EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

Methyl-N-methylanthranilate (Phototoxicity only)

Scientific Committees
on consumer safety
on emerging and newly identified health risks
on health and environmental risks

The SCCS adopted this opinion at its 13th plenary meeting

of 13-14 December 2011

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

The fragrance substance Methyl-N-methylanthranilate (CAS n° 85-91-6 and EC n° 201-642-6) is one of the substances regulated by IFRA[1] and has therefore also been subject to a further evaluation by the SCCNFP[2] in the opinion (SCCNFP/0392/00) "An Initial List of Perfumery Materials which must not form part of Cosmetic Products except subject to the restrictions and conditions laid down". Methyl-N-methylanthranilate was mentioned as entry 21.

An updated IFRA recommendation led to submission II for this substance.

A SCCP opinion (SCCP/1068/06) on photo-toxicity only was adopted during the 10th SCCP plenary meeting of 19 December 2006 with the following 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 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."

The current submission III, a compilation of studies based on a complete literature search was provided in May 2008 by EFFA[3]. The submission should provide toxicity data in order to allow the substance to be use in concentration up 0.1% in leave-on products including deodorants and antiperspirant. For rinse-off products the applicant has applied for a concentration up to 0.2%.

2. TERMS OF REFERENCE

1. *Does SCCS consider Methyl-N-methylanthranilate safe for use in leave-on products including deodorants and antiperspirants in a concentration up to 0.1% taken into account the scientific data provided?*

2. *Does SCCS consider Methyl-N-methylanthranilate safe for use in rinse-off products in a concentration up to 0.2% taken into account the scientific data provided?*

3. *And/or does the SCCS have any further scientific concerns with regard to the use Methyl-N-methylanthranilate cosmetic products?*

[1] IFRA International Fragrances Association
[2] SCCNFP Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers
[3] EFFA European Flavour & Fragrance Association

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

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

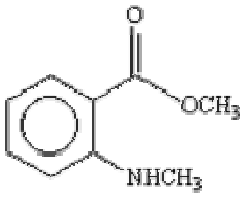
3.1.1.3.	Trade names and abbreviations

/

3.1.1.4.	CAS / EC number

CAS: 85-91-6
EC: 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 g/mol

3.1.4.	Purity, composition and substance codes

/

3.1.5. Impurities / accompanying contaminants

/

3.1.6. Solubility

Miscible in all proportions with ethanol 96%, DMSO and diethyl ether

257 mg/L at 25°C (calculated, solvent not specified)

0.0053 mol/L in unbuffered Water, pH 7.32

3.1.7. Partition coefficient (Log P_{ow})

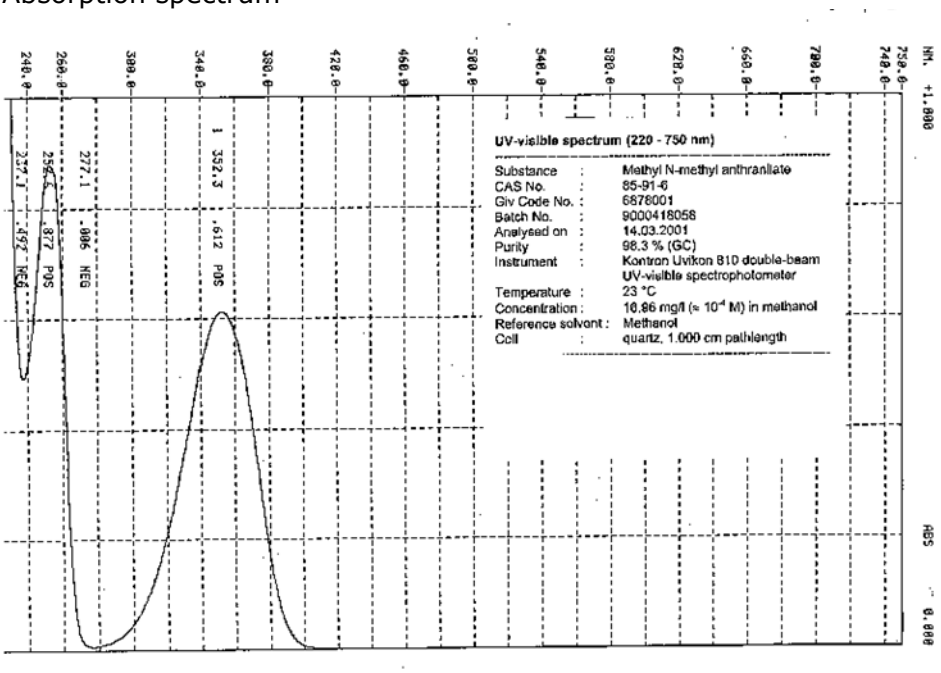
Log P_{ow}: 2.8 (calculated)

3.1.8. Additional physical and chemical specifications

Melting point:	19 °C
Boiling point:	256 °C
Flash point:	> 110 °C
Vapour pressure:	0.01mm Hg at 20 °C (calculated)
Density:	1.12 – 1.13 at 25 °C
Viscosity:	/
pKa:	/
Refractive index:	1.57900 - 1.58100 at 20 °C

Potential Nitrosamine Formation:
Methyl N-methyl anthranilate may potentially form nitrosamines under certain conditions that could cause nitrosamine formation.

