAN ZEELAND 153, 1984
G	ECW	E. COLI WP2 UVRA, REVERSE MUTATION	+	0	5.0000	41766	PANI, BABUDRI, VENTURINI 407, 1981
G	EC2	E. COLI WP2, REVERSE MUTATION	+	0	100.0000	46206	PESHEKHONOV, TARASOV 1274, 1981
G	EC2	E. COLI WP2, REVERSE MUTATION	+	0	40.0000	34772	ASHWOOD-SMITH, POULTON, BARKER 407, 1980
G	EC2	E. COLI WP2, REVERSE MUTATION	+	0	40.0000	401	ASHWOOD-SMITH, TOWERS, ABRAMOWSKI 401, 1982
G	EC2	E. COLI WP2, REVERSE MUTATION	+	0	5.0000	41766	PANI, BABUDRI, VENTURINI 407, 1981
G	EC2	E. COLI WP2, REVERSE MUTATION	+	0	100.0000	6198	IGALI, BRIDGES, ASHWOOD-SMITH 21, 1970
G	ECR	E. COLI (OTHER), REVERSE MUTATION	+	0	100.0000	46705	ASHWOOD-SMITH, NATARAJAN, POULTON 189, 1982
G	ECR	E. COLI (OTHER), REVERSE MUTATION	+	0	12.0000	29382	PESHEKHONOV, TARASOV 1274, 1981
G	ECR	E. COLI (OTHER), REVERSE MUTATION	+	0	10.0000	29382	FUJITA, SANO & SUZUKI 35, 1978
G	ECR	E. COLI (OTHER), REVERSE MUTATION	+	0	10.0000	27845	SEKI, NOZU & KORDO 19, 1978
R	SCH	S. CEREVISIAE, HOMOZYGOSIS	+	0	10.0000	28042	HENRIQUES, CHANET, AVERBECK 63, 1977
R	SCH	S. CEREVISIAE, HOMOZYGOSIS	+	0	10.0000	34006	AVERBECK & MOUSTACCHI 133, 1979
R	SCH	S. CEREVISIAE, HOMOZYGOSIS	+	0	1.0800	60317	AVERBECK 217, 1985
G	SCF	S. CEREVISIAE, FORWARD MUTATION	+	0	10.0000	28042	HENRIQUES, CHANET, AVERBECK 63, 1977
G	SCF	S. CEREVISIAE, FORWARD MUTATION	+	0	10.8000	42254	AVERBECK & AVERBECK 453, 1979
G	SCF	S. CEREVISIAE, FORWARD MUTATION	+	0	10.0000	21620	AVERBECK, CHANDRA & BISWAS 241, 1975
G	SCF	S. CEREVISIAE, FORWARD MUTATION	+	0	10.8000	40196	CASSIER, CHANET, HENRIQUES 841, 1980
G	SCF	S. CEREVISIAE, FORWARD MUTATION	+	0	2.2000	19556	SWANBECK & THYRESSON 242, 1974
G	SCF	S. CEREVISIAE, FORWARD MUTATION	+	0	10.8000	38895	AVERBECK, BISAGNI, MARQUET 547, 1979
G	SCF	S. CEREVISIAE, FORWARD MUTATION	+	0	1.0800	58467	AVERBECK, AVERBECK, BISAGNI 47, 1985
G	SCR	S. CEREVISIAE, REVERSE MUTATION	+	0	10.8000	39098	AVERBECK & MOUSTACCHI 475, 1980
G	SCR	S. CEREVISIAE, REVERSE MUTATION	+	0	10.8000	40196	CASSIER, CHANET, HENRIQUES 841, 1980
G	SCR	S. CEREVISIAE, REVERSE MUTATION	+	0	10.0000	21620	AVERBECK, CHANDRA & BISWAS 241, 1975
G	SCR	S. CEREVISIAE, REVERSE MUTATION	+	0	1.0800	58467	AVERBECK, AVERBECK, BISAGNI 47, 1985
