EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

OPINION ON

METHYL-N-METHYLANTHRANILATE

(photo-toxicity only)

Opinion adopted by the SCCP during the 10th plenary of 19 December 2006

ACKNOWLEDGEMENTS

Members of the working group are acknowledged for their valuable contribution to this opinion. The members of the working group are:

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Keywords: SCCP, scientific opinion, fragrance ingredient, methyl-N-methylanthranilate, Directive 768/76/EEC, CAS 85-91-6

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1. BACKGROUND

The Scientific Committee on Cosmetics and Non-Food Products (SCCNFP) adopted an "Initial List of Perfumery Materials which must not form part of Cosmetic Products except subject to the restrictions and conditions laid down" (SCCNFP/0392/00, final, adopted by the SCCNFP during the 18th Plenary meeting of 25 September 2001). The opinion was 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 its 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 in its opinion. For Methyl-*N*-methylanthranilate is mentioned under entry no 21 with the restriction: "*For applications on areas of the skin exposed to sunlight, excluding bath preparations, soaps and other wash-off products, limit to 10 % in the finished cosmetic product*."

An updated IFRA[1] recommendation was submitted in December 2002. However, the SCCNFP requested additional data. Industry has now provided additional information in Submission II.

The substance is used as an ingredient in fragrances, and the substance is the main part of the ingredient Petitgrain Mandarin Oil (Citrus reticulate leaf oil (CAS 8014-17-3)).

2. TERMS OF REFERENCE

1. *Does the SCCP consider the use of Methyl-N-methylanthranilate to be safe for the consumers, when used as an ingredient in perfumes in leave-on products in a concentration less than 0.1 % in the finished cosmetic product taking into consideration the provided data?*

2. *Does SCCP recommend any restrictions in the use of Petitgrain Mandarin oil due to its content of Methyl-N-methylanthranilate in cosmetic products?*

3. *Does SCCP recommend any restriction for the use of the substance in rinse-off products?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1.	Chemical identity

3.1.1.1.	Primary name and/or INCI name

Methyl-N-methylanthranilate

[1] Research Institute for Fragrance Materials, Inc

3.1.1.2. Chemical names

Benzoic acid, 2-(methylamino)-, methyl ester (CAS)
Dimethyl anthranilate
2-Methylamino methyl benzoate
N-Methylanthranilic acid, methyl ester
Methyl 2-methylaminobenzoate
Methyl o-methylaminobenzoate

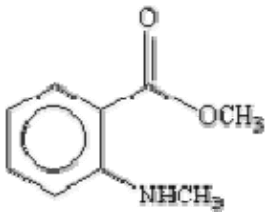
3.1.1.3. Trade names and abbreviations

/

3.1.1.4. CAS / EINECS number

CAS : 85-91-6
EINECS : 201-642-6

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_9H_{11}NO_2$

3.1.2. Physical form

Clear pale yellow to yellow liquid with a bluish fluorescence having a grape-like odour

3.1.3. Molecular weight

Molecular weight: 165.2

3.1.4. Purity, composition and substance codes

/

3.1.5. Impurities / accompanying contaminants

/

3.1.6. Solubility

/

3.1.7.	Partition coefficient (Log P_{ow})

Log P_{ow} : /

3.1.8.	Additional physical and chemical specifications

Melting point	:	19 °C
Boiling point	:	256 °C
Flash point	:	> 110 °C
Vapour pressure	:	/
Density	:	1.12 – 1.13 at 25 °C
Viscosity	:	/
pKa	:	/
Refractive index	:	1.57900 - 1.58100 at 20 °C

3.2. Function and uses

Methyl-N-methylanthranilate is used as a fragrance ingredient in concentrations up to 0.1% in the finished cosmetic product.

3.3. Toxicological Evaluation

3.3.1.	Acute toxicity

Not applicable

3.3.2.	Irritation and corrosivity

3.3.2.1.	Skin irritation

Not applicable

3.3.2.2.	Mucous membrane irritation

Not applicable

3.3.3.	Skin sensitisation

Not applicable

3.3.4.	Dermal / percutaneous absorption

Not applicable

3.3.5.	Repeated dose toxicity

Not applicable

3.3.6.	Mutagenicity / Genotoxicity

Not applicable

3.3.7. Carcinogenicity

Not applicable

3.3.8. Reproductive toxicity

Not applicable

3.3.9. Toxicokinetics

Not applicable

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

Guideline:	/
Species:	human
Group:	10 (both sexes, unknown ratio)
Substance:	dimethyl anthranilate
Batch:	/
Purity:	/
Dose:	5µl/cm^2 of 5% dimethyl anthranilate in hydrophilic ointment, over 2 x 2 cm^2 area of skin of mid back, applied for 6 hours.
Light:	20 J/cm^2 UVA
GLP:	/

In the experiment described, observations were made immediately after UVA exposure and at 24 and 48 hours later. 8 of 10 subjects produced a reaction.

The authors considered that 5% dimethyl anthranilate is phototoxic under the conditions of the test.

Ref.: 1995

Guideline:	/
Species:	human
Group:	27 females (26 completed the study)
Substance:	dimethyl anthranilate
Batch:	/
Purity:	/
Dose:	Sample A: 0.3 ml of 0.5% dimethyl anthranilate in 25% diethyl phthalate/75% ethanol, placed in 25 mm Hill Top Chambers. Sample B: Saline Sample C: vehicle
Light source:	model 16S solar UV simulator
GCP:	in compliance

Induction: 2 applications per week for 3 weeks onto same skin site. Within 10 minutes of patch removal, 2 MED (previously determined, with UVA component being about 5% of the light) given from mixed light source giving UVA/B.
Rest period: 2 weeks.
Challenge: Preparations applied in duplicate to naïve skin sites. After approximately 24 hours, one site was irradiated with 16 J/cm^2 UVA followed by 0.75 MED UVB. Observations were made at 1, 24, 48 and 72 hours.

The majority of the responses observed in response to UV challenge of skin treated with Test Articles A, B and C consisted of slight to mild erythema. This was slightly higher than the responses observed at the non-irradiated sites.

The authors concluded that while these responses may represent mild photo-allergic reactions, they were not accompanied by oedema, vesicles, papules or spreading beyond the test site nor were they maintained beyond the 48-hour evaluation.

Ref.: 36789

Comment
The dose (concentration) of dimethyl anthranilate was too low for a 'maximisation'-type test.

Guideline:	/
Species:	human
Group:	5 male, 5 female
Substance:	dimethyl anthranilate
Batch:	/
Purity:	/
Dose:	5 µl/cm^2 of dimethyl anthranilate 'as is' applied to skin, allowed to dry and then covered with Webril. After 6 and 24 hours, sites irradiated with UVA and observations made immediately and at 24 and 48 hours.
Light:	150W Solar simulator with Schott WG345 filter to eliminate UVB. UVA irradiance 25 mW/cm^2
GCP:	/

In the described experiment (and it is not stated whether the supplied dimethyl anthranilate was pure or a diluted sample), 8 of 10 subjects reacted and the authors considered that dimethyl anthranilate is phototoxic.

Ref.: 1788

Guideline:	/
Species:	human
Group:	25 (both sexes, unknown ration)
Substance:	dimethyl anthranilate
Batch:	/
Purity:	/
Dose:	5 µl/cm^2 of dimethyl anthranilate 'as is' applied under occlusion to skin for 24 hours then 3 MED given. Procedure repeated twice weekly for 3 weeks (6 applications) but in the last two applications, 4 MED given. After rest period of 10 days, 5 µl/cm^2 of dimethyl anthranilate 'as is' applied under occlusion to skin for 24 hours then 3 minutes UVA given Xenon Solar simulator with Schott WG345 filter to eliminate UVB. Sites examined at 24, 48 and 72 Hours.
Light:	150W Xenon Solar simulator with Schott WG345 filter to eliminate UVB. UVA irradiance 25 mW/cm^2
GCP:	/

Under the above test conditions, 18 of 25 subjects developed reactions which the study authors considered to be phototoxic.

Ref.: 1788

Guideline:	/
Species:	human

Group:	35 females
Substance:	dimethyl anthranilate
Batch:	/
Purity:	/
Doses:	Sample A; 1.0% dimethyl anthranilate w/v in 25% v/v diethyl phthalate in ethanol.
Light Source:	1000W Xenon arc solar simulator
GCP:	in compliance

0.2 ml of the test substances (with vehicle and blank controls) were applied in duplicate in 25 mm Hill Top Chambers under occlusive conditions for 24 hours. 10 minutes after patch removal, 16 J/cm^2 UVA was given then 0.75 MED UVB to the sites for irradiation. Observations were made at 1, 24, 48 and 144 hours.

At 1, 24, 48, and 144 hours post-irradiation 54%, 46%, 40%, and 26% (respectively) of the subjects tested with 1.0% dimethyl anthranilate received a score of 1 or 2. The non-irradiated results for the subjects receiving a score of 1 or 2 were 6% at 1 hour, 3% at 24 hours, 3% at 48 hours and 0% at 144 hours.

Under the conditions of the study, 1.0% dimethyl anthranilate was considered to be phototoxic.

Ref.: 34769

Guideline:	/
Species:	human
Group:	34 (of which 29 (24 females and 5 males) completed the study)
Substance:	dimethyl anthranilate
Batch:	/
Purity:	/
Doses:	Sample A; 0.5% dimethyl anthranilate w/v in 25% v/v diethyl phthalate in ethanol. Sample B; 0.3% dimethyl anthranilate w/v in 25% v/v diethyl phthalate in ethanol. Sample C; 0.1% dimethyl anthranilate w/v in 25% v/v diethyl phthalate in ethanol.
Light Source:	1000W Xenon arc solar simulator
GCP:	in compliance

0.3 ml of the test substances (with vehicle and blank controls) were applied in duplicate in 25 mm Hill Top Chambers under occlusive conditions for 24 hours. 10 minutes after patch removal, 16 J/cm^2 UVA was given then 0.75 MED UVB to the sites for irradiation. Observations were made at 1, 24, 48 and 72 hours.

Under the conditions of the study, the test articles did not induce a phototoxic reaction.

Ref.: 34768

Guideline:	3T3 Neural Red Uptake Phototoxicity Assay
Substance:	methyl-N-methylanthranilate
Batch:	99AC93 / Sample G
Purity:	/
Controls:	positive: chlorpromazine; negative: blank
GLP:	in compliance

The purpose of the study was to evaluate the phototoxicity and cytotoxicity potential of methyl-N-methylanthranilate as measured by a reduction in neutral red uptake in cultures of normal Balb/c 3T3 mouse fibroblasts.

In this 3T3 Neutral Red Uptake (NRU) Phototoxicity Assay, duplicate 96 well mono-layers of 3T3 fibroblast were exposed to dilutions of methyl N-methylanthranilate; one plate was exposed to 5 J/cm2 UVA irradiation (phototoxicity), the other not exposed (cytotoxicity). The treatment medium was then replaced by culture medium and at approximately 24 hrs post treatment the number of viable cells determined by Neutral Red Uptake. The number of viable cells present for each concentration of test article was compared to that of untreated controls and the percent inhibition of growth calculated. The IC_{50} concentration (i.e. the concentration producing 50% inhibition of growth) was calculated and expressed as µg/m1 for both the phototoxicity and cytotoxicity plates.

Substance	Dose spacing	Concentration +UVA (µg/ml)	Concentration -UVA (µg/ml)	IC_{50} (without UVA) (µg/ml)	IC_{50} (with UVA) (µg/ml)	MPE	PIF
Sample G	¼ Log	9.96 – 0.176	100 – 1.77	> 100	4.39	0.525	> 22.85
Sample G	¼ Log	100 – 0.557	100 – 0.556	> 100	3.81	0.362	> 26.25

Mean Photo Effect (MPE): a material is considered non phototoxic if the MPE is <0.1 (including negative MPE values) and phototoxic if the MPE is 0.1.
Photo-Irritancy Factor (PIF): a material is considered phototoxic if the PIF> 5.0.

The study indicated that methyl-N-methylanthranilate is phototoxic.

Ref.: 40277

Guideline: /
Species: hairless mice
Group: /
Substance: dimethyl anthranilate
Methyl-N-methyl anthranilate (ICI 1752)
Batch: /
Purity: /
Dose: 20 µl on 5 cm^2 skin followed, 30 minutes later, by UV exposure (or no exposure control)
Light: Osram XBF 6000W Xenon Lamp with Schott WG320 filter. Dose "that required to produce perceptible erythema"
GLP: /

Observations were made at 2, 4, 25 and 48 hours after exposure.

The authors reported that both samples produced phototoxic effects although the raw data was not provided.

Ref.: 2042

In vitro yeast test for phototoxicity

Guideline: /
Substance: dimethyl anthranilate
Methyl-N-methylanthranilate (ICI 1752)
Batch: /
Purity: /

GLP: /

A brewer's yeast suspension was streaked across dextrose agar Petri dishes in duplicate with dishes containing or not containing the test substances. The dishes were irradiated or not irradiated with UV. Other details are sparse in the provided document.

The authors reported that both samples produced phototoxic effects although the raw data was not provided.

Ref.: 2042

Guideline: /
Matrix: SKIN^2™ in 6-well Millicell™ plates
Substance: methyl-N-methylanthranilate
Batch: Fluka Chemika 292244/1 193
Purity: /
GLP: /

25µl methyl-N-methylanthranilate aliquots, at 5 test concentrations (with blank and untreated controls) were placed in 2 tissue plates per dilution. Irradiation was with a Dr Honle Mercury Halide solar simulator with H1 UVA transmitting filter to give a dose of 2.9 J/cm^2.

Following irradiation, the plates were placed in the incubator for 30 minutes. The tissues were then removed from both the irradiated and non- irradiated plates and rinsed with phosphate buffered solution (PBS) and placed in another set of 6-well MILLICELL® plates containing serum-free assay medium. These plates were incubated overnight (16—24 hours).
On the third day, a viability assay was conducted based on the mitochondrial enzyme reduction of the tetrazolium salt MTT (3-(4,5-di- methylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide). Tissues were incubated with 2 ml of a 2 mg/ml solution of MTT in serum-free assay medium for 2 hours. After incubation, each tissue was washed with PBS.

The amount of MTT reduced by a culture is proportional to the number of viable cells. The converted MTT was extracted from the tissues and quantified using a Molecular Devices Vmax™ kinetic microplate reader (at an optical density of 540 nm using the automix function) in conjunction with Soft- max/MAC software application program. Blank extraction aliquots were used to subtract non-specific binding of MTT to nylon mesh. The reported results were adjusted for readings observed with the blank control.

In a first experiment, the material was evaluated at concentrations ranging from 0.05—5%. Although not statistically significant, the highest concentration (a 5% solution) exhibited a phototoxic trend (69.1% MTT viability). Since no cytotoxicity was observed, a second experiment was conducted using higher test concentrations (0.5—25%). In this experiment, the wide divergence in the CD readings for the control tissue sets and the disparity in the CD readings for the low-dose levels (irradiated and non-irradiated) invalidated the results from this experiment. Therefore, the data from this experiment are not being considered.

A third experiment was conducted and an additional test concentration between 25% and 10% was selected (17.5%) and the lowest test concentration (0.5%) was not included. In this third experiment there was no significant intrinsic toxicity at any dose level (between 82% and 95% viability).

Exposure to UV light caused a decrease in viability at dose levels greater than 1%. Phototoxicity was first exhibited at the 5% test concentration ($p < 0.05$); the three higher dose levels (10%, 17.5%, and 25% solutions of methyl-N-methylanthranilate) were

phototoxic (significance p < 0.001). Increases in concentration corresponded to dose-dependent decreases in viability.

Ref.: 32077

***In vitro* yeast test for phototoxicity**

Guideline: /
Substance: dimethyl anthranilate
Batch: /
Purity: /
GLP: /

25µl aliquots of various dilutions using methanol as a solvent were placed on ¼ inch blank paper discs which were then dried. They were then placed, 4 discs per test concentration, onto plates growing *Saccharomyces cerevisiae*. 8-methoxypsoralen was used as the positive control. Irradiation was with bulbs providing UVA 320-400 nm. The dose of light was not stated.

Evaluation of the zone of inhibition provided information on phototoxicity.

The raw data was not provided but the study authors state that 0.05% dimethyl anthranilate, the lowest dilution tested, was phototoxic.

Ref: 9196

Methyl N-methylanthranilate, which occurs naturally in many citrus oils, is used in both fragrances and flavours. Earlier studies reported that methyl N-methylanthranilate was phototoxic in hairless mice at a concentration of 50% in methanol and in humans at a concentration of 5% in hydrophilic ointment. Further studies were conducted to determine if a no-effect level for phototoxic effects in humans could be established. Phototoxicity was evaluated using a 24-hour occluded application of methyl N- methylanthranilate to naive sites on the back followed by immediate exposure of the test sites to UVB and UVA from a Solar Simulator. Phototoxic effects were observed in 14/35 female volunteers with 1% methyl N-methylanthranilate in 75% ethanol/25% diethyl phthalate; no phototoxic effects were observed in 29 volunteers with 0.1%, 0.3% or 0.5% in 75% ethanol/25% diethyl phthalate.

A study to determine the photo-allergic potential of methyl N-methylanthranilate was conducted in 26 female volunteers using a modified human photo-maximization procedure (six 24-hour occluded induction applications with each application followed immediately by UVB/UVA exposure from a Solar Simulator, after a 2-week rest period, a 24-hour occluded challenge application was immediately followed by exposure to UVA/UVB); phototoxicity was also evaluated during the induction phase of this study. No photo-allergic or phototoxic reactions were observed with 0.5% in 75% ethanol/25% diethyl phthalate. Based on the findings in these studies, it can be concluded that the NOEL for methyl N-methylanthranilate for phototoxic effects in humans is 0.5%; and under the conditions of the above study, methyl N methylanthranilate is not photo-allergic in humans at a concentration of 0.5%.

Ref.: 41706 (an abstract)

An irrelevant reference concerns an unrelated compound.

Ref.: 2675

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

See point 3.3.10.1

3.3.11. Human data

See point 3.3.10.1

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Not applicable

3.3.14. Discussion

Methyl-N-methylanthranilate has a photo-toxic potential.

4. CONCLUSION

Methyl-N-methylanthranilate is phototoxic as demonstrated by both *in vivo* and *in vitro* experiments. Although the action spectrum of the phototoxicity has not been provided, phototoxicity is normally within the UVA spectrum.

The lowest NOAEL in humans was at 0.5% with 16 J UVA/cm² (with 0.75 MED UVB) (ref 34768). However, an *in vitro* test indicated that it was phototoxic at 0.05%, the lowest dilution tested (ref 9196). Phototoxicity is related to the product of dose and UV exposure.

Because of the phototoxicity, methyl-N-methylanthranilate should not be deliberately added to leave-on cosmetic products, as there is always the potential for light exposure.

Until appropriate toxicity data on the substance are available, including information on the possible nitrosamine formation by this secondary amine, up to 0.1% can be used in rinse-off finished cosmetic products.

The above opinion applies also to the presence of methyl-N-methylanthranilate in essential oils, including Petitgrain Mandarin.

5. MINORITY OPINION

Not applicable

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