Scientific Committee on Consumer Safety

SCCS

OPINION ON

the fragrance ingredients *Tagetes minuta* and *T. patula* extracts and essential oils (phototoxicity only)

The SCCS adopted this Opinion at its 9th plenary meeting

on 25 March 2015

The Corrigendum was adopted by written procedure on 13 December 2017

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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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ISSN 1831-4767 ISBN 978-92-79-35666-7

Doi:10.2772/54225 ND-AQ-14-019-EN-N

http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

ACKNOWLEDGMENTS

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The corrigendum is adding a clarification that the conclusion refers to sunscreen products and products marketed for exposure to natural/artificial UV light. The Opinion has been amended by written procedure on 13 December 2017.

Keywords: SCCS, scientific opinion, fragrance ingredients, *Tagetes minuta*, *T. patula*, Regulation 1223/2009, CAS 91722-29-1, 91770-75-1, EC 294-431-3, 294-862-7, Corrigendum

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on the fragrance ingredients: *Tagetes minuta* and *T. patula* extracts and essential oils, SCCS/1551/15, 25 March 2015, Corrigendum 13 December 2017

TABLE OF CONTENTS

1. BACKGROUND 5

2. TERMS OF REFERENCE 5

3. OPINION 6

3.1 Chemical and Physical Specifications 6

3.1.1 Chemical identity 6
3.1.2 Physical form 6
3.1.3 Molecular weight 6
3.1.4 Purity, composition and substance codes 7
3.1.5 Impurities / accompanying contaminants 7
3.1.6 Solubility 7
3.1.7 Partition coefficient (Log P_{ow}) 7
3.1.8 Additional physical and chemical specifications 7
3.1.9 Homogeneity and Stability 7

3.2 Function and uses 8

3.3 Toxicological Evaluation 8

3.3.1 Photo-induced toxicity 8
3.3.2 Human data 17
3.3.3 Safety evaluation (including calculation of the MoS) 34
3.3.4 Discussion 34

4. CONCLUSION 37

5. MINORITY OPINION 37

6. REFERENCES (from the submission) 38

1. BACKGROUND

The Tagetes spp. extracts and oils CAS n. 91722-29-1 are widely used fragrance ingredients of many fragrance compounds used in perfumery. Because of industry assessment that Tagetes spp. extracts and oils cause photo-toxicity, the IFRA standards recommend:

"For applications on areas of skin exposed to sunshine, excluding bath preparations, soaps and other products which are washed off the skin, oils and absolutes obtained from *Tagetes minuta L.*, syn. *Tagetes glandulifera Schrank* and *Tagetes patula L.* should not be used such that the level in the consumer product exceeds 0.01%."

The Scientific Committee on Consumer Products (SCCP) adopted at its 4th plenary meeting the 21 of June 2005 the opinion (SCCP/0869/05) on *Tagetes erecta*, *T. minuta* and *T. patula* Extracts and Oils (phototoxicity only) with the following conclusion:

According to the data submitted, Tagetes extracts and oils are phototoxic. As no safe limit of use in cosmetic products has been demonstrated, it is recommended that Tagetes erecta, Tagetes minuta and Tagetes patula extracts and oils should not form part of cosmetic products.

In August 2013 the Commission received an update dossier by the International Fragrance Association (IFRA) on the safety assessment of Tagetes extracts and essential oils. According to the applicant, Tagetes extracts and essential oils were examined for its phototoxic potential in vitro systems as well as *in vivo* in experimental animals and human volunteers.

This submission is intended to demonstrate the safety of the ingredients when used as fragrance in cosmetic leave-on products with a concentration limit of 0.01%.

2. TERMS OF REFERENCE

1. On the basis of data submitted, does the SCCS consider Tagetes minuta and T. patula extracts and essential oils safe for use as fragrance ingredients in cosmetic leave-on products with a maximum concentration limit of 0.01%?

2. Does the SCCS have any further scientific concerns with regard to the use of Tagetes minuta and T. patula extracts and essential oils as fragrance ingredients in cosmetic products?

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

INCI Names
Tagetes minuta flower extract
Tagetes minuta flower oil
Tagetes patula flower extract
Tagetes patula flower oil

3.1.1.2 Chemical names

3.1.1.3 Trade names and abbreviations

3.1.1.4 CAS / EC number

Tagetes minuta flower extract
CAS: 91770-75-1
EC: 294-862-7

Tagetes minuta flower oil
CAS: 91770-75-1/8016-84-0
EC: 294-862-7

Tagetes patula flower extract
CAS: 91772-29-1
EC: 294-431-3

Tagetes patula flower oil
CAS: 91772-29-1/8016-84-0
EC: 294-431-3/-

3.1.1.5 Structural formula

Not applicable

3.1.1.6 Empirical formula

Not applicable

3.1.2 Physical form

Liquid

3.1.3 Molecular weight

Not applicable

3.1.4 Purity, composition and substance codes

Tagetes spp extracts are widely used fragrance ingredients of many fragrance compounds used in perfumery and perfumed cosmetics. In leave-on cosmetics, tagetes extracts and tagetes oils are used at a maximum concentration up to 0.01%. Tagetes minuta flower extract, Tagetes minuta flower oil, Tagetes patula flower extract and Tagetes patula flower oil are mixtures of many substances. Major constituents of these extracts/oils are limonene, (E)-β-ocimine, β-phelandrene, p-cymene, β –caryophyllene, α-muurolene, terpinolene, α-terpineol, (Z-)-tagetone, (Z)-tagetenone, (E)-tagetenone, dihydrotagtenone, (E)-ocimenone, verbenone, piperitone, pepritenone.

Chemical composition of these extracts/oils varies depending upon the harvesting location, growth stage of the plant, and harvesting time of the budding.
No information was found on the levels of alpha-terthienyl, a phototoxic thiophene compound responsible for the phototoxicty of tagetes extracts/oils (Rampone 1986), in the tagetes extracts/oils used in perfumes and cosmetic products. However, the tagetes extracts/oil used for phototoxicity testing are reported to contain from below detection limit to 2.45% alpha-terthienyl (see 3.3.1).

3.1.5 Impurities / accompanying contaminants

Not applicable

3.1.6 Solubility

Not applicable

3.1.7 Partition coefficient (Log P_{ow})

Log P_{ow}: /

3.1.8 Additional physical and chemical specifications

Melting point:
Boiling point:
Flash point:
Vapour pressure: /
Density: /
Viscosity: /
pKa: /
Refractive index: /
pH: /
UV_Vis spectrum (….. nm): /

3.1.9 Homogeneity and Stability

Not applicable

General Comments to physico-chemical characterisation

No information was found on the levels of alpha-terthienyl, a phototoxic thiophene compound responsible for the phototoxicty of tagetes extracts/oils (Rampone 1986), in the tagetes extracts/oils used in perfumes and cosmetic products. However, the tagetes

extracts/oil used for phototoxicity testing are reported to contain from below detection limit to 2.45% alpha-terthienyl (see 3.3.1).

3.2 Function and uses

Tagetes spp extracts are widely used fragrance ingredients of many fragrance compounds used in perfumery and perfumed cosmetics. In leave-on cosmetics, tagetes extracts and tagetes oils are used at a maximum concentration up to 0.01%.

3.3 Toxicological Evaluation

3.3.1 Photo-induced toxicity

3.3.1.1 Phototoxicity in vitro

New studies since the 2004 submission and the SCCP 2005 opinion.

A.
(Taken from Submission IV)

References:	RIFM 2013f, g, h, i, j, RIFM# 65839, 65840, 65841, 65842, 65843, Certificates of analysis (Firmenich 2012a, b, Robertet 2012 a, b, c)
Date of reports:	2013
Method:	Human skin model test, Procedures according to MatTek Corporation 1997, Phototoxicity Protocol for use with EpiDerm™ Model (EPI-200).
Test system:	*In vitro* reconstituted human fully differentiated epidermis (EST-1000).
Replicates:	Duplicate plates
Test substances:	a) Tagetes patula absolute (EG) b) Tagetes minuta absolute (ZA) c) Tagetes minuta essential oil (EG) d) Tagetes minuta essential oil (ZA) e) Tagetes minuta essential oil - Low terthiophene (ZA)
Batches:	a) 1000881306 (CoA, TTP: no data) b) 2079040 (CoA, TTP: 0.31%) c) 1001063725 (CoA, TTP: no data) d) 2108166 (CoA, TTP: 0.018%) e) 1802615 (CoA, TTP: below limit of detection)
Concentrations:	0.1, 0.316, 1.0, 3.16, 10% (v/v, with/without light irradiation)
Vehicle/negative control:	Sesame oil
Positive control:	/
Duration of exposure:	24 h on skin tissue equivalents
Irradiation:	
Source of light:	Sunlight simulator (Dr. Hoenle SOL 500 solar simulator in combination with the solar standard filter H1 to keep UV-B irradiation as low as possible, spectrum: >320 nm)
Intensity of irradiation:	UV-A: 6 J/cm² (= 1.7 – 1.8 mW/cm²)
Duration of irradiation:	60 min.
Negative Control:	Sesame oil water
GLP:	Yes
Published:	No

Methods:
Tagetes extracts and essential oils (Tagetes patula absolute (EG), Tagetes minuta absolute (ZA), Tagetes minuta essential oil (EG), Tagetes minuta essential oil (ZA), Tagetes minuta essential oil - Low terthiophene (ZA)) were tested for their photo-toxic potential on the three dimensional human epidermis model (EpiDerm^{TM}). The test substances were diluted in sesame oil and concentrations of 0.1, 0.316, 1.0, 3.16, 10% (v/v) were examined. The test solutions were applied onto filter pads, which were then applied onto the skin tissue equivalents for 24 hours. Sesame oil water was used as negative control. Each concentration including negative control was tested at a volume of 20 µL per tissue in duplicates. One test group of skin equivalents treated with the test substance concentrations and the negative control was irradiated with artificial sunlight for 60 minutes at 1.7 – 1.8 mW/cm^2 UV-A corresponding to an irradiation dose of 6 J/cm^2 UV-A. The other test group of skin equivalents treated with the test substance concentrations and the negative control were kept in the dark for 60 minutes. Tissues were then rinsed with PBS to remove test material, transferred to new 6 well plates with fresh medium and incubated over night. The next day, assay medium was replaced by MTT-medium and tissues were incubated for 3 hours with MTT. Tissues were then rinsed with PBS, and the formazan was extracted with isopropanol]. Optical density (OD) was determined at 540/570 nm in a plate spectrophotometer and cell viability was calculated for each tissue as % of the corresponding vehicle control either irradiated or unirradiated.