Absorption spectrum



General Comments

Water solubility and Log P_{ow} were reported as calculated values, but not determined according to EC Methods A.6 and A.8 respectively.

- Calculated Log P_{ow} values are not acceptable. The Log P_{ow} strongly depends on the pH, especially for ionisable molecules, zwitterions etc. Therefore, a single calculated value of Log P_{ow}, usually without any reference to the respective pH, cannot be correlated to physiological conditions and to the pH conditions of the percutaneous absorption studies.

3.2. Function and uses

Methyl N-methylanthranilate is a fragrance ingredient used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 10 to 100 metric tonnes per annum.

In a use level survey the ten highest concentrations used in fragrance compounds were from 1.285 - 4.8%. 4065 fragrance compounds contained the ingredient. (IFRA 2004)

Methyl-N-methylanthranilate has an IFRA Standard restricting its use to 0.1% for leave-on products. There are no restrictions for its use in non skin-contact products or on rinse-off products including household cleaning products. The Standard is set due to the phototoxic effects of the material.

Reported to occur in orange peel oil (200 ppm); mandarin peel oil (3800-8500 ppm); tangerine peel oil (720 ppm); shima-mikan peel oil (6700 ppm) and in grapefruit juice, bergamot oil, honey and starfruit (TNO, 2008).

Petitgrain Mandarinier (Citrus reticulata blanco): range 4-55% (ISO 8898)
Mandarine oil Italian (Citrus reticulata blanco): range 0.3-0.6% (ISO 3528)
Petitgrain bigaradier (Citrus aurantium amara): traces

Main natural food occurrence is in Mandarin oil (6500 mg/kg). (Council of Europe 2000)

The daily oral intake in man was stated as 10.1 mg per day (Bar; 29590)

In Europe, daily oral intake is estimated at 60μg/day (1 μg/Kg bw/day). An ADI of up to 0.2 mg/kg bw was established (JECFA 2005)

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

The acute oral LD50 of methyl-N-methylanthranilate in rats (n=10/dose) was reported to be 3.7 (3.0 – 4.5) g/kg bw. Mortality was 0, 3, and 6 of 10 rats at 2.50, 3.18, and 4.0 g/kg bw, respectively. No additional details were reported (RIFM, 1974a).

In a range finding study for a subchronic study, the acute oral LD50 of methyl-N-methylanthranilate in rats (n=4 females/dose) was determined using oral doses of 1 – 11.3 g/kg bw. The animals were observed for 7 days following dosing. There was no mortality at 2.25 g/kg bw or less, but 100% mortality at the next higher dose of 3.38 g/kg. There were no clinical signs of adverse effects and no abnormal gross autopsy finding in survivors. At the higher doses there was a short period (15 minutes) of increased exploratory behaviour followed by a decrease in motor activity and failure to respond to painful stimuli. Death occurred 18-48 hours after dosing without recovery of consciousness. There was piloerection from 4 hr and some red-coloured nasal discharge in the last hours before death. The only abnormal gross autopsy finding was slight reddening of the lungs (Gaunt, 1970).

The acute dermal LD_{50} of methyl-N-methylanthranilate in rabbits (n=4) was reported to be >5 g/kg. There was no mortality. No additional details were reported (RIFM, 1974a).

Route	Species	No. animals / dose group	LD_{50}	Reference
oral	rat	10	3.7 g/kg	RIFM 1974a
oral	rat	4	>2.5 <3.38 g/kg	Gaunt 1970
dermal	rabbit	4	>5 g/kg	RIFM 1974

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

In a hairless mouse (Skh:hairless-1) phototoxicity study, an open application of 100% methyl-N-methylanthranilate (20 µl/5cm^2) was not irritating at non-irradiated locations. Evaluations were done at 2.5, 4.5, 24.5, and 48.5 hours after application (RIFM, 1978).

No irritation was reported during the pre-screen phase of two human maximization studies of 10% methyl-N-methylanthranilate. There were five subjects (sex not specified) for the pre-screen in each study (RIFM, 1974b; RIFM, 1974c).

In 2 human phototoxicity studies of 0.1 to 0.5% (n=24 females, 5 males) or 1% (n=35 females) methyl-N-methylanthranilate in 25%:75% diethyl phthalate:ethanol (DEP:EtOH), there was no difference between test material or either vehicle or blank controls in severity (+ to 1+) or incidence of response at non-irradiated sites at any observation time (1, 24, 48, or 72 hours after patch removal). The incidence was approximately 25% at 1 hour, and decreased with increasing time to complete resolution in all subjects by the end of the study (72 or 144 hours). A single 24 hour occluded application of duplicate patches was made to naïve sites. One of the duplicate sites was exposed to UVB and UVA radiation for evaluation of phototoxic potential, and one was used to evaluate primary irritation (RIFM, 1998; RIFM, 1999).