G	SCR	S. CEREVISIAE, REVERSE MUTATION	+	0	10.8000	34006	AVERBECK & MOUSTACCHI 133, 1979
G	STP	S. COELICOLOR, FORWARD MUTATION	+	0	100.0000	13160	TOWNSEND, WRIGHT & HOPWOOD 799, 1971
G	ANF	A. NIDULANS, FORWARD MUTATION	+	0	100.0000	40491	SCOTT & MALEY 63, 1981
G	ANF	A. NIDULANS, FORWARD MUTATION	+	0	100.0000	6369	ALDERSON & SCOTT 569, 1970
G	ANR	A. NIDULANS, REVERSE MUTATION	+	0	50.0000	37866	MURONETS, KOVTUNENKO & KAMENOVA 741, 1980
D	DTA	STRAND BREAKS/X-LINKS, ANIMAL CELLS IN VITRO	+	0	1.0800	60324	PAPADOPOULOU & AVERBECK 281, 1985
D	UTA	UDS, OTHER ANIMAL CELLS IN VITRO	+	0	2.0000	42977	WENIGER 22, 1981
G	GCO	MUTATION, CHO CELLS IN VITRO	+	0	0.5000	43036	SCHENLEY & HSIE 179, 1981
G	G9H	MUTATION, CHL V79 CELLS, HPRT	+	0	0.0300	52165	SWART, BECKERS & SCHOETHORST 271, 1983
G	G9H	MUTATION, CHL V79 CELLS, HPRT	+	0	10.8000	52167	PAPADOPOULOU, SAGLIOCCO & AVERBECK 287, 1983
G	G9H	MUTATION, CHL V79 CELLS, HPRT	+	0	0.5000	48900	UWAIFO, BILLINGS & HEIDELBERGER 1054, 1983
G	G9H	MUTATION, CHL V79 CELLS, HPRT	+	0	0.5000	31541	BURGER & SIMONS 381, 1979
G	G9H	MUTATION, CHL V79 CELLS, HPRT	+	0	5.0000	41766	PANI, BABUDRI, VENTURINI 407, 1981
G	G9H	MUTATION, CHL V79 CELLS, HPRT	+	0	5.0000	41566	FRANK & WILLIAMS 307, 1982
G	G9H	MUTATION, CHL V79 CELLS, HPRT	+	0	2.2000	40030	ARLETT, HEDDLE, BROUGHTON 147, 1980
G	G9H	MUTATION, CHL V79 CELLS, HPRT	+	0	10.0000	39715	ARLETT, HEDDLE, BROUGHTON 147, 1980
G	G5T	MUTATION, L5178Y CELLS, TK LOCUS	+	0	10.0000	39715	ARLETT, HEDDLE, BROUGHTON 147, 1980
G	G51	MUTATION, L5178Y CELLS, ALL OTHER LOCI	+	0	10.0000	49440	ARLETT 545, 1981
G	G51	MUTATION, L5178Y CELLS, ALL OTHER LOCI	+	0	10.0000	39715	ARLETT, HEDDLE, BROUGHTON 147, 1980
G	GIA	MUTATION, OTHER ANIMAL CELLS IN VITRO	-	0	0.0300	52165	SWART, BECKERS & SCHOETHORST 271, 1983
S	SIC	SCE, CHINESE HAMSTER CELLS IN VITRO	+	0	6.5000	41551	SAHAR, KITTREL, FULGHUM 91, 1981
S	SIC	SCE, CHINESE HAMSTER CELLS IN VITRO	+	0	40.0000	34772	ASHWOOD-SMITH, POULTON, BARKER 407, 1980
S	SIC	SCE, CHINESE HAMSTER CELLS IN VITRO	+	0	1.6000	37287	CASSEL & LATT 15, 1980
S	SIC	SCE, CHINESE HAMSTER CELLS IN VITRO	+	0	5.0000	401	ASHWOOD-SMITH, TOWERS, ABRAMOWSKI 401, 1982