Results
Tagetes patula absolute (EG):
Dose-dependent cytotoxicity was observed in the presence and absence of irradiation with artificial sunlight. Without irradiation only the highest tested concentration of 10% induced cytotoxic effects (mean absorbance value: 31.9%). Under irradiation, all of the tested concentrations induced clear cytotoxicity. The mean relative absorbance values versus the negative control ranged from 12.7% to 0.1%. Since the decrease of the viability compared to the non-irradiated test groups was >30% of all tested concentrations, the test item showed a phototoxic potential at 0.1, 0.316, 1.0, 3.16, 10% (v/v).

Tagetes minuta absolute (ZA):
Dose-dependent cytotoxicity was observed in the presence and absence of irradiation with artificial sunlight. Starting with the concentration of 0.1%, the mean absorbance value was reduced to 76.8%, the highest tested concentration of 10% induced a mean absorbance value of 2.2% upon irradiation. In the absence of artificial sunlight, only the highest tested concentration caused a relevant reduction of the mean relative absorbance value to 46.3%, while the lower concentrations did not induce a decrease of mean absorbance values. Since the decreases of the viability compared to the non irradiated test groups was ≥30% at 0.316%, 1.0%, 3.16%, and 10%, the test item is considered to have a phototoxic potential to skin. However, the lowest concentration of 0.1% did not meet this criterion and consequently can be considered a threshold and not phototoxic.

Tagetes minuta essential oil (EG):
Dose-dependent cytotoxicity was observed in the presence and absence of irradiation with artificial sunlight. With and without irradiation the three highest tested concentrations of 1.0%, 3.16% and 10% induced cytotoxic effects. The mean absorbance values were within the range of 18.3% to 0.6% under irradiation for these concentrations. Without irradiation, the mean absorbance values of the cytotoxicity inducing concentrations were in the range of 47.1% to 2.1%. However, the decrease of the viability compared to the non-irradiated test groups was <30% after exposure to the different test item concentrations.

Tagetes minuta essential oil (ZA):
Dose-dependent cytotoxicity was observed in the presence and absence of irradiation with artificial sunlight. Under irradiation the three highest tested concentrations of 1.0%, 3.16% and of 10% induced a mean absorbance value of 12.8%, 1.7%, and 0.5%, respectively. In the absence of artificial sunlight, the three highest tested concentrations also caused a relevant reduction of the mean relative absorbance value to 19.3%, 7.1%, and to 0.2%, respectively. The lower tested concentrations did not induce a relevant decrease of mean absorbance values. However, the decrease of the viability compared to the non-irradiated test groups was <30% at any tested concentration.

Tagetes minuta essential oil - Low terthiophene (ZA):
Dose-dependent cytotoxicity was observed in the presence and absence of irradiation with artificial sunlight. Under irradiation the two highest tested concentrations of 3.16% and of 10% induced a mean absorbance value of 9.7% and 4.9%, respectively. In the absence of artificial sunlight also the two highest tested concentrations led to a relevant reduction of the mean relative absorbance value to 16.0% and to 21.3%, respectively. The lower tested concentrations did not induce a relevant decrease. Since the decrease of the viability compared to the non irradiated test groups was <30% at 3.16% and 10%, which caused clear cytotoxic effects, the test item was considered to have no potential to induce phototoxicity to the skin.

The validity of the studies was confirmed as the mean OD of the negative control for irradiated tissues and non-irradiated tissues based on optical density was ≥0.8.

Conclusion
a) Tagetes patula absolute (EG) revealed a phototoxic effect on the skin tissues equivalents at all tested concentrations.
b) Tagetes minuta absolute (ZA) revealed a phototoxic effect on the skin tissues equivalents at concentrations ≥0.316% but not at 0.1%.
c) Tagetes minuta essential oil (EG) revealed no phototoxic effect on the skin tissues equivalents up to the highest tested concentration of 10%.
d) Tagetes minuta essential oil (ZA) revealed no phototoxic effect on the skin tissues equivalents up to the highest tested concentration of 10%.
e) Tagetes minuta essential oil - Low terthiophene (ZA) revealed no phototoxic effect on the skin tissues equivalents up to the highest tested concentration of 10%.

SCCS comment:
Although not an official guideline study, this *in vitro* model is now commonly used as a follow-up of the Neutral red uptake assay (OECD 432). Extrapolation of the results obtained from this *in vitro* assay to humans should be done with caution: underprediction has been demonstrated in certain cases, and a factor of about 10 is suggested (EVCAM 2014, Kejlova 2010).
The vehicle sesame oil seems to decrease phototoxicity in the Episkin model, compared to an aequous vehicle (Kejlova 2014), thus it is essential to have the correct controls included in the study. There are no data available from a Neutral Red Uptake Phototoxicity Assay.

B
(taken from Submission IV)

Reference:	RIFM 2007, RIFM# 53455
Date of report:	2007
Guideline/method:	Human skin model test, Procedures according to MatTek Corporation 1997, Phototoxicity Protocol for use with EpiDerm™ Model (EPI-200).
Test system:	Three-dimensional human epidermis model (EpiDerm™).
Replicates:	Duplicate plates
Test substances:	a) Tagetes minuta essential oil (EG) b) Tagetes minuta essential oil (ZA) c) Tagetes minuta absolute (ZA) d) Tagetes patula absolute (EG)
Batches:	a - d) no data (TTP: no data)
Concentrations:	0.01%, 0.05%, 0.1% (v/v, with/without irradiation)
Vehicle:	Diethyl phthalate:Ethanol (DEP:EtOH, 3:1) in Hanks balanced salt solution (HBSS, 10%)
Duration of exposure:	Trial 1: 24 h Trial 2: 12-h
Irradiation:	
Source of light:	Dermalight SOL 3 solar simulator, equipped with a UVA H1 filter (320 — 400 nm)
Intensity of irradiation:	UV-A: 1 J/cm² (= 1.7 ± 1 mW/cm²)
Duration of irradiation:	60 min.
Negative Control:	Blank, DEP:EtOH in HBBS, DMSO
Positive control:	Chlorpromazine (0.02% in HBBS)
GLP:	Yes
Published:	No

Material and methods:
Tagetes extracts and essential oils (Tagetes minuta essential oil (EG), Tagetes minuta essential oil (ZA), Tagetes minuta absolute (ZA), Tagetes patula absolute (EG)) were tested for its photo-toxic potential on the three-dimensional human epidermis model (EpiDerm™). The test substances were diluted in Diethyl phthalate:Ethanol (DEP:EtOH, 3:1) in Hanks balanced salt solution (HBSS, 10%) and concentrations of 0.01, 0.05, 0.1% (v/v) were examined. The test solutions were applied onto the skin tissue equivalents for 24 hours. However, the test article exposures resulted in excessive cytotoxicity, precluding the ability to make accurate phototoxicity determinations. Based upon the viability results obtained from the first trial, a second trial was conducted, where the test article exposures were reduced to 12 hours. The solvent, DMSO and blank wells were used as negative controls, while Chlorpromazine at 0.02% in HBBS was used as positive control. In both trials, half of the cultures in each treatment group were subjected to UVA light for 60 minutes, which resulted in an exposure of 6 J/cm². The remaining half of each treatment group was held at room temperature in the dark for the same time period. All of the tissues were rinsed immediately after the UVA/dark exposures. The cultures were returned to the incubator for a post-exposure period of approximately 21 hours. The MTT conversion assay was used to assess cellular metabolism after exposure.