There were no reactions at non-irradiated sites in phototoxicty (n=5 males & 5 females) and photoallergy (n=8 males and 10 females) studies of undiluted methyl-N-methyl anthranilate. Samples (5 ul/cm^2) were applied under occlusion to the back for 6 and 24 hours. Reactions were graded immediately and 24 and 48 hours after patch removal in the phototoxicity study and at 24, 48, and 72 hours after challenge patch removal in the photoallergy study (RIFM, 1978a).

METHOD	DOSE	RESULTS		REFERENCES
		Reactions	Incidence	
Maximization pre-screen	10% in petrolatum	none	0%	RIFM, 1974b
Maximization pre-screen	10% in petrolatum	none	0%	RIFM, 1974c
24-hour occluded patch; irritation control in phototoxicity study	0.1 – 0.5% in 25:75 DEP:EtOH	+ to 1+ reactions at all levels, including vehicle and blank control	No difference from controls	RIFM, 1998
24-hour occluded patch; irritation control in phototoxicity study	1.0% in 25:75 DEP:EtOH	+ to 1+ reactions at all levels, including vehicle and control	No difference from controls	RIFM, 1998
Phototoxicity Photoallergy	100%	No reactions at unirradiated sites	No difference from controls in either study	RIFM, 1978a

Comment
Methyl-N-methylanthranilate is non-irritating to skin.

3.3.2.2. Mucous membrane irritation

No data submitted

3.3.3. Skin sensitisation

Human maximization tests (Kligman, 1966; Kligman and Epstein, 1975) were carried out with 10% methyl-N-methylanthranilate in petrolatum on various panels of volunteers. Application was under occlusion to the same site on the forearms or backs of all subjects for five alternate day, 48-hour periods. Patch sites were pre-treated for 24 hours with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a 10 – 14 day rest period, challenge patches were applied under occlusion to fresh sites for 48 hours. Reactions were read at patch removal and again at 24 hours. The following results were obtained:

• In the first study, 2 subjects reacted to methyl-N-methyl anthranilate Four unrelated materials were tested simultaneously on this group of 25 volunteers. One of these 4 materials, citral produced severe reactions in 14 subjects. The reactions that were seen with methyl-N-methyl anthranilate were considered to be false positive reactions because of a spillover effect from the strong reactions produced by citral.

Ref.: RIFM, 1974b

• Because of these positive reactions, the same sample of methyl-N-methyl anthranilate was subsequently retested in another maximization test and produced no reactions.

Ref.: RIFM, 1974c

• In a photoallergenicity study, undiluted methyl-N-methylanthranilate was not allergenic at non-irradiated sites in a study with 8 male and 10 female subjects. A sample of 100% methyl-N-methylanthranilate was applied occluded to duplicate sites on the back at a dose of 5 µl/cm2. Challenge was 10 days after the last exposure. Methyl-N-methylanthranilate was applied at the same dosage and in the same way as the induction doses. Sites were examined at 24, 48, and 72 hours after patch removal. (RIFM, 1978; Kaidbey, 1978).

METHOD	CONCENTRATION	RESULTS		REFERENCES
		Reactions	Incidence	
Maximization	10% (6900 μg/cm^2)	2/25	8%	RIFM, 1974b
Maximization	10% (6900 μg/cm^2)	0/25	0%	RIFM, 1974b
Sensitization control during photoallergy study	100% (5 ul/cm2)	0/18 at non-irradiated sites	0	RIFM, 1978a

Methyl-N-methylanthranilate was not sensitizing when administered to guinea pigs in a modified maximization test. In this study 0.1 ml of a 5% solution was co-administered with 0.1 ml of Freund's Complete Adjuvant (FCA) twice and 250 mg was applied dermally with an occluded patch at 25% in petrolatum. Challenge dose was given on day 21 dermally in an occluded 24 hour application of a subirritant concentration (not specified). Reactions were read at 24 and 48 hours (Klecak, 1977).

Methyl-N-methylanthranilate at 10% in a "suitable solvent" such as ethanol, acetone, water, petrolatum or PEG was not sensitizing in a guinea pig open epicutaneous test (OET). On Day 0, 0.1 ml was applied to an area measuring 8 cm^2 on the clipped flank of 6 – 8 guinea pigs per concentration. Applications were repeated daily for 21 days to the same site, unless necrotic or ulcerating reactions were provoked. Application sites were left uncovered. Reactions were read 24 hours after each application. Animals were treated to a challenge dose on Days 21 and 35 on the contralateral flank with 0.025 ml of the minimal irritating concentration (3%) to a skin area measuring 2 cm^2. Reactions were read at 24, 48, and 72 hours. Minimal irritating concentration was determined by open dermal application for 21 days of 0.025 ml of serially diluted concentrations to 100%. (Klecak, 1977; Klecak 1979; Klecak, 1985).