S	SIC	SCE, CHINESE HAMSTER CELLS IN VITRO	+	0	0.1000	48661	LIRMAIMAA & WOLFF 239, 1982
S	SIC	SCE, CHINESE HAMSTER CELLS IN VITRO	+	0	0.3000	30805	LATT & LOVEDAY 184, 1978
S	SIC	SCE, CHINESE HAMSTER CELLS IN VITRO	+	0	40.0000	41682	NATARAJAN, VERDEGAAL-IMMERZEEL 113, 1981
S	SIS	SCE, SYRIAN HAMSTER CELLS IN VITRO	+	0	0.0200	37352	MACRAE, CHAN, WAT 1096, 1980
S	SIT	SCE, TRANSFORMED CELLS IN VITRO	+	0	11.0000	52800	LIU-LEE, HEDDLE, ARLETT 139, 1984
M	MIA	MICRONUCLEUS TEST, ANIMAL CELLS IN VITRO	+	0	11.0000	52800	LIU-LEE, HEDDLE, ARLETT 139, 1984
C	CHM	CHROM ABERR, MOUSE CELLS IN VITRO	+	0	10.8000	46228	HOOK, HEDDLE & MARSHALL 100, 1983
C	CIS	CHROM ABERR, SYRIAN HAMSTER CELLS IN VITRO	+	0	0.0200	37352	MACRAE, CHAN, WAT 1096, 1980
T	TCM	CELL TRANSFORMATION, C3H10T1/2 CELLS	+	0	1.0000	48900	UWAIFO, BILLINGS & HEIDELBERGER 1054, 1983
D	DIH	STRAND BREAKS/X-LINKS, HUMAN CELLS IN VITRO	-	0	20.0000	45164	BREDBERG, LAMBERT & SODERHALL 221, 1982
D	DIH	STRAND BREAKS/X-LINKS, HUMAN CELLS IN VITRO	+	0	0.1000	37881	COHEN, KRAEMER, WATERS 347, 1981
D	RIH	OTHER DNA REPAIR, HUMAN CELLS IN VITRO	+	0	5.0000	38635	KAYE, SMITH, HANAWALT 696, 1980
D	UHF	UDS, HUMAN FIBROBLASTS IN VITRO	+	0	50.0000	27625	BADEN, PARRINGTON, DELHANTY 247, 1972
D	UHF	UDS, HUMAN FIBROBLASTS IN VITRO	+	0	80.0000	45472	WOTTAWA & VIERNSTEIN 279, 1982
D	UHF	UDS, HUMAN FIBROBLASTS IN VITRO	-	0	0.5000	44011	BILLOULAC, DENECHAUD, DUBUISSON 285, 1980
D	UHF	UDS, HUMAN FIBROBLASTS IN VITRO	-	0	0.6000	42966	HONIGSMANN, JAEINICKE, BRENNER 491, 1981
D	UHF	UDS, HUMAN FIBROBLASTS IN VITRO	+	0	10.0000	43535	GRUENERT & CLEAVER 163, 1981
D	UIH	UDS, OTHER HUMAN CELLS IN VITRO	-	0	50.0000	27625	BADEN, PARRINGTON, DELHANTY 247, 1972
D	UIH	UDS, OTHER HUMAN CELLS IN VITRO	+	0	10.0000	43535	GRUENERT & CLEAVER 163, 1981
G	GIH	MUTATION, HUMAN CELLS IN VITRO	+	0	0.2500	34297	BURGER & SIMONS 371, 1979
S	SHF	SCE, HUMAN FIBROBLASTS IN VITRO	+	0	0.2000	47091	LAMBERT, BREDBERG & MCKENZIE 62, 1982
S	SHF	SCE, HUMAN FIBROBLASTS IN VITRO	+	0	1.0000	50835	BREDBERG & LAMBERT 191, 1983
S	SHF	SCE, HUMAN FIBROBLASTS IN VITRO	+	0	40.0000	41682	NATARAJAN, VERDEGAAL-IMMERZEEL 113, 1981