Results:
In the first trial with culture exposure of 24 h, the relative viability values of the dark-exposed tissues was less than 50% for all Tagetes compounds and concentrations and according to the evaluation criteria the reactions were graded as too toxic to determine (TTTD) phototoxicity (data not shown).The results of the second trial with exposure of 12 hours are presented in the table below:

Table xx Comparative viability results after irradiation and in the dark in percentage of control after exposure of 12 hours

Substance	Concentration (w/v)	Viability (% of Control)		Result Remark
		+uvA	Dark	
Tagetes minuta essential oil (EG) in (DEP:EtOH, 3:1) in HBSS	0.1%	126.8	94.3	Negative
	0.05%	106.6	44.8	TTTD
	0.01%	88.8	89.6	Negative
Tagetes minuta essential oil (ZA) in (DEP:EtOH, 3:1) in HBSS	0.1%	115.6	90.4	Negative
	0.05%	76.4	96.2	Negative
	0.01%	112.3	99.3	Negative
Tagetes minuta absolute (ZA) in (DEP:EtOH, 3:1) in HBSS	0.1%	14.9	79.9	Phototoxic
	0.05%	19.9	39.7	TTTD
	0.01%	55.2	47.4	TTTD
Tagetes patula absolute (EG) in (DEP:EtOH, 3:1) in HBSS	0.1%	17.8	68.4	Phototoxic
	0.05%	11.5	80.9	Phototoxic
	0.01%	27.2	15.5	TTTD
Chlorpromazine (in HBSS)	0.02%	50.4	101.3	Phototoxic

TTTC = to toxic to determine (measure of cytotoxicity), HBSS = Hank's Balanced Salt Solution

As can be deduced from the table above, the test articles, Tagetes minuta absolute (ZA at 0.1% concentration only and Tagetes patula absolute (EG) at 0.1% and 0.05% concentrations did indicate the potential for phototoxic effects.

Discussion and conclusion:
The reduced exposure time of 12 hours resulted in notably higher viability in the dark exposure groups such that a prediction of phototoxic potential could presumably have been made. However, the reproducibility of the exposure responses appeared to be generally poor, presumably due to the poor reproducibility and uniformity of the dosing solutions. Since all test article dosing solutions contained immiscible oil droplets, and the droplets were observed to be present on the tissue in the first trial, it is probable that the tissues were exposed to varying amounts of the intended dose. This may have directly affected the reproducibility of the test article responses.
Finally, due to the limitations with regards to test material and vehicle linked cytotoxicity, replicate variability, non reliable dose response reactions, insolubility of the Tagetes fragrance materials dissolved 3:1 in Diethyl phthalate:Ethanol (DEP:EtOH) and other deficiencies (e.g., missing certificate of analysis) the study was finally considered as not reliable.

Note to File (taken from the original report (RIFM 2007, report 53455):
The results indicate the potential for phototoxic effects for Absolute Tagetes Minuta (South Africa) and Absolute Tagetes Patula (Egypt) and indicate no phototoxic potential for Essential Oil Tagetes Minuta (Egypt) and Essential Oil Tagetes Minuta (South Africa). However, REXPAN is of the opinion that the results of the assays were not robust enough to

determine no-observed-effect-levels for phototoxicity due to problems encountered in the conduct of assay. REXPAN recommended an *in vivo* mouse phototoxicity assay to determine the no-observed-effect-levels.
The problems encountered included:
- test material-linked cytotoxicity
- vehicle-linked cytotoxicity
- tissue replicate variability
- dose response not reliable
- insolubility of the Tagetes fragrance materials (in 3:1 DEP:EtOH) in the aqueous HBSS vehicle.

SCCS comment:
The SCCS agrees with the above-mentioned comments about the reliability of the results of this study.

Overall SCCS comment
UV/vis absorption spectra of the different test items should be present, even before biological testing.
Only 2 tissue replicates have been used per condition in the different tests performed.
Information on the solubility of the test items in the vehicle (sesame oil) is not provided in the study reports. A potential (phototoxic) effect of the vehicle used cannot be ruled out as no untreated control group has been included in the studies. Information on the phototoxic potential is provided at concentrations higher than the anticipated use concentration of 0.01%. In addition, no positive control group was included in the different studies. Therefore the results cannot be used by SCCS to assess the phototoxic potential of Tagetes patula absolute (EG), Tagetes minuta absolute (ZA), Tagetes minuta essential oil (EG), Tagetes minuta essential oil (ZA) and Tagetes minuta essential oil - Low terthiophene (ZA).

3.3.1.2 Phototoxicity in vivo – animal studies

New studies since the 2004 submission and the SCCP 2005 opinion.

(taken from Submission IV):

Reference:	RIFM 2008, RIFM# 55511
Date of report:	2008
Guideline/method:	Guidance for Industry: photosafety testing; May 2003. Rockville (MD): US Dept of Health and Human Services Food and Drug Administration, (CDER)
Species/strain:	Hairless mice/ Crl:SKH1-*hr*
Group size:	a) primary irritancy phase: 3 females per group b) phototoxicity phase: 6 females per group
Test substances:	a) Tagete minuta essential oil (ZA) b) Tagete minuta absolute (ZA) c) Tagete minuta essential oil (EG) d) Tagete patula absolute (EG)
Batches:	a) NFS (TTP: 0.024%) b) NFS (TTP: 0.35%) c) NFS (TTP: 0.024%) d) NFS (TTP: 2.45%)
Concentration:	0.001, 0.01, 0.1 %
Volume:	100 µL
Route:	Open epicutaneous application
Carrier:	Methanol

Negative control:	No exposure
Positive control:	8-MOP
Source of light:	Solar simulator (6.5 kW long-arc xenon water-cooled lamp in combination with filter to attenuate UV-B)
Intensity of irradiation:	About 0.5 Minimal Erythema Doses (MED) was delivered
Duration of irradiation:	30 ± 5 min.
Observations:	Clinical signs/skin findings: prior to administration, 60 ± 10 min., 4 hours ± 10 min. after UV exposure, constantly during UV exposure, one, two and three days after UV exposure.
GLP:	Yes
Published:	No

(taken from study report RIFM 2008, RIFM# 55511):

Methods:
Primary Irritancy Phase:
Thirty-nine female Crl:SKH1-hr hairless mice were assigned to thirteen groups (Groups 1 through 13), three mice per group. The test article formulations, Essential oil Tagete minuta (South Africa), Absolute Tagete minuta (South Africa), Essential oil Tagete minuta (Egypt) or Absolute Tagete patula (Egypt), at concentrations of 0.001, 0.01 or 0.1%, and/or the vehicle, Methanol, were topically administered (100 mcL per mouse) once. Mice were observed for viability, clinical observations, skin observations and body weights. Necropsy occurred on day 4 of study. Necropsy observations were not recorded.

Phototoxicity Phase:
Ninety female Crl:SKH1-hr hairless mice were assigned to fifteen groups (Groups 14 through 28), six mice per group, for the phototoxicity phase of the study. Formulations of the test article, Essential oil Tagete minuta (South Africa), Absolute Tagete minuta (South Africa), Essential oil Tagete minuta (Egypt) or Absolute Tagete patula (Egypt), at concentrations of 0.001, 0.01 or 0.1%, comparator article, 8-methoxypsoralen at a concentration of 1 mg/mL, and/or the vehicle, Methanol, were topically administered (100 mcL per mouse) once. All mice were lightly anesthetised via intraperitoneal injection of chloral hydrate and then positioned on plastic tubing with laboratory tape. An aluminum foil mask with a single hole (1.3 cm2) was placed over the mid-dorsal area of each mouse before UVR exposure. UVR exposure began 30 minutes +\- 5 minutes after completion of formulation administration of each group for Groups 16 through 28 and approximately 60 ± 10 minutes after completion of the comparator article formulation administration for Group 15. Group 14 mice were exposed to UVR on the same day as the mice in Groups 15 through 28. The UVR exposure duration was approximately 30 ± 5 minutes for all groups.

Results (taken from study report)
Primary Irritancy:
All mice survived to scheduled sacrifice. No skin reactions indicative of primary irritancy occurred. No clinical observations related to the Tagete extracts administration were observed throughout the study. Body weights and body weight changes were unremarkable.
Phototoxicity:
All mice survived to scheduled sacrifice. Skin reactions indicative of phototoxicity occurred in the 0.1% absolute Tagete extract groups. All six mice administered the 0.1% Absolute Tagete minuta (South Africa) had erythema grade 1. Five of the 6 mice in this dosage group had edema grade 1 and 3 of the 6 mice had flaking grade 1. All six mice administered the 0.1% Absolute Tagete patula (Egypt) had edema grade 1 and 5 of the 6 mice in this dosage group had erythema grade 1. In addition, 4 of the 6 mice in this dosage group had flaking grade 1 and 4 of the 6 mice had flaking grade 2.

There were no skin reactions in the untreated mice or the mice administered the vehicle formulation followed by a single exposure to UVR. No skin reactions related to Tagete extract administration occurred in the remaining dosage groups [Essential oil Tagete minuta (South Africa) and Essential oil Tagete minuta (Egypt) at concentrations of 0.001%, 0.01% and 0.1% or Absolute Tagete minuta (South Africa) and Absolute Tagete patula (Egypt) at concentrations of 0.001% and 0.01%]. No clinical observations related to the Tagete extracts administration were observed throughout the study. Mean body weight and body weight changes were comparable among the fifteen dosage groups. All six mice administered the comparator article, 8-MOP, had skin reactions indicative of phototoxicity validating the assay.

Conclusion (according to the study report):
Skin reactions indicative of phototoxicity (edema, erythema and flaking) occurred in the 0.1% Tagete absolute extract groups followed by a single exposure to UV in Crl:SKH1-*hr* hairless mice. No adverse findings indicative of phototoxicity occurred after topical administration of Tagete minuta essential oil (ZA) and Tagete minuta essential oil (EG) at concentrations of 0.001%, 0.01% and 0.1% or Tagete minuta absolute (ZA) and Tagete patula absolute (EG) at the 0.001% and 0.01% concentrations followed by a single exposure to UV in Crl:SKH1-*hr* hairless mice.

Based on these results, a No-Observed-Effect-Concentration (NOEC) for the induction of phototoxicity in mice of greater than or equal to 0.1% was determined for Tagete minuta essential oil (ZA) and Tagete minuta essential oil (EG). A NOEC of 0.01% was determined for Tagete minuta absolute (ZA) and Tagete patula absolute (EG).

SCCS comment:
The referenced guideline does not state recommendations about type and number of animals, UV dosing schedules and scoring of test reactions. The positive control test substance (8-MOP) is highly phototoxic; a less strong phototoxic agent might have been a better control to evaluate the UV dosing schedule. Hairless mice are more sensitive in photoxicity testing than humans (Forbes 1977).

Summary of the data: occurrence of skin reactions indicative of photoxicity in mice.

	0,001%	0.01%	0,1%
T. Minuta Essential Oil ZA; TTP 0.024%	–	–	–
T. Minuta Absolute ZA; TTP 0.35%	–	–	+
T. Minuta Essential EG; TTP 0.024%	–	–	–
T. Patula Absolute EG; TTP 2.45%	–	–	+

Studies from the 2004 submission which report testing with Tagetes 0.01% (text taken from the SCCP 2005 opinion)

A.
Reference: RIFM 1986a, RIFM 1986b (RIFM# 4344, 4342)
Material tested : Tagetes minuta Flower Oil
Sample : Tagetes oil (T. minuta - S. Africa) C-988-86

Controls : Methanol (negative); 8-MOP on methanol (positive)
Species/strain : male Skh: HR hairless mice (6/group)
Concentration : 0.01%, 0.1%, 1% (range 1), 3.125%, 6.25%, 12.5% (range 2), 25% 50% and 100% (range 3).
Solvent: methanol
Dilution Assay with 3 decimal dilutions in each of 3 concentration ranges.

A 20 µl aliquot of the test material was applied to a 5cm2 area of dorsal skin of each mouse. Approximately 30 minutes after treatment, each animal was covered by an aluminium foil mask taped to the mouse and restraining tray, and then exposed to a bank of 11 fluorescent black light lamps (F4OTlBL PUVA, Sylvania) (Phosphor type BL-O(*) providing a broad output band centered near 350nm, that was placed 0.27 meters from exposure trays. A measured intensity of 0.5 S.U/hr (**) was delivered for 60 minutes. The area to be exposed to light was defined by a 1 cm diameter hole in the foil centered over the treatment area. Reactions were read 4 hours after irradiation and again at 1, 2, 3 and 4 days. The treatment areas were scored for presence or absence of erythema, oedema, scaling, ulceration or fissuring. Animals exhibiting one or more of these symptoms at any examination period were considered positive for phototoxicity if the response was confined to the light-exposed area.

(*) Forbes, F.D. et al, Emission spectrum differences in fluorescent blacklight lamps. Photochem. Photobiol.24: 613, 1976.
(**) Berger, D.S., The Sunburning Ultraviolet Meter: Design and Performance, Photochem Photobiol. 24: 587-593 (1976).

Results
Range 1 : No reactions at 0.01 % (0/6), 5/6 reactions at 0.1 %, 5/5 reactions at 1 %.
Range 2 : 6/6 reactions at 3.125%, 5/5 reactions at 6.25%, 6/6 reactions at 12.5%.
Range 3 : 6/6 reactions at 25%, 6/6 reactions at 50%, 6/6 reactions at 100%.
The test article induced a phototoxic response in a all animals at a level of 1% or higher and in most animals at 0.1%, but no response at 0.01%. The positive control (8-MOP) was phototoxic at levels of 0.0025% (6/6), 0.00125% (4/6), 0.000625% (3/6), but not at 0.0003125% (0/6). The Phototoxic Index is > 62.5.

SCCS comment:
The TTP levels in the test articles are not documented.

B.
Reference: RIFM 1985c (RIFM# 3362)
Material tested : T. minuta Flower Extract
Sample : Tagetes minuta absolute (Egypt) (Marigold abs. Lot # 101076)
Species/strain : 10 Himalayan white spotted guinea pigs/dose level
Concentration : 0.001 %, 0.01% and 3% in ethanol

A 0.025 ml aliquot of the test material was applied to 2 cm2 test sites on the shaved flanks. Thirty minutes after application of the test material, the left flank was exposed to nonerythemogenic UV-A irradiation (20 J/cm2) from Westinghouse FS 40 Black Lamps. The test sites on the right flanks remain unexposed and serve as control sites. Test sites were examined at 4, 24 and 48 hours after application of the test material.

Results
No reactions at 0.001 % (0/10)
8/10 reactions at 0.01 %,
10/10 reactions at 3%

SCCS comment
All reactions on the 0.01% were graded 1 ('slight'). At 4 hrs, there were 4 animals that also reacted to the non-irradiated 0.01% application. At 24 hrs, two animals reacted to the non-irradiated 0.01% application. Therefore the ratio of positives at this concentration is 6/10, respectively 7/10.
The 0.001% applications can be regarded as a surrogate for the vehicle-irradiated control.
The TTP levels in the test articles are not documented. The test protocol describes 5 test areas (and 5 UV-unexposed), while results of 4 are documented.
The 3% test article applications showed the same degree of positive reactions on the non-irradiated areas in all animals. This sheds doubt on the appropriateness of the test model.

C.

Reference:	RIFM 1986d (RIFM# 4343)
Material tested :	T. patula Flower Extract
Sample:	Tagetes patula absolute (sample #D-1245-86)
Controls:	Methanol (negative); 8-MOP in methanol (positive)
Species/strain:	male Skh: HR hairless mice (6/group)
Concentration:	0.01%, 0.1% and 1%

Phototoxicity Screen after epicutaneous application to Hairless Mice. The study was performed as described in the previous study on Tagetes minuta absolute (RIFM# 3365).

Results
2/6 reactions at 0.01 %
6/6 reactions at 0.1 %
6/6 reactions at 1 %
The responses at levels 1% and 0.1% were more severe than that produced by the reference 8-MOP solution at 0.0025%. The positive control (8 -MOP) was phototoxic at levels of 0.0025% (6/6), 0.00125% (4/6) and 0.000625% (2/6), but not at 0.0003125%. The Phototoxic Index is > 62.5

SCCS comment:
The TTP levels in the test articles are not documented.

3.3.2 Human data

New studies since the 2004 submission and the SCCP 2005 opinion.

A. (taken from Submission IV)

References:	RIFM 2013a, b, c, d, e, RIFM# 65844, 65845, 65846, 65847, 65848, Certificates of analysis (Firmenich 2012a, b, Robertet 2012 a, b, c)
Date of reports:	2013
Guideline/Method:	Modified photo-toxicity test according to approved study protocol and standard operating procedures
Species:	Human
Group size:	28 enrolled volunteers/27 completed (14 males/13 females, age range: 18 – 65)
Test substances:	a) Tagetes patula absolute (EG = Tag-1) b) Tagetes minuta absolute (ZA = Tag-2) c) Tagetes minuta essential oil (EG = Tag-8) d) Tagetes minuta essential oil (ZA = Tag-3) e) Tagetes minuta essential oil - Low terthiophene (ZA = Tag-4, Tag-6, Tag-7)

Batches:	a) 1000881306 (TTP: no data) b) 2079040 (TTP: 0.31%) c) 1001063725 (TTP: no data) d) 2108166 (TTP: 0.018%) e) 1802615 (TTP: below limit of detection)
Concentrations:	a – d) 0.01% e) 0.01% (Tag-6), 0.05% (Tag-4), 0.1% (Tag-7)
Vehicle:	Diethyl phthalate:ethanol (3:1 = Tag-5)
Controls	vehicle
Route:	Occlusive epicutaneous application
Patch:	Webril/adhesive patches (25 mm Hill Top Chamber System®)
Scoring system:	Modified scoring scale of the International Contact Dermatitis Research Group System (Fisher, Alexander A., Contact Dermatitis, Lea & Febiger, 2008, 27)
Source of light:	Harrison Research Laboratory custom made light sources using four Philips F40BL fluorescent tubes (peak: 369 nm; half-power bandwidth: 16 nm (362 – 379 nm)
UV dose:	UV-A: 4.6±0.2 mW/cm² UV-B: 1.4 ±0.2 mW/cm² (based on minimal erythema dose (MED)
Duration of irradiation:	about 17 min.
Testing schedule:	Day 1: Duplicate patches occlusively applied for 24 h Day 2: 24 h post-patching removal of patches, irradiation with UV-A and UV-B, scoring prior to and after irradiation, non-irradiated sites protected from light and scored after patch removal Day 3/4: about 48/72 h post patching scoring of irradiated and non-irradiated sites
GLP:	Yes
Published:	No

Material and methods:

Tagetes extracts and essential oils (Tagetes patula absolute (EG), Tagetes minuta absolute (ZA), Tagetes minuta essential oil (EG), Tagetes minuta essential oil (ZA), Tagetes minuta essential oil - Low terthiophene (ZA)) were tested for its photo-toxic potential in a modified photo-toxicity test on human volunteers as a result of a single application and UV-B and UV-A irradiation to induce a phototoxic response in humans according to approved study protocol and standard operating procedures. Twenty-eight were enrolled and 27 volunteers completed the study (14 males/13 females, age range: 18 – 65). The materials were 0.01% preparations in diethyl phthalate:ethanol (3:1) except Tagetes minuta essential oil - Low terthiophene (ZA), which was tested at 0.01%, 0.05% and 0.1%. The vehicle was used as control. Each volunteer was occlusively patched with two patches (Webril/adhesive patches (25 mm Hill Top Chamber System®)), one for the non-irradiated site, the other for subsequent irradiation. For UV-A irradiation a total dose of 4.6±0.2 mW/cm² in a wavelength range between 320 nm and 400 nm and for UV-B irradiation a wavelength range of 280 nm to 320 nm and UVB irradiation of 1.4 ±0.2 mW/cm² was used. The light sources consisted of four Philips F40BL fluorescent tubes. Prior to the application the minimal erythemal dose (MED) was determined. On study day 1, duplicate patches were occlusively applied for 24 h and on day 2 the patches were removed and the skin was irradiated with UV-A and UV-B. The skin sites were scored prior to and after irradiation. The non-irradiated sites were protected from light and were also scored after patch removal. On days 3 and 4 (about 48/72 h post patching) the irradiated and non-irradiated sites were scored again.

Results:

Tagetes patula absolute (EG):
One volunteer exhibited low-level (±/1) reactions on both the irradiated and the non-irradiated contact sites. Twenty-four volunteers exhibited low-level (±/1) reactions on the

irradiated contact site only. One volunteer exhibited a slight tanning response on the irradiated contact site.
Tagetes minuta absolute (ZA):
All volunteers, 27 in total, exhibited low-level (±/1) reactions on the irradiated contact site only. One volunteer exhibited a slight tanning response on the irradiated contact site.
Tagetes minuta essential oil (EG):
One volunteer exhibited low-level (±/1) reactions on both the irradiated and the non-irradiated contact sites. All other volunteers, 26 in total, volunteers exhibited low-level (±/1) reactions on the irradiated contact site only. One volunteer exhibited a slight tanning response on the irradiated contact site.
Tagetes minuta essential oil (ZA):
Twenty-six volunteers exhibited low-level (±/1) reactions on the irradiated contact site only. One volunteer exhibited a slight tanning response on the irradiated contact site.
Tagetes minuta essential oil - Low terthiophene (ZA):
0.01%: One volunteer exhibited low-level (±) reactions on both the irradiated and the non-irradiated contact sites. Twenty-six volunteers exhibited low-level (±/1) reactions on the irradiated contact site only. One volunteer exhibited a slight tanning response on the irradiated contact site.
0.05%: Twenty-five volunteers exhibited low-level (±/1) reactions on the irradiated contact site only.
0.1%: Two volunteers exhibited low-level (±/1) reactions on both the irradiated and the non-irradiated contact sites. Twenty-five volunteers exhibited low-level (±/1) reactions on the irradiated contact site only. One volunteer exhibited a slight tanning response on the irradiated contact site. Twenty-four volunteers exhibited low-level (±/1) reactions and one volunteer exhibited a slight tanning response on the irradiated (no test material) control site.
Vehicle control: One volunteer exhibited low-level (±/1) reactions on both the irradiated and the non-irradiated contact sites. Twenty-five volunteers exhibited low-level (±/1) reactions on the irradiated contact site only. One volunteer exhibited a slight tanning response on the irradiated contact site. Twenty-four volunteers exhibited low-level (±/1) reactions and one volunteer exhibited a slight tanning response on the irradiated (no test material) control site.

Conclusion:
No serious adverse events related to the test material preparations occurred during this test. Low levels effects seen at all concentrations were also noted in the vehicle control groups.
Tagetes extracts and essential oils (Tagetes patula absolute (EG), Tagetes minuta absolute (ZA), Tagetes minuta essential oil (EG), Tagetes minuta essential oil (ZA), Tagetes minuta essential oil - Low terthiophene (ZA)) tested at 0.01% or up to 0.1% (Tagetes minuta essential oil - Low terthiophene (ZA)) did not induce a photo-toxic response on the skin of human volunteers under the conditions of this modified phototoxicity test.

SCCS comment:
Most subjects reacted at 24 hours after irradiation (UVA 4.6 J/cm2 and UVB approx 175-259 mJ) with grade 1 (Erythema) on all test substances, including the DEP/Ethanol vehicle and the non-exposed irradiated area. There were no reactions of grade 2 (Intense erythema).
If the results of the individuals with grade 1 reaction (Erythema) on the vehicle or blank irradiated area are discarded (i.e. assumed to be negative), then the number of positive (grade 1, Erythema) reactions could be summarized:
0.01% Tagetes patula absolute no TTP data (EG = Tag-1): 0/27
0.01% Tagetes minuta absolute TTP 0.31% (ZA = Tag-2): 2/27
0.01% Tagetes minuta essential oil TTP 0.018% (ZA = Tag-3): 2/27
0.05% Tagetes minuta essential oil – TTP below detection limit (ZA = Tag-4): 4/27
0.01% Tagetes minuta essential oil – TTP below detection limit (ZA = Tag-6): 3/27
0.1% Tagetes minuta essential oil – TTP below detection limit (ZA = Tag 7): 4/27

0.01% Tagetes minuta essential oil no TTP data (EG = Tag-8): 4/27
The abovementioned positive reactions occurred in the same 2-4 subjects

Besides grade 1 reactions (Erythema) on the vehicle, there were also grade 1 reactions on the irradiated (without test solution) control sites. This sheds doubt on the adequacy of the UV dose.

B. (taken from Submission IV)

References:	RIFM 2012a, RIFM# 63321
Date of report:	2008
Guideline/Method:	Photo-toxicity test according to approved study protocol and standard operating procedures
Species:	Human
Group size:	96 volunteers (20 males/76 females)
Test substances:	a) Tagetes minuta essential oil (EG, A) b) Tagetes minuta essential oil (ZA, B) c) Tagetes minuta absolute (ZA, C) d) Tagetes patula absolute (EG, D) e) Tagetes low TTP (G, H, I)
Batches:	a – e) no data (TTP: no data)
Concentrations:	a – d) 0.01% e) 0.01% (G), 0.05% (H), 0.1% (I)
Vehicle:	Diethylphthalate:ethanol (3:1, E)
Controls:	Vehicle (E) and blank patch (F)
Route:	Occlusive epicutaneous application
Patch:	25 mm Hill Top Chamber System® (completed by Durapore®, and in order to ensure adhesion may be covered by and secured on all sides by hypoallergenic tape (Blenderm™)
Scoring system:	According to laboratory methodology
Source of light:	150-watt Berger Solar Ultraviolet Simulator (Solar Light Co., Philadelphia, PA), WG-320 UV filter and UG-11 UV (UV-A/UV-B), additional filter (WG-335, WG-345, or WG-360) to provide UV-A
UV dose:	UV-A: 10 J/cm² (0.5 MED), UV-A/UV-B: 0.75 MED
Duration of irradiation:	about 17 min.
Testing schedule:	Day 1: Duplicate patches occlusively applied for 24 h Day 2: 24 h post-patching removal of patches, irradiation with UV-A/UV-B and UV-A, scoring after irradiation (irradiated and non-irradiated sites) Day 3/4: scoring about 48/72 h post patching (irradiated and non-irradiated sites)
GLP:	Yes
Published:	No

Material and methods:
Tagetes extracts and essential oils (Tagetes minuta essential oil (EG, A), Tagetes minuta essential oil (ZA, B), Tagetes minuta absolute (ZA, C), Tagetes patula absolute (EG, D) and Tagetes low TTP (G, H, I)) were tested for its photo-toxic potential in a photo-toxicity test on Human volunteers following a randomized, evaluator-blinded test design. The study consisted of a single 24-hour application of duplicate patches to naive sites. One of the duplicate patch sites was exposed to UV-B/UV-A and UV-A radiation for evaluation of phototoxic potential, while the other site was used to evaluate primary irritation potential or to serve as non-irradiated control. All patch sites were located on the paraspinal region of the back. For multiple test articles, the test sites were alternated sequentially among the individual panelists according to the assigned identification number. At Visit 4, each subject received duplicate patches, 2-3 patches with the test article, 1 vehicle patch and 1 control patch, placed on both sides of the spine on naive sites. Patches were prepared fresh and the contact

time was approximately 24 hours. A minimum of 31 volunteers completed the study in each of the following groups and each group was exposed to a different set of test articles:
Group 1 (33 volunteers): 0.01% (A), 0.01% (B), Vehicle control (E), Blank patch (F)
Group 2 (32 volunteers): 0.01% (C), 0.01% (D), Vehicle control (E), Blank patch (F)
Group 3 (31 volunteers): 0.01% (G), 0.05% (H), 0.1% (I), Vehicle control (E), Blank patch (F)
The minimum erythema dose (MED) was determined for each volunteer by a series UV-B/UV-A and UV-A exposures the site that produced uniform redness to the borders of the exposure site (erythema grade of 1) using the smallest dose of energy was considered the subject's inherent MED (IMED). Approximately 24 hours after application, the patches on the left paraspinal region were removed. The test sites were then marked with a skin marker and irradiated with 10 J/cm² or 0.5 MED of UV-A within ten minutes of patch removal. After UVA irradiation, the sites were irradiated with 0.75 MED of UV-B/UV-A. Patches were removed from the non-irradiated test sites on the right paraspinal region after the UV-A/UV-B dosing was complete, and also marked as above. The non-irradiated sites were used as controls. All test sites were evaluated at approximately 1, 24, 48, and 72 hours after the final patch was removed. A 150-watt Berger Solar Ultraviolet Simulator (Solar Light Co., Philadelphia, PA) was used as the ultraviolet radiation source. The WG-320 UV filter and UG-11 UV filter were used to provide a basic solar-like spectrum. An additional filter (WG-335, WG-345, or WG-360) was used to provide a UV-A spectrum for UV-A exposures. Inflammatory responses (erythema and reactions) or superficial effects were scored. The scorer was blinded as to treatment assignments and any previous scores. In addition, at the 72 hour evaluation a Dermatologist also evaluated the test sites.

Results, discussion and conclusion:
The results of were summarized by the authors as follows:

Group 1: There were a total of three subjects that experienced reactions that may be indicative of a phototoxic response. Two volunteer exhibited a reaction to 0.01% Tagetes minuta essential oil (EG) strongly and slightly indicative of a phototoxic response. But also 1 volunteer of the vehicle control showed a skin reaction indicative of a phototoxic response.
Group 2: There were a total of three subjects that experienced reactions that may be indicative of a phototoxic response. One volunteer exposed to 0.01% Tagetes minuta absolute (ZA) showed a skin reaction that was indicative of a phototoxic response, while two others showed responses considered as indicative for a strong phototoxic response.
Group 3: There were a total of four subjects that experienced reactions that may be indicative of a phototoxic response. Three volunteers exhibited a reaction to 0.1% Tagetes low TTP that had a slight potential that these responses were indicative of a phototoxic response. One subject exhibited a reaction to 0.05% Tagetes low TTP that had a potential that this response was indicative of a phototoxic response.

However, the assessment of the findings and the evaluation of the observed skin responses had several deficiencies and limitations as some volunteers as well as questionable and 0.5 designation reactions were not included as they were not considered to be significant. There were additional volunteers indicative of potentially phototoxic reactions based on the mentioned criteria of at least a 1-reaction in the irradiated area and thus disregarding 0.5-reactions. The obtained results of the vehicle control exposed volunteers as well as the results of the blank patches were not appropriately considered. In addition, there is no information on the tested materials (no certificate of analysis) and especially no information on the batch, the purity and TTP content.
Finally, based on the above mentioned limitations and deficiencies in substance characterization and in data presentation and assessment this study was considered as not appropriate to evaluate the phototoxic potential of the Tagetes extracts and essential oils.

SCCS comment:
The UV dose was almost double the dose in study A above.

The report does not have a comment on the surprising observation that in some individuals there was a positive reaction at the site that had been patched with a blank patch, while the other sites in these individuals did not or barely react (grade 0.5).
Assuming that the tests are negative in the participants who also had a (grade 1 or more) reaction (at least mild erythema or mild tanning) on the vehicle or the blank patch, the results can be summarised:
Tagetes minuta essential oil (EG, A) 0.01%: 2/33 had a positive reaction
Tagetes minuta essential oil (ZA, B) 0.01%: none
Tagetes minuta absolute (ZA, C) 0.01%: 3/32 had a positive reaction
Tagetes patula absolute (EG, D) 0.01%: none
Tagetes low TTP (G) 0.01%: none
Tagetes low TTP (H) 0.05%: 1/31 reacted; if a grade 2 reaction compared to a grade 1 for vehicle is accepted, then 2/31 reacted
Tagetes low TTP (I) 0.1%: 2/31 reacted
Nevertheless, in view of the reactions at the irradiated blank patch and the vehicle, a firm conclusion from this study cannot be drawn.

C.

Reference:	RIFM 2011 (RIFM nr 62941)
Study period:	nov-dec 2009
Guideline/Method:	Photo-toxicity test procedure according to Marzulli & Maibach (1996)
Species:	Human
Group size:	97 enrolled volunteers; 96 completed (three groups of 32 each); 42 males, 54 females
Test substances:	A: 0.01% Tagetes minuta Egypt essential oil (TTP 0.035%) B: 0.01% Tagetes minuta South Africa essential oil (TTP 0.031%) C: 0.01% Tagetes minuta South Africa absolute (TTP 0.33%) D: 0.01% Tagetes patula Egypt absolute (TTP 2.40%) E: Vehicle control (DEP/Ethanol 3:1) F: Blank patch G: 0.01% Tagetes low TTP H: 0.05% Tagetes low TTP I: 0.1% Tagetes low TTP
Batches:	Certificates of analysis with batch nrs provided by suppliers
Vehicle:	Diethyl phthalate:ethanol (3:1), also test article E
Controls:	Vehicle and blank patch
Route:	Single 24 hr occlusive epicutaneous application in duplicate
Patch:	Nonwoven cotton Webril patches, approx 2 cm2, covered by hypoallergenic Blenderm tape with 0.3 ml test solution.
Scoring system:	Blinded scorer, grading on erythema, reaction and superficial effects.
Source of light:	Berger Solar Ultraviolet Simulator 150-watt. Filters to provide UVB 290-320 nm, UVA 320-400 nm, with additional filters for UVA exposure
UV dose:	10 Joules/cm2 or 0.5 MED of UVA, followed by 0.75 MED of UVB/UVA
Testing schedule:	Pre-test determination of MED. Day 1: Duplicate patches occlusively applied for 24 h Day 2: 24 h post-patching removal of patches on left paraspinal area, irradiation with UV-A and UV-B, scoring 1 hr after irradiation. Right paraspinal area (non-irradiated control) remains occluded until after irradiation, followed by scoring 1 hr after removal. Day 3 and 4: about 48 and 72 h post patching scoring of irradiated and non-irradiated sites
GLP:	Yes

Methods (taken from the submission, with minor textual adaptation):

The study was conducted following a randomized, evaluator-blinded test design. The study consisted of a single 24-hour application of duplicate patches to naive sites. One of the duplicate patch sites was exposed to UVB/UVA and UVA radiation for evaluation of phototoxic potential while the other site was used to evaluate primary irritation potential or to serve as non-irradiated control. All patch sites were located on the paraspinal region of the back. For multiple test articles, the test sites were alternated sequentially among the individual panelists according to the assigned identification number.
On Day 1, each subject received duplicate patches, 2-3 patches with the test article, 1 vehicle patch and 1 control patch, placed on both sides of the spine on naive sites. Patches were prepared fresh and were applied and removed by a trained technician. The patch contact time was approximately 24 (+/-1) hours. Any excess test article, which is opaque, was removed from the skin with a Kimwipe prior to irradiation. Thirty two subjects completed the study in each of the following groups. Each group of subjects tested a different set of test articles:

Group 1 (32 subjects)
0.01% essential oil Tagetes minuta from Egypt (A)
0.01% essential oil Tagetes minuta from South Africa (B)
Vehicle control (3:1 diethyl phthalate:ethanol) (E)
Blank patch (F)
Group 2 (32 subjects)
0.01% absolute Tagetes minuta from South Africa (C)
0.01% absolute Tagetes patula from Egypt (D)
Vehicle control (3:1 diethyl phthalate:ethanol) (E)
Blank patch (F)
Group 3 (32 subjects)
0.01% Tagetes low TTP (G)
0.05% Tagetes low TTP (H)
0.1% Tagetes low TTP (I)
Vehicle control (3:1 diethyl phthalate:ethanol) (E)
Blank patch (F)

UV Exposure: The minimum erythema dose (MED) was determined for each subject by exposing unprotected, naive skin to a series of five UVB/UVA exposures each 25 percent greater than the previous dose. An additional five exposures of UVA were also done, directly below the first set of exposures. Approximately 22-24 hours after the irradiation, the sites were illuminated by a 100 watt incandescent white bulb and visually evaluated for erythema. In each set of exposures, the site that produces uniform redness to the borders of the exposure site (erythema grade of 1) using the smallest dose of energy was considered the subject's inherent MED (IMED).
Approximately 24 (+/- 1) hours after application, the patches on the left paraspinal region were removed. The test sites were then marked with a skin marker and irradiated with 10 Joules/cm2 or 0.5 MED of UVA, (whichever was greater) of UVA irradiation within ten minutes of patch removal. After UVA irradiation, the sites were irradiated with 0.75 MED of UVB/UVA. Patches were removed from the non-irradiated test sites on the right paraspinal region after the UVA/UVB dosing was complete, and also marked as above. The non-irradiated sites were used as controls to assess non-phototoxic reactions (i.e., the test article's inherent irritation potential). All test sites were evaluated at approximately 1 (+/- 0.25), 24 (+/- 1), 48 (+/- 2), and 72 (+/- 2) hours after the final patch was removed.
Instruments: A 150-watt Berger Solar Ultraviolet Simulator (Solar Light Co., Philadelphia, PA) was used as the ultraviolet radiation source in this study. The 1mm WG-320 UV filter and 1mm UG-11 UV filter were used to provide a basic solar-like spectrum (UVB: 290 to 320 nanometers and UVA: 320 to 400 nanometers). An additional filter (WG-335, WG-345, or WG-360) was used to provide a UVA spectrum for UVA exposures.
Prior to treatment exposure for each new subject, an intensity measurement was taken from the solar simulator using a radiometer/photo detector. For MED exposures, intensity

measurements were taken hourly. The measurements were taken at the same distance from the lamp as the subject's skin during exposures.
Evaluations: Inflammatory responses (erythema and reactions) or superficial effects (if observed) were scored according to the scales as specified. In cases where the patch area was larger than the irradiated area, only the irradiated areas were scored unless reactions outside irradiated area exhibited unusual responses. Scores represent the presence of clinically significant effects (on at least 25% of the patch site). Questionable (barely perceptible, minimal or involving less than 25% of the test site) reactions as well as the 0.5 designation were not considered to be significant.
For erythema, the scoring was: No visible reaction (0); Slight, confluent or patchy erythema or tanning (0.5); Mild erythema (pink) or mild tanning (1); Moderate erythema (definite redness) or moderate tanning (2); Strong erythema (very intense redness) or strong tanning (3).
Scoring of irritation was conducted using handheld lamp with a 100-watt incandescent blue bulb as the artificial light source to illuminate the patch areas. The scorer was blinded as to treatment assignments and any previous scores. All reasonable attempts were made to ensure that the same individual did all scoring of reactions to the test articles during the course of the study. In addition, at the 72 hour evaluation a Dermatologist also evaluated the test sites.

Results (taken from the submission, with minor textual adaptations for clarity):
Group 1
A total of 32 subjects completed the study for Group 1. A total of two subjects experienced reactions at an individual patch site that may be indicative of a potentially phototoxic response and one subject experienced reactions that may be indicative of pre-sensitization.
Subject No. 2 exhibited a level 1 reaction at the site patched with test substance A: 0.01% Essential oil Tagetes minuta from Egypt under UV irradiated conditions. All other sites for this subject (both UV and non-irradiated sites) had no reactions (grade 0). Based upon the responses for this test substance A compared to no responses for the other sites, there is a slight potential that this response was indicative of a possible phototoxic response.
Subject No. 15 exhibited strong reactions at both UV-irradiated and non-irradiated sites for test substance A (0.01% Essential oil Tagetes minuta from Egypt), B (0.01% essential oil Tagetes minuta from South Africa) and E (vehicle control). These reactions are consistent with a presensitization response. The results for this subject were not included in the Frequency Summary Table.
Subject No. 55 exhibited a level 1 reaction at the site patched with test substance B (0.01% essential oil Tagetes minuta from South Africa) under UV irradiated conditions that persisted through 72 hours. All other sites for this subject (both UV and non-irradiated sites) had no reactions (grade 0), slight reactions (grade 0.5) or mild reactions (grade 1). Based upon the greater severity or duration of the responses for Test substance B compared to the other sites, there is a slight potential that this response was indicative of a phototoxic response.

Group 2
A total of 32 subjects completed the study for Group 2. A total of four subjects experienced reactions at an individual patch site that may be indicative of a potentially phototoxic response.
Subject No. 32 exhibited level 1 reactions at the site patched with Test substance F (blank patch) under UV irradiated conditions that persisted through 72 hours. All other sites for this subject (both UV and non-irradiated sites) had no reactions (grade 0), slight reactions (grade 0.5) or mild reactions (grade 1).
Subject No. 36 exhibited a level 2 reaction at the site patched with Test substance E (vehicle control) under UV irradiated conditions. All other sites for this subject (both UV and non-irradiated sites) had no reactions (grade 0) or slight reactions (grade 0.5). Based upon the greater severity of the responses for Test substance E compared to the other sites, there is a potential that this response was indicative of a phototoxic response to the vehicle.
Subject No. 48 exhibited a level 2 reaction at the site patched with Test substance C (0.01% absolute Tagetes minuta from South Africa) under UV irradiated conditions. All

other sites for this subject (both UV and non-irradiated sites) had no reactions (grade 0), slight reactions (grade 0.5) or mild reactions (grade 1). Based upon the responses for Test substance C compared to the responses for the other sites, there is a slight potential that this response was indicative of a phototoxic response.
Subject No. 54 exhibited a level 2 reaction at the site patched with Test substance E (vehicle control) under UV irradiated conditions. All other sites for this subject (both UV and non-irradiated sites) had no reactions (grade 0), slight reactions (grade 0.5) or mild reactions (grade 1). Based upon the greater severity of the responses for Test substance E compared to the other sites, there is a slight potential that this response was indicative of a phototoxic response to the vehicle.
Subject No. 60 exhibited a level 1 reaction at the site patched with Test substance C (0.01% absolute Tagetes minuta from South Africa) under UV irradiated conditions. All other sites for this subject (both UV and non-irradiated sites) had no reactions (grade 0) or slight reactions (grade 0.5). Based upon the greater severity or duration of the responses for Test substance C compared to the other sites, there is a potential that this response was indicative of a phototoxic response.

Group 3
A total of 32 subjects completed the study for Group 3. A total of four subjects experienced reactions at an individual patch site that may be indicative of a potentially phototoxic response.
Subject No. 66 exhibited a level 1 reaction at the site patched with Test substance G (0.01% Tagetes low TTP) under UV irradiated conditions. All other sites for this subject (both UV and nonirradiated sites) had no reactions (grade 0) or slight reactions (grade 0.5). Based upon the greater severity of the responses for Test substance G compared to the other sites, there is a slight potential that this response was indicative of a possible phototoxic response.
Subject No. 71 exhibited a level 1 reaction at the site patched with Test substance I (0.1% Tagetes low TTP) under UV irradiated conditions that persisted through 72 hours. All other sites for this subject (both UV and non-irradiated sites) had no reactions (grade 0), slight reactions (grade 0.5) or mild reactions (grade 1). Based upon the greater severity or duration of the responses for Test substance I compared to the responses for the other sites, there is a slight potential that this response was indicative of a phototoxic response.
Subject No. 75 exhibited a level 1 reaction at the site patched with Test substance E (vehicle control) under UV irradiated conditions. All other sites for this subject (both UV and non-irradiated sites) had no reactions (grade 0) or slight reactions (grade 0.5). Based upon the greater severity of the responses for Test substance E compared to the other sites, there is a slight potential that this response was indicative of a possible phototoxic response to the vehicle.
Subject No. 96 exhibited a level 1 reaction at the site patched with Test substance H (0.05% Tagetes low TTP) under UV irradiated conditions. All other sites for this subject (both UV and non- irradiated sites) had no reactions (grade 0) or slight reactions (grade 0.5). Based upon the greater severity or duration of the responses for Test substance H compared to the other sites, there is a slight potential that this response was indicative of a possible phototoxic response.

SCCS comment:
The submitted report does not give a summary conclusion. In the text of the results paragraph of the report, it is not clear what is meant by level 1 (or level 2) reaction; presumably it is the grading for erythema (the report defines grading for Erythema (from 0 to 3), Reaction (R) and Superficial effects (s)).
Reactions at 1 hour after irradiation seem to have been disregarded.
All test substances (i.e. also the vehicle and even the blank patch) gave a reaction in some individuals. Discarding the positive tests in the subjects that also reacted to the vehicle, the results could be summarised:

Test substance A (0.01% Tagetes minuta Egypt essential oil (TTP 0.035%): 0/32 (although one individual reacted, he/she also had an erythematous reaction on the non-irradiated patch)
Test substance B (0.01% Tagetes minuta South Africa essential oil (TTP 0.031%): 1/32
Test substance C (0.01% Tagetes minuta South Africa absolute (TTP 0.33%): 2/32
Test substance D (0.01% Tagetes patula Egypt absolute (TTP 2.40%): 0/32 (there were reactions, in the same individuals these were also to the vehicle)
Test substance G (0.01% Tagetes low TTP): 1/32
Test substance H (0.05% Tagetes low TTP): 1/32
Test substance I (0.1% Tagetes low TTP): 1/32

In each group one subject reacted to the irradiation on the blank patch, which raises concern whether the UV-B dose might have been too high. Because in several subjects there were also reactions to the vehicle, a conclusion cannot be drawn from the test results.

D.

Reference:	RIFM 2009 (RIFM nr 57515)
Study period:	august 2008
Guideline/Method:	Photo-toxicity test procedure according to Marzulli & Maibach (1996)
Species:	Human
Group size:	83 screened, 35 enrolled volunteers; 5 males, 30 females
Test substances:	A: 0.01% Tagetes minuta Egypt essential oil (TTP 0.025%) B: 0.01% Tagetes minuta South Africa essential oil (TTP 0.024%) C: 0.01% Tagetes minuta South Africa absolute (TTP 0.36%) D: 0.01% Tagetes patula Egypt absolute (TTP 2.42%) E: Vehicle control (DEP/Ethanol 3:1)
Batches:	Certificates of analysis with batch nrs provided by suppliers
Vehicle:	Diethyl phthalate:ethanol (3:1), also test substance E
Controls	Vehicle, also test substance E
Route:	Single 24 hr occlusive epicutaneous application in duplicate
Patch:	Nonwoven cotton Webril patches, approx 2 cm2, covered by hypoallergenic Blenderm tape with 0.3 ml test solution.
Scoring system:	Blinded scorer, grading on erythema, reaction and superficial effects.
Source of light:	Berger Solar Ultraviolet Simulator 150-watt. Filters to provide UVB 290-320 nm, UVA 320-400 nm, with additional filters for UVA exposure
UV dose:	16 Joules/cm2 or 0.5 MED of UVA, followed by 0.75 MED of UVB/UVA
Testing schedule:	Pre-test determination of MED. Day 1: Duplicate patches occlusively applied for 24 h Day 2: 24 h post-patching removal of patches on left paraspinal area, irradiation with UV-A and UV-B, scoring 1 hr after irradiation. Right paraspinal area (non-irradiated control) remains occluded until after irradiation, followed by scoring 1 hr after removal. Day 3 and 4: about 48 and 72 h post patching scoring of irradiated and non-irradiated sites.
GLP:	Yes

Methods: (taken from the submitted report)
The study was conducted following a randomized, evaluator-blind test design. The study consisted of a single 24-hour application of duplicate patches to naïve sites. One of the duplicate patch sites was exposed to UVB/UVA and UVA radiation for evaluation of phototoxic potential while the other site was used to evaluate primary irritation potential or to serve non-irradiated control.
As part of screening, the minimum erythema dose (MED) was determined for each subject by exposing unprotected, naïve skin to a series of five UVB/UVA exposures each 25 hours

after the irradiation, the sites were illuminated by a 100-watt incandescent white bulb and visually evaluated for erythema. The site that produced uniform redness to the borders of the exposure site (erythema grade of 1) using the smallest dose of energy was considered the subject's inherent MED (IMED).

On Day 1, each subject received duplicate patches – 4 patches with the test articles and 1 control patch – to be placed on each side of the spine on naïve sites. Patches were prepared fresh and applied and removed to by a trained technician. All patch sites were located on the paraspinal region of the back. The test sites were alternated sequentially among the individual panelists according to the assigned identification number. The patch contact time was approximately 24 (+/-1) hours. Any excess test article or test article which was opaque was to be removed from the skin with the appropriate solvent prior to irradiation.
Approximately 24 (+/-1) hours after application, the patches on the left paraspinal region were removed. The test sites were marked with a skin marker an irradiated with 16 Joules/cm2 of UVA irradiation with ten minutes of patch removal. After UVA irradiation, the sites were irradiated with 0.75 MED of UVB/UVA.

Patches were removed from the non-irradiated test sites on the right paraspinal region after the UVA/UVB dosing was complete, and also marked as above.
The non-irradiated sites were used as controls to assess non-phototoxic reactions (i.e., the test article's inherent irritation potential). All test sites were evaluated at approximately 1 (+/-0.25), 24 (+/-1), 48 (+/-2) hours after the final patch was removed.

A 150-watt Berger Solar Ultraviolet Simulator (Solar Light Co., Philadelphia, PA) was used as the ultraviolet radiation source in this study. The 1mm WG-320 UV filter and 1 mm UG-11 UV filter was used to provide a basic solar-like spectrum (UVB: 290 to 320 nanometers). An additional filter (WG-335, WG-345, or WG-360) was used to provide a UVA spectrum for UVA exposures.
Prior to treatment exposure for each new subject, an intensity measurement was taken from the solar simulator using a radiometer/photo detector. For MED exposures, intensity measurements were taken hourly. The measurements were taken at the same distance from the lamp as the subject's skin during exposures.

Inflammatory responses (erythema and reactions) of superficial effects (if observed) were scored according to the following scale. In cases where the patch area was larger than the irradiated area, only the irradiated areas were scored unless reactions outside irradiated area exhibited unusual responses. Scores represented the presence of clinically significant effects (on at least 25% of the patch site). Questionable (barely perceptible, minimal of involving less than 25% of the test site) reactions as well as the 0.5 designation were not considered to be significant.
Scoring of irritation was conducted using a handheld lamp with a 100-watt incandescent blue bulb as the artificial light source to illuminate the patch areas. The scorer was blinded to the treatment assignments and any previous scores. The same individual performed all scoring during the course of the study. In addition, at the 72-hour evaluation a Dermatologist evaluated the test sites.

Results and conclusions (taken from the submitted report):
None of the five unexposed test articles produced reactions observable at the 72 hour reading.
At the 72 hour reading, barely perceptible erythema (0.5) was observed in exposed test sites of 16, 14, 13, 9, and 12 subjects for test articles A, B, C, D and E, respectively
Positive reactions, erythema grades greater than or equal to 1, were observed in eight subjects at the 72 hour reading. To test article A, 2 subjects exhibited mild (1) reactions, 1 subject exhibited a moderate (2) reaction, and 1 subject exhibited a severe (3) reaction. To test article B, 2 subjects exhibited moderate (2) reactions. To test article C, 2 subjects

exhibited mild (1) reactions. To test article D, 2 subjects exhibited mild (1) reactions and 1 subject exhibited a moderate (2) reactions.

Conclusions (taken from the submitted report):
No irritant reactions were observed at unexposed test sites. Under the conditions of the study, positive phototoxic reactions of greater than or equal to 1 were observed for all test articles exposed to UV, thus No-Observed-Effect-Levels (NOELs) could not be determined.

SCCS comment
The summary of the report is based on the 72 hrs reading; scorings at 24 and 48 hrs are considered by the SCCS as informative and should have been summarised.
Discarding the results of the individuals that reacted with at least a score 1 to the vehicle, and taking into account the 24 hr and 48 hr readings, then positive reactions (score 1 or more) were observed in 10 individuals. It can be summarised als follows:
0.01% Tagetes minuta Egypt essential oil (TTP 0.025%): 5/35 reacted
0.01% Tagetes minuta South Africa essential oil (TTP 0.024%): 6/35
0.01% Tagetes minuta South Africa absolute (TTP 0.36%): 4/35
0.01% Tagetes patula Egypt absolute (TTP 2.42%): 5/35

To the vehicle the number of reactions at 24 and 48 hrs can be summarised:
Slight, confluent or patchy erythema: 9/35
Mild erythema (pink): 7/35
Moderate erythema (definite redness): 3/35
Because of the reactions to the vehicle, no firm conclusion can be drawn from this study.

E.

Reference:	RIFM 1987c (RIFM nr 5752)
Study period:	December 1986
Guideline/Method:	No specific method referenced
Species:	Human
Group size:	10 subjects; 2 males, 8 females
Test substances:	A: Tagetes absolute (not further specified, conc not specified) B: Vehicle control 75% Ethanol, 25% Diethylphthalate)
Batches:	Only product ID mentioned: 2-1235-84-0.5. No certificate of analysis
Vehicle:	Presumably 75% ethanol 25% Diethyl phthalate
Controls	Vehicle, also test substance B
Route:	Single 24 hr occlusive epicutaneous application in duplicate, preceded by 3 to 4 times tape stripping
Patch:	Parke-Davis Ready-Bandage with 0.2 ml test solution during 24 hrs.
Scoring system:	Grading of erythema and edema.
Source of light:	Four 40W fluorescent bulbs Sylvania/GTE 350 Blacklight. Continuous UVA spectrum between 320 and 400 nm, delivering approx 4,400 microwatt/cm2 at distance 10 cm.
UV dose:	15 – 20 Joules (presumably per cm2)
Testing schedule:	Day 1: Tape stripping and application of test material on designated skin area (inner forearm), (presumably ?) followed by UV irradiation and then occlusion with a patch with 0.2 ml of additional test material. On contralateral forearm application of patches with 0.2 ml test material, without irradiation. Day 2 (24 hrs): removal of patches and scoring.

	One week: scoring
GLP:	Unclear

Results:
There were no skin reactions observed at any time during the study.

SCCS comment:
The methods described in the report are unclear some essential points: it seems that the UV irradiation was performed immediately after the application of the test solution. The copies of the CRF's show 4 test areas on each volar forearm; this is not explained in the methods. The test concentration of the test article is not disclosed. Therefore the test results cannot be evaluated for this Opinion.

F.

Reference:	RIFM 1987[a] (RIFM nr 5742)
Study period:	Feb – June 1987
Guideline/Method:	No specific method referenced.
Species:	Human
Group size:	10 subjects, all females
Test substances:	A: Tagetes absolute 2% (open test) B: Tagetes absolute 1% (open test) C: Tagetes absolute 0.5% (open test) D: Vehicle control: 75% Ethanol, 25% Diethylphthalate (open) E: Tagetes absolute 0.5% (patched) F: Tagetes absolute 0.25% (patched) G: Tagetes absolute 0.1% (patched) H: Vehicle control: 75% Ethanol, 25% Diethylphthalate (patched)
Batches:	No information. No certificate of analysis.
Vehicle:	Presumably 75% ethanol 25% Diethyl phthalate
Controls:	Within same subjects. Vehicle, test substances without irradiation and 8-Methoxypsoralen as positive control. Irradiation of skin area without application of any test substance.
Route:	Test substances A-D open application (20 microliter), E-H closed application (0.2 ml) (see Test substances, above). Applications on each side of the back, preceded by 3 times tape stripping.
Patch:	1.25" Lintene disc, Filter Fabrics, with Dermicel tape with 0.2 ml test solution during 24 hours.
Scoring system:	Blinded scorer. Grading on signs of erythema, infiltration, edema, blistering/ulceration from 0 - 4.
Source of light:	Solar simulator 150W xenon compact arc, with Schott filters to eliminate UVB and UVC, and to attenuate infrared.
UV dose:	Approx 14 (13.8 – 14,1) J/cm2 UVA, followed by 0.5 MED of UVA plus UVB.
Testing schedule:	Pre-test determination of MED Day 1: tape stripping (3 times) of all test areas. Application of test materials on designated skin