Methyl-N-methylanthranilate was not sensitizing in guinea pigs when injected intradermally at a dose of 0.05 ml at a concentration of 0.1% in isotonic saline on day 0 and subsequently at a dose of 0.1 ml on 9 alternate days for a total induction dose of 0.95 mg. Challenge dose was 0.05 ml of 0.1% delivered intradermally on days 35 and 49 (Klecak, 1977).

Methyl-N-methylanthranilate was not sensitizing in guinea pigs when injected intradermally 5 times (total dose 250 mg) at doses of 0.05 ml of undiluted compound mixed with the same volume of FCA. Challenge doses were administered dermally in occluded 24 hour applications at a subirritant concentration (not specified) on days 21 and 35 (Klecak, 1977).

METHOD	INDUCTION CONCENTRATION	CHALLENGE CONCENTRATION	REACTIONS	REFERENCES
Draize intradermal injection	0.5 ml of 0.1% in isotonic saline 9 times	Same as induction, on days 35 and 49	none	Klecak, 1977
Modified Maximization	0.1 ml of 5% w and wo Freunds Complete Adjuvant twice intradermal plus 250 mg at 25% in petrolatum dermal	Subirritant, not otherwise specified	none	Klecak, 1977
Freunds Complete Adjuvant	0.05 ml plus 0.5 ml FCA, intradermal five times	Subirritant, not otherwise specified	none	Klecak, 1977
OET	Serial dilutions to 100% 21 times	3%	none	Klecak, 1977
OET	10%	Not specified	none	Klecak, 1979 Klecak 1985

Local Lymph Node Assay (LLNA): No data submitted.

Comment
No experiment conformed to current guidelines and no LLNA is available. However, the available information suggests that methyl-N-methylanthranilate is not a sensitiser.

3.3.4. Dermal / percutaneous absorption

No data submitted

3.3.5. Repeated dose toxicity

Two to 30-day studies: No data submitted.

In a 13 week feeding study in rats (n=15/sex/dose), the NOAEL of methyl-N-methylanthranilate was reported to be 300 ppm (~21 mg/kg/day). Animals were housed 5 to a cage and fed, *ad libitum*, diets containing 0, 300, 1200, or 3600 ppm (~ 21, 82, and 244 mg/kg/day) methyl-N-methylanthranilate.
There were no mortality and no adverse clinical signs, and food and water intake and body weights were similar for all groups. There were no differences among groups in results of serum or urine analysis at week 6 or at study termination. During week 6, but not at termination, haemoglobin and red blood cell levels were lower in males and females in the mid- and high-dose groups. At termination, males in the mid- and high-dose groups had higher absolute kidney weights and both males and females in these two groups had greater relative kidney weights. No treatment related gross or histological abnormalities were seen at necropsy (Gaunt, 1970).

There was no effect when 20.3 mg/kg methyl-N-methylanthranilate (19.9 for males, 22.2 for females) was administered to rats (n= 15 males and 15 females and equal number of controls) in the diet for 90 days. At termination of the study, there were no significant differences from controls in body weight gain, efficiency of feed utilization, haematocrit, red blood cell count, white blood cell count, % neutrophils, % lymphocytes, blood urea nitrogen, or blood glucose. There were no gross or histological pathological findings attributable to test compound administration. The NOAEL was 20mg/kg bw/day, the only dose tested (Oser, 1965; reported in Bar, 1967 and JECFA 2005).

	Dose			Reference
90 day diet	21, 82, 244 mg/kg/day	rat	NOAEL 21mg/kg/day based on increased absolute and relative kidney weights	Gaunt 1970
90 day diet	Average daily intake 19.9mg/kg bw for males and 22.2mg/kg bw for females	rat	NOAEL 20.3mg/kg/day	Oser 1965

Chronic (90+ days) studies: No data submitted.

3.3.6. Mutagenicity / Genotoxicity

Bacterial Gene Mutation Assay

Method:	OECD 471
Substance:	dimethyl anthranilate
CAS:	85-91-6
Batch	9000427273
Purity	95.3%
GLP	in compliance
Date	2003

Dimethyl anthranilate was tested in DMSO in the *Salmonella typhimurium* reverse mutation assay with 5 histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535, and TA1537). The test was performed in two separate experiments in the presence and absence of S9-mix.

In the preincubation assay dimethyl anthranilate was tested in a dose range finding study up to 5000μg/plate in TA98 and TA100 but it precipitated on the plates at 3330 and 5000μg/plate. Toxicity was observed at dose levels of 333 μg/plate and up.

When tested at up to 1000 μg/plate in TA1535, TA1537 and TA102, toxicity was observed in all tester strains.

In the direct plate assay, it was tested in a dose range finding study up to 5000 μg/plate in TA98 and TA100. It precipitated on the plates at doses of 3330 and 5000 μg/plate and toxicity was observed at these dose levels.

The substance was tested at up to 3330 μg/plate in TA1535, TA1537 and TA102 but it precipitated at this upper level. Toxicity was observed in all tester strains.

Dimethyl anthranilate did not induce a dose-related increase in the number of revertant (His^+) colonies in each of the 5 tester stains both in the absence and presence of S9-metabolic activation. These results were confirmed in a separate experiment.

Based on the above, dimethyl anthranilate is not mutagenic in the *Salmonella typhimurium* reverse mutation assay.

Ref.: RIFM, 2003

Studies in Mammalian Cells

Methyl-N-methylanthranilate at 0.00001 to 0.001 M (0.16 – 165 μg/ml calculated) did not induce unscheduled DNA synthesis in cultured hepatocytes from ACI rats. (Yoshimi, 1988).

Comment

The SCCS points out that not all genotoxic end-points have been addressed.

3.3.7. Carcinogenicity

No data available on this material.

3.3.8. Reproductive toxicity

There are no adequate data available.

One study is reported in an abstract in which mice were injected intraperitoneally. Since only an abstract is available, the description of methodology is incomplete, the quality of the data is poor, and no maternal data were provided.

Pregnant AJ mice were injected intraperitoneally with 25-75 mg/kg bw/day methyl-N-methylanthranilate on gestational days 10.5-12.5 or 11.5-13.5 (gestation day 0 was not identified). The authors note that while not conclusive, there was an increase in the incidence of cleft lip and/or palate to 20%, compared to 4% for controls. It was also reported that preliminary results indicated that methyl-N-methylanthranilate elevated the level of protein carboxymethylation in cultured palate cells. A full report has not been published.

Ref.: Clark, 1980

Comment
The above study appears not to have been published in full. However, the mode of administration needs to be considered in relation to the foreseeable systemic exposure from cutaneous application (see 3.3.11.) and the rapid metabolism of methyl-N-methylanthranilate after oral administration. Therefore, although this data is of concern, it may not be useful for risk assessment

No adequate study on reproductive toxicity is available.

3.3.9. Toxicokinetics

No data submitted on the distribution of this material.

Intestinal absorption was examined after doses of 25 to 260 ppm into the duodenal lumen of male Dunkin-Hartley rats. Samples of portal blood taken up to 30 min after administration revealed rapid absorption at all concentrations. At 25ppm, no unhydrolysed ester was detected, indicating that methyl N-methylanthranilate was absorbed as the hydrolysed form. No unhydrolysed ester was observed after 10 min at 40ppm or after 20 min at 120ppm. At 260ppm, the unhydrolysed ester was detected at all times, peaking at 5 min (Pelling, 1980).

The metabolism of methyl-N-methylanthranilate is consistent with that of anthranilic acid esters. The ester function undergoes hydrolysis, principally in the liver, followed by excretion of N-methylanthranilic acid in the urine (JECFA, 2005).

In rats and humans, the main reaction of methyl N-methylanthranilate is hydrolysis to N-methylanthranilic acid, with little N-demethylation, to yield anthranilic acid (ratio of N-methylanthranilic acid:Anthranilic acid, approximately 20:1); the metabolites are eliminated in the urine (Morgareidge, 1963; JECFA, 2005).

Methyl-N-methylanthranilate was metabolized by guinea pig liver microsomes to N-methyl anthranilic acid. The hydrolytic activity at a substrate concentration of 1000 uM was 35 nmol/min/mg protein. Methyl-N-methylanthranilate was also demethylated to methylanthranilate. The N-demethylase activity at 1000 μg substrate concentration was 3.9 nmol/min/mg protein. Kinetic analysis indicated that Vmax/Km values were 7.4 fold higher in microsomes than in cytosol. The hydrolytic activity was markedly inhibited by diisopropyl fluorophosphate, phenylmethylsulfonyl fluoride, and bis(p-nitrophenyl)phosphate but not by physostigmine. Hydrolytic activity was suppressed by aspirin, a substrate of carboxylesterase, in a concentration dependent manner. Demethylation was inhibited by SKF 525-A, a non-selective inhibitor of cytochrome P450 (Yamaori, 2005).

The carboxylic ester bond of methyl-N-methyl anthranilate was hydrolyzed by pig liver and pig jejunum homogenates. At a substrate concentration of 250 µl/l, in 2 hours the liver and

jejunum homogenates hydrolyzed >99% and 20%, respectively, of the compound (Grundschober, 1977; RIFM, 1974d).

Cytochrome-P-450 mediated metabolism of 15mM methyl-N-methylanthranilate by nasal and liver microsomes from Fischer-344 rats was inhibited 77% and 16%, respectively, by 3 mM heliotropin (Dahl, 1982).

Formaldehyde was produced when methyl-N-methylanthranilate was metabolized by microsomes from rat nasal mucosa. The rate of formaldehyde production exceeded 1000 pmol/mg microsomal protein per minute (Dahl, 1983).

TEST SYSTEM *in vitro*	SPECIES	CONCENTRATION	RESULTS	REFERENCES
	Guinea pig liver microsomes	1000 µM	Ester hydrolysis at 35 mg/min/mg protein and N-demethylation at 3.9 mg/mg protein/min	Yamaori, 2005
	Rat nasal mucosal microsomes		Rate of formaldehyde release >1000 pmol/mg protein/min	Dahl, 1983
	Pig liver and jejunum hydrolysates	250 ul/l	Ester hydrolysis in 2 hr >99% for liver and ~20% for jejunum	Grundschober, 1977 RIFM, 1974d
	Nasal and liver microsomes from F-344 rat	15 mM	Cyt P-450 mediated metabolism inhibited by 3 mM heliotropin, 77% for nasal and 16% for liver	Dahl, 1982

Comment
During ester-cleavage of the molecule, methanol will be formed. This could be the cause of formaldehyde production observed in the study of Dahl, 1982.

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; Xenon arc solar simulator with Schott WG 345 filter to eliminate <320nm.
GLP: /

Phototoxicity was evaluated on 10 volunteers (5 male/5 female). Methyl –N-methylanthranilate at a concentration of 5% in hydrophilic ointment was applied at a dose

of 5 µl/cm2 to a 2 cm^2 area. The test sites were covered with nonwoven cotton cloth and Blenderm tape was used to secure the patches to the skin. Six hours later, a 1-cm circle in the test area was exposed to 20 J/cm^2 UVA from a 150 watt Xenon-arc solar simulator with a Schott WG 345 filter (UVA irradiance, 29 - 35 mW/cm^2). Reactions were read immediately after the irradiation, and also at 24 and 48 hours after the irradiation. Phototoxic reactions were observed in 8/10 subjects

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

Ref.: RIFM, 1978a; Kaidbey, 1980

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.: Pagnoni et al, 2001; RIFM, 2001.

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.: Kaidbey, 1978

Guideline:	/
Species:	human
Group:	25 (both sexes, unknown ratio)
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.: Kaidbey, 1978

A photoallergy study using the photomaximization procedure was conducted on 18 volunteers (8 male/10 female). A sample of 5% methyl N-methylanthranilate in hydrophilic ointment was applied at a dose of 5 µl/cm² for 24 hours under occlusion to skin sites over the mid-back. At patch removal, the site was immediately exposed to 3 MED using a 150 Watt Xenon-arc Solar Simulator. This procedure was repeated 48 hours later and subsequently thereafter to the same test site for a total of six exposures (two exposures per week). Challenge was conducted 10 days after the last induction exposure. An application with 5% methyl N-methylanthranilate in hydrophilic ointment at a dose of 5 µl/cm² was made for 24 hours under occlusion to a normal skin site. Application was followed by 3 minutes of long ultraviolet light (UVA) from the Solar Simulator with a Schott WG 345 filter. A non-irradiated treated site and an irradiated vehicle treated site served as the controls. The reactions were read at 24, 48 and 72 hours after the irradiation. No photoallergic reactions were observed; however, phototoxic reactions were observed in 14/18 subjects.

Ref: RIFM, 1978b; Forbes et al., 1978

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 and produced 14/35 reactions

Ref.: Berger et al, 1999; RIFM, 1999.

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.: Berger et al, 1998; RIFM, 1998

CONCENTRATION	RESULTS		REFERENCES
	Reactions	Incidence	
5% in hydrophilic ointment	14/18	78%	RIFM, 1978a
0.5% in 75% EtOh/25% DEP	0/29	0%	RIFM, 1998
0.3% in 75% EtOh/25% DEP	0/29	0%	RIFM, 1998
0.1% in 75% EtOh/25% DEP	0/29	0%	RIFM, 1998
1% in 75% EtOh/25% DEP	14/35	40%	RIFM, 1999
0.5% in 75% EtOh/25% DEP (phototoxicity evaluated during the induction phase of a photoallergy test)	0/26	0%	RIFM, 2001

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.: Letizia et al., 2003 (abstract)

Comment
In the above experiments, the test substance was applied under occlusive conditions for 24 hours before irradiation. It is unknown what the retention and metabolism of the test substance is in the skin during this period.
The appropriateness of the vehicle used in the above studies is questionable.

Guideline: /
Species: hairless mice, Skh:hairless-1
Group: /
Substance: dimethyl anthranilate
Methyl-N-methyl anthranilate (ICI 1752) (100% and at 50% in methanol)
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.: Forbes et al., 1978; RIFM, 1978b

***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.: Forbes et al., 1978

***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: Bagley, 1988

Methyl-N-methylanthranilate was reported to be phototoxic in an in vitro study with brewers' yeast (*Saccharomyces cerevisiae*). Filter paper disks (8 mm diameter) were saturated with test material and placed on dextrose agar plates streaked with the yeast. With styrene covers in place, plates were exposed at room temperature to a bank of F40T12BL black light lamps and examined for 4 days. Replicate plates were maintained in the dark. Growth inhibition adjacent to a disk was interpreted as phototoxicity when seen in light-exposed plates only. No additional details were provided (RIFM, 1978c).

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.: Api, 1997

***In vitro* Phototoxicity**

Guideline:	3T3 Neutral 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.: Harbell et al, 2002

Guideline: OECD 432; 3T3 Neutral Red Uptake Phototoxicity Assay
Test substance: methyl anthranilate methyle (09-EE-138)
Code: 7403039108
Batch: 9R01
Purity: 100%
Positive Control: chlorpromazine
Negative Control: Earl's balanced salt solution
Test Substance: "*pre-diluted at 0.1% in EtOH as requested by the sponsor and then tested at the highest concentration allowed by the OECD guideline 432 considering its limit of solubility in the suggested solvents*"
GLP: in compliance
Date: December 2009 – January 2010

Results on the test item
PIF /
MPE 0.058
IC5 $_{50}$ value (µg/ml) +UV = / ; -UV = / ;
Classification Non Phototoxic

Conclusion
The test item is considered as Non Phototoxic up to 0.1% under the experimental conditions used.

Ref: Tailhardat, 2010

Comment: It is unclear from the report what the actual dilutions of the test material were.

PIF <2 or MPE <0.1	Not phototoxic		PIF; photo-irritation factor MPE; Mean photo-effect
PIF 2-5 or MPE 0.1-0.15	Probably phototoxic		
PIF >5 or MPE >0.15	Phototoxic		

Guideline: OECD 432; 3T3 Neutral Red Uptake Phototoxicity Assay
Test substance: methyl anthranilate methyle (09-EE-139)
Code: 7403039108
Batch: 9R01
Purity: 100%
Positive Control: chlorpromazine
Negative Control: Earl's balanced salt solution
Test Substance: "*pre-diluted at 0.05% in EtOH as requested by the sponsor and then tested at the highest concentration allowed by the OECD guideline 432 considering its limit of solubility in the suggested solvents*"
GLP: in compliance
Date: December 2009 – January 2010

Results on the test item
PIF /
MPE 0.022
$IC5_{50}$ value (μg/ml) +UV = / ; -UV = / ;
Classification Non Phototoxic

CONCLUSION
The test item is considered as Non Phototoxic up to 0.05% under the experimental conditions used.

Ref: Tailhardat (60099), 2010

Comment
It is unclear from the report what the actual dilutions of the test material were.

Guideline: OECD 432; 3T3 Neutral Red Uptake Phototoxicity Assay
Test substance: methyl anthranilate methyle (09-EE-140)
Code: 7403039108
Batch: 9R01
Purity: 100%
Positive Control: chlorpromazine
Negative Control: Earl's balanced salt solution
Test Substance: "*pre-diluted at 0.01% in EtOH as requested by the sponsor and then tested at the highest concentration allowed by the OECD guideline 432 considering its limit of solubility in the suggested solvents*"
GLP: in compliance
Date: December 2009 – January 2010

Results on the test item
PIF /
MPE 0.009
IC5 $_{50}$ value (μg/ml) +UV = / ; -UV = / ;
Classification Non Phototoxic

CONCLUSION
The test item is considered as Non Phototoxic up to 0.01% under the experimental conditions used.

Ref: Tailhardat, 2010

Comment: It is unclear from the report what the actual dilutions of the test material were.

Essential Oils

3T3 Neutral Red phototoxicity test

Petitgrain Mandarin	8014-17-3	PIF 10.08 MPE 0.45 IC5 $_{50}$ value (μg/ml) +UV = 31.8 -UV = 320	Phototoxic	Baylac S. Petitgrain Mandarin EO 021500
Petitgrain Bitter Orange	72968-50-4	PIF 0.89 MPE -0.02 IC5 $_{50}$ value (μg/ml) +UV = 360.9 -UV = 318.1	Not Phototoxic	Baylac S. Petitgrain Bitter Orange
Mandarin Peel	8008-31-9	PIF 0.79 MPE 0.03 IC5 $_{50}$ value (μg/ml) +UV = 104.2 -UV = 80.8	Not Phototoxic	Baylac S. Mandarin peel oil
Petitgrain Citronnier	8048-51-9	PIF 1.87 MPE 0.017 IC5 $_{50}$ value (μg/ml) +UV = 73.93 -UV = 138.4	Not Phototoxic	Baylac S. Petitgrain Citronnier

Petitgrain Manarinier	84929-38-4	Methyl N methyl anthranilate 50%	3T3 NRU positive
Petitgrain Bigarade	8014-17-3	0.01%	negative
Petitgrain Citonnier	8008-57-9	0.04%	negative
Petitgrain Zeste	8016-85-1 8008-31-9	0.05%	negative

Ref: unknown

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

Calculation of the total human skin exposure from the use of multiple cosmetic products containing Methyl N-methylanthranilate. The table below was provided by the applicant.

Product Type	Grams Applied	Applications per day	Retention factor	Mixture/ Product	Ingredient/ Mixture[a]	Ingredient mg/kg/day[c]
Anti-perspirant	0.5	1.00	1	0.01	0.21	0.0002
Bath Products	17	0.29	0.001	0.02	0.21	0.000003
Body Lotion	8	0.1	1	0.004	0.1[b]	0.0004
Eau de Toilette	0.75	0.1	1	0.08	0.1[b]	0.0010
Face Cream	0.8	0.1	1	0.003	0.1[b]	0.0001
Fragrance Cream	5	0.1	1	0.04	0.1[b]	0.0010
Hair Spray	5	2	0.01	0.005	0.21	0.00002
Shampoo	8	1	0.01	0.005	0.21	0.00001
Shower Gel	5	1.07	0.01	0.012	0.21	0.00002
Toilet Soap	0.8	6	0.01	0.015	0.21	0.00003
Total						**0.0027**

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] IFRA Standard restricting its use to 0.1% for leave-on-products. The limit only applies to applications on skin, excluding rinse-off products.
[c] Based on a 60 kg adult

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Not applicable

3.3.14. Discussion

In Europe, an ADI up to 0.2 mg/kg bw has been established. Using information on potential cutaneous exposure to methyl-N-methylanthranilate from industry data, a maximum systemic exposure of 0.0027 mg/kg bw/day may be estimated. No specific data on percutaneous absorption is available.

The substance is not irritant to skin. No information on mucous membrane irritation is available.

Although not conforming to current guidelines, the available data suggests that the substance is not a contact allergen.

90 day rat studies suggest a NOAEL of circa 20mg/kg bw/day, based on increased absolute and relative kidney weights at 80 mg/kg bw.

Dimethyl anthranilate is not mutagenic in the *Salmonella typhimurium* reverse mutation assay. Methyl-N-methylanthranilate (0.16 – 165 μg/ml) did not induce unscheduled DNA synthesis in cultured rat hepatocytes. Not all genotoxic end-points have been addressed.

There is no adequate reproductive toxicity study available.
Intestinal absorption in doses of 25 to 260 ppm into the duodenal lumen of rats revealed rapid absorption at all concentrations. At 25ppm, no unhydrolysed ester was detected, indicating that methyl N-methylanthranilate was absorbed as the hydrolysed form. No unhydrolysed ester was observed after 10 min at 40 ppm or after 20 min at 120 ppm. At 260 ppm, the unhydrolysed ester was detected at all times, peaking at 5 min.

Methyl-N-methylanthranilate has an established phototoxic potential.

- 1.0% dimethyl anthranilate (w/v in 25% v/v diethyl phthalate / ethanol) was considered to be phototoxic and produced reactions in 14/35 humans.
- 0.5% dimethyl anthranilate (w/v in 25% v/v diethyl phthalate / ethanol) produced reactions in 0/26 humans.
- 3T3 Neutral Red phototoxicity test (used for hazard identification) indicated that methyl-N-methylanthranilate is non-phototoxic at 0.1% under the experimental conditions used.
- With an *in vitro* yeast toxicity study (*Saccharomyces cerevisiae*), 0.05% dimethyl anthranilate was phototoxic.

In the above experiments in humans, the test substance was applied under occlusion for 24 hours before irradiation. It is unknown what the retention and metabolism of the test substance is under these conditions.

- The experiment with 1% dimethyl anthranilate (w/v in 25% v/v diethyl phthalate / ethanol) indicates that the test substance was present in sufficient quantity to cause a phototoxic reaction..
- There is no information on the scenario of application of ≤0.5% methyl-N-methylanthranilate with UV irradiation following soon afterwards.
- There is also no information on repeated low dose exposures to methyl-N-methylanthranilate with irradiation.

Essential oils containing methyl-N-methylanthranilate may be phototoxic.

4. CONCLUSION

1. *Does SCCS consider Methyl-N-methylanthranilate safe for use in leave-on products including deodorants and antiperspirants in a concentration up to 0.1% taken into account the scientific data provided?*

Methyl-N-methylanthranilate is phototoxic and this is the toxicological endpoint of concern. Whilst up to 0.1% methyl-N-methylanthranilate may be safe for use in many leave-on cosmetic products, including deodorants and antiperspirants, the SCCS considers that for the use in sunscreen/sun care products or products (including fragrances) intended for use on areas exposed to light (especially face and neck), a risk cannot be excluded. This is because there is no information on UV irradiation given soon after application of methyl-N-methylanthranilate or the effects of repeated low dose exposures with UV irradiation.

2. *Does SCCS consider Methyl-N-methylanthranilate safe for use in rinse-off products in a concentration up to 0.2% taken into account the scientific data provided?*

The available information suggests that there is no safety concern on the use of methyl-N-methylanthranilate at up to 0.2% in rinse-off products.

3. *And/or does the SCCS have any further scientific concerns with regard to the use Methyl-N-methylanthranilate cosmetic products.*

Methyl-N-methylanthranilate is a secondary amine, and thus prone to nitrosation. It should not be used in combination with nitrosating substances. The nitrosamine content should be < 50 ppb.

There is no information on the possible combination effects of the presence of more than one phototoxic substance in cosmetic products.

The presence of methyl-N-methylanthranilate in essential oils is considered in the above.

5. MINORITY OPINION

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