S	SHL	SCE, HUMAN LYMPHOCYTES IN VITRO	+	0	25.0000	28791	WAKSVIK, BROGGER & STENE 195, 1977
S	SHL	SCE, HUMAN LYMPHOCYTES IN VITRO	+	0	0.0040	25236	MOURELATOS, FAED, JOHNSON 1091, 1977
S	SHL	SCE, HUMAN LYMPHOCYTES IN VITRO	+	0	0.0100	49190	CARTER, LYONS, WINDHORST 239, 1982
S	SHL	SCE, HUMAN LYMPHOCYTES IN VITRO	+	0	4.0000	29698	MOURELATOS, FAED, GOULD 649, 1977
S	SHL	SCE, HUMAN LYMPHOCYTES IN VITRO	+	0	25.0000	36230	WAKSVIK, BROGGER & THUNE 259, 1979
S	SHL	SCE, HUMAN LYMPHOCYTES IN VITRO	+	0	0.6000	30486	LAMBERT, MORAD, BREDBERG 13, 1978
S	SHL	SCE, HUMAN LYMPHOCYTES IN VITRO	+	0	0.1000	24299	CARTER, WOLFF & SCHNEIDL 548, 1976
S	SHL	SCE, HUMAN LYMPHOCYTES IN VITRO	+	0	0.0140	31160	GAYNOR & CARTER 257, 1978
S	SHL	SCE, HUMAN LYMPHOCYTES IN VITRO	-	0	0.2500	32961	BROGGER, WAKSVIK & THUNE 287, 1978
S	SHL	SCE, HUMAN LYMPHOCYTES IN VITRO	+	0	21.6000	45033	ABEL & SCHIMMER 451, 1981
S	SHL	SCE, HUMAN LYMPHOCYTES IN VITRO	+	0	0.0100	43268	KRAEMER, WATERS & COHEN 80, 1981
S	SHT	SCE, TRANSFORMED HUMAN CELLS	+	0	0.1000	43268	KRAEMER, WATERS & COHEN 80, 1981
S	SIH	SCE, OTHER HUMAN CELLS IN VITRO	+	0	0.0200	43919	WEST, JOHANSEN & FAED 67, 1982
S	SIH	SCE, OTHER HUMAN CELLS IN VITRO	+	0	0.2160	58355	BILLARDON, LEVY & MOUSTACCHI 63, 1984
S	SIH	SCE, OTHER HUMAN CELLS IN VITRO	+	0	40.0000	41682	NATARAJAN, VERDEGAAL-IMMERZEEL 113, 1981
C	CHF	CHROM ABERR, HUMAN FIBROBLASTS IN VITRO	+	0	1.0000	28558	OMAR, WIESMANN & KREBS 65, 1977
C	CHF	CHROM ABERR, HUMAN FIBROBLASTS IN VITRO	+	0	40.0000	41682	NATARAJAN, VERDEGAAL-IMMERZEEL 113, 1981
C	CHL	CHROM ABERR, HUMAN LYMPHOCYTES IN VITRO	+	0	0.1000	22617	SWANBECK, THYRESSON-HOK, BREDBERG 55, 1975
C	CHL	CHROM ABERR, HUMAN LYMPHOCYTES IN VITRO	+	0	25.0000	36230	WAKSVIK, BROGGER & THUNE 259, 1979
C	CHL	CHROM ABERR, HUMAN LYMPHOCYTES IN VITRO	+	0	25.0000	28791	WAKSVIK, BROGGER & STENE 195, 1977
C	CHL	CHROM ABERR, HUMAN LYMPHOCYTES IN VITRO	+	0	0.1000	15669	SASAKI & TONOMURA 1829, 1973
C	CHL	CHROM ABERR, HUMAN LYMPHOCYTES IN VITRO	-	0	0.2500	32961	BROGGER, WAKSVIK & THUNE 287, 1978
C	CIH	CHROM ABERR, OTHER HUMAN CELLS IN VITRO	+	0	0.1000	15669	SASAKI & TONOMURA 1829, 1973

Ref. : 4

The lowest doses giving a genotoxic response are given in ppm in column 6. It is apparent that for 30 of the 93 tests listed, the lowest dose giving a genotoxic response was of the order of 1 ppm or lower. The lowest doses giving response were found for mutation in CHL V79 cells (0.03 ppm) and sister chromatid exchange in human lymphocytes (three different experiments 0.004 – 0.01 ppm).

Solutions of either calf thymus DNA or poly(dA-dT) containing 5-MOP or 8-MOP (0.18 mM) were exposed to 419 nm light for 2 h at 4°C. The psoralen intercalate with DNA based pairs and the studies showed that the photochemical properties of 5-MOP and 8-MOP were similar. The primary photoproduct was 4',5'-monoadducts. The 4',5'-monoadducts were converted to crosslinks when the samples were subsequently exposed to UVA radiation (1 and 2 J/cm²).

Ref. : 6

2.10.3.2. Photomutagenicity/genotoxicity, *in vivo*

5-MOP

Four skin type I volunteers and three skin type II volunteers received, to previously untreated sites (approximately 1 cm²) on their buttocks, 10 daily tanning exposure of 0.7 MED (minimal erythema dose) over 14 days using either a) SSR (solar simulated radiation) alone, b) sunscreen + SSR, or c) sunscreen + 5 MOP (30 ppm) + SSR; 1 week later there were challenge with 2 MED SSR. The sunscreen lotion contained 2-ethylhexyl-4'-methoxycinnamate (5%). DNA damage was measured on fixed skin sections by use of monoclonal antibodies to thymidine dimers. Unscheduled DNA synthesis (UDS) was measured by incubation of sliced skin with methyl³H thymine followed by autoradiography. The result showed a significant level of protection by the 5-MOP sunscreen tanning regimes. The protection was seen for both UDS and thymine dimers formation and was observed both with skin type II and I volunteers.

Ref. : 7

Sites of previously unexposed buttock skin of eight human volunteers (skin type II) were treated daily for 3, 5, 8, or 10 days with suberythemogenic doses of solar-simulated radiation (SSR) in the presence of a UVB sunscreen containing 2-ethylhexyl-4'-methoxycinnamate (2%), 1-7,7-trimethyl-3-(4-methylbenzylidene)bicyclo-[2,2,2]-2-heptanone (1%) and 5-MOP (30 ppm), or daily for 10 days with SSR + the same sunscreen without 5-MOP. One week after cessation of treatment, these sites together with a control-unexposed site, were challenged with 2 minimal erythema doses (2 MED) of SSR. Biopsy samples were taken within 15 min of the challenge dose and were incubated for 1 h in tritiated thymidine. UV-induced DNA damage was measured indirectly by unscheduled DNA synthesis (UDS), and directly using a monoclonal antibody to thymine dimers. The level of pigmentation was assessed in sections in a semiquantitative fashion with Masson-Fontana staining, and the number of layers in the stratum corneum was used to assess changes in epidermal thickness. It was found that 5-MOP showed a photochemoprotection after three to five daily exposures with a maximum after eight daily exposures. The onset of this protection coincided with increases in melanin and in stratum corneum thickness. In an extension of this study, it was found that 10 daily exposures, 5-MOP photochemoprotection declined at a rate of about 5% per week. The authors point out that there was a good correlation between the photochemoprotection endpoints of UDS and thymine dimer levels.

Ref. : 8

Comment

The above experiments were unable to provide information on the question of whether 5-MOP photochemoprotection was mediated by an increase in melanin pigmentation or stratum corneum thickness or a combination of both.

IARC has reported 6 experiment *in vivo* with 8-MOP

Injection 8-MOP at a dose of 5 mg/kg bw together with UVA irradiation enhanced sister chromatid exchange in hamster. In 4 experiments with humans at doses up to between 0.6 and 0.8 mg/kg bw 8-MOP together with UVA, no enhancement in sister chromatid exchange was found. Likewise in 1 experiment with 0.7 mg/kg bw 8-MOP together with UVA, no enhancement in chromosome aberrations was found.

Ref. : 4

2.10.4. Photocarcinogenicity

2.10.4.1. Animal studies

5-MOP

5-Methoxypsoralen was tested in mice by skin application in combination with ultraviolet A radiation or solar-simulated radiation, producing skin papillomas and carcinomas; in these studies, no or few skin tumors were observed with ultraviolet A radiation or solar-simulated radiation alone. IARC concludes that there is *sufficient evidence* for carcinogenicity of 5-MOP + UVA radiation in experimental animals.

Ref. : 1, 3

A study on the effect of UVA irradiation on mice after application of a UVB sunscreen containing 5-MOP is described in more details below.

Mouse

Groups of 15 female HRS/J hairless albino mice, eight weeks old, received skin application of 4 $\mu\text{L}/\text{cm}^2$ Sun System III oil (containing 25 ppm 5-MOP and ethyl hexyl-*p*-methoxycinnamate as UVB screen) on their backs on five days per week for 20 weeks. The mice were immobilized for 60 min after each application and were irradiated with UVA. Groups received 2.5×10^4 , 5×10^4 or 10×10^4 J/m^2 UVA radiation. One control group of 15 mice received applications of Sun System III oil only, and another group of six mice received UVA irradiation only (10×10^4 J/m^2). The number of tumors > 1 mm in diameter per surviving mouse at 44 weeks of observation was dependent on the dose of radiation: 0.8 tumors at 2.5, 3.5 tumors at 5 and 8 tumors at 10×10^4 J/m^2 ; the percentages of animals with two or more tumors at that time were 13 at 2.5, 86 at 5 and 100 at 10×10^4 J/m^2 . At 44 weeks, 15/15, 14/15 and 9/15 animals were still alive in the three groups, respectively. By 52 weeks, some of the tumors had developed into large invasive tumors fixed to underlying tissue, especially in the group receiving the highest dose of radiation. Histological examination showed that atypical squamous-cell papillomas had progressed to invasive squamous-cell carcinomas. A shorter latency period for tumour development was seen with larger UVA doses. Control groups (SS III without UVA and UVA without SS III) remained free of tumours.

Ref. : 9

Comment

The experiment showed that a sunscreen (UVB screen) containing 5-MOP enhanced tumour formation in mice exposed to UVA radiation.

8-MOP

8-Methoxypsoralen was tested by oral and intraperitoneal administration and by skin application in combination with ultraviolet A radiation in mice, producing epidermal and dermal tumors. IARC concludes that there is *sufficient evidence* for carcinogenicity of 8-MOP + UVA radiation in experimental animals.

Ref. : 2, 3

A study with both 5-MOP and 8-MOP is described in more details below.

Mouse

Groups of 20 male and 20 female hairless albino mice, eight to ten weeks old, received topical applications of 0.01% (100 ppm) or 0.03% (300 ppm) solutions of 5-MOP or 8-MOP (purity, 100%) in 70:30 (v/v) BP-grade arachis oil and isopropyl myristate on both flanks on five days per week for up to 37 weeks. After each application, the animals were immobilized for 30 min and then received simulated solar irradiation (SSR) for 50 min. The estimated daily dose of irradiation was $1.7 \times 10^4 \text{ J/m}^2$, of which approximately 400 J/m^2 were UVB radiation. Seven control groups were either left untreated or were treated with vehicle alone, arachis oil alone, simulated solar irradiation alone, vehicle plus irradiation, or applications of 0.03% 5-MOP or 0.03% 8-MOP without irradiation. Mortality rates and mean numbers of skin tumours > 1 mm in diameter per surviving animal after 26 weeks of treatment are summarized in Table 4. A random sample of tumours was examined histologically, and 83% of the skin tumours in the group treated with 5-MOP and irradiation were found to be papillomas. Trend tests using life-table methods indicated a positive dose-related trend in incidence for treatment with either 5-MOP ($p < 0.01$) or 8-MOP ($p < 0.01$) in combination with simulated solar radiation.

Table 4. Total and mean no. of skin tumours per mouse surviving at 26 weeks

Treatment	<u>Total no. of tumors</u> No. of surviving mice	Mean no. of tumors/mice
No treatment	2/40	0.05
Vehicle alone	1/31	0.03
Arachis oil alone	8/39	0.2
SSR alone	18/33	0.5
Vehicle + SSR	14/39	0.4
8-MOP alone (0.03%)	6/26	0.2
5-MOP alone (0.03%)	5/31	0.2
8-MOP (0.03%) + SSR	372/38	9.8
5-MOP (0.03%) + SSR	179/31	5.8
8-MOP (0.01%) + SSR	69/36	1.9
5-MOP (0.01%) + SSR	84/34	2.5

Ref. : 10

Comment

The results indicate a linear relationship between the concentrations of furocoumarines and mean no. of tumors induced by SSR. Application of a solution containing 100 ppm increased the no. of tumors per mice more than 5 times. It should be noted that the treatment only lasted about 1/4 to 1/3 of the lifespan of the animals.

2.10.4.2. Human studies

5-MOP

IARC concludes that there is *inadequate evidence* from epidemiological studies that 5-MOP + UV radiation is carcinogenic to humans. The overall evaluation by IARC is that 5-MOP + UV is *probably carcinogenic to humans (Group 2A)*.

Ref. : 3

8-MOP

The development of nonmelanocytic skin cancer (basal- and squamous-cell skin cancers) has been reported in patients treated with 8-MOP and UVA (PUVA) for psoriasis or mycosis fungoides. Three cases of malignant melanoma of the skin have been reported in patients with psoriasis treated with PUVA. The strongest evidence for a causal association between PUVA treatment and nonmelanocytic skin cancer comes from the follow-up of 1380 psoriatic patients treated in the USA. The standardized incidence ratio (SIR) for squamous-cell carcinoma increased from 4.1 (95% confidence interval, 2.3-6.8) at low doses to 22.3 (13.5-34.1) at medium doses, and 56.8 (42.7-74.2) at high doses; this effect was independent of possible confounding effects of therapy with ionizing radiation and topical tar. The effect on basal-cell cancer incidence was much weaker (high doses: SIR, 4.5; 2.8-6.9)⁸. One cohort study of 525 psoriatic patients treated with PUVA did not suggest an increase in the incidence of skin cancer (mean follow-up period, 2.1 years). This 'negative' result could have been due to lack of statistical power and to the low doses used in the study. Another study with a five-year follow up showed no skin tumour in 94 patients treated with PUVA for psoriasis or mycosis fungoides.

Ref. : 3

IARC concludes that there is *sufficient evidence* from epidemiological studies that 8-MOP + UV radiation is carcinogenic to humans. The overall evaluation by IARC is that 8-MOP + UV is *carcinogenic to humans (Group 1)*.

Ref. : 3

2.10.4.3. 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. In studies on 5-MOP and 8-MOP, photogenotoxic and photomutagenic effects *in vitro* have been observed at concentrations below 0.01 and 0.1 ppm, respectively. The data submitted is not adequate for evaluation of the safety at a higher limit for furocoumarins in sun protection and bronzing products. The industry submitted two articles indicating that the presence of 30 ppm 5-MOP in a UVB sunscreen reduced the formation of thymine dimers and unscheduled DNA synthesis in human skin after solar-simulated radiation. In contrast, in mouse experiments 25 ppm 5-MOP in a UVB sunscreen enhanced tumour formation after UVA irradiation. Mice experiments suggest that the risk of skin tumour formation increases linearly with the amount of 5-MOP or 8-MOP applied to the skin prior to solar-simulated radiation.

The International Agency for Research on Cancer (IARC) has classified 5-MOP and 8-MOP plus ultraviolet radiation in Group 2A (*probably carcinogenic to humans*) and in Group 1 (*carcinogenic to humans*), respectively.

2.12. Opinion

SCCNFP concludes that the data available from *in vitro* short-term studies, experimental studies in animals, and epidemiological studies on humans on the effect of furocoumarins in sun protection and sun bronzing product do not justify a higher limit than 1 ppm (not to be intentionally added) for furocoumarins in cosmetics. This Opinion should be interpreted in conjunction with the Opinion on CMR substances (SCCNFP/0474/01, final, Adopted 25 September 2001)

2.13. References

1. IARC Furocoumarins. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans 40: 289-376, 1986.
2. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemical to Humans. Methoxsalen. 24: 101-124, 1980.
3. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 5-Methoxypsoralen and 8-Methoxypsoralen plus ultraviolet irradiation. Suppl 7: 242-245, 1987.
4. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Suppl 6: 1-729, 1987.
5. Youssefi AA, Arutyunyan R, Emerit I. Chromosome damage in PUVA-treated human lymphocytes is related to active oxygen species and clastogenic factors. *Mutat Res* 309: 185-191, 1994.
6. Amici LA, Gasparro FP. 5-Methoxypsoralen photoadduct formation: conversion of monoadducts to crosslinks. *Photodermatol Photoimmunol Photomed* 11: 135-139, 1995.
7. Potten CS, Chadwick CA, Cohen AJ, Nikaido O, Matsunaga T, Schipper NW, Young AR. DNA damage in UV-irradiated human skin *in vivo*: automated direct measurement by image analysis (thymine dimers) compared with indirect measurement (unscheduled DNA synthesis) and protection by 5-methoxypsoralen. *Int J Radiat Biol* 63: 313-324, 1993.

8. Chadwick CA, Potten CS, Cohen AJ, Young AR. The time of onset and duration of 5-methoxypsoralen photochemoprotection from UVR-induced DNA damage in human skin. *Br J Dermatol* 131: 483-494, 1994.
9. Cartwright LE, Walter JF. Psoralen-containing sunscreen is tumorigenic in hairless mice. *J Am Acad Dermatol* 8: 830-836, 1983.
10. Young AR, Magnus IA, Davies AC, Smith NP. A comparison of the phototumorigenic potential of 8-MOP and 5-MOP in hairless albino mice exposed to solar simulated radiation. *Br J Dermatol* 108: 507-518, 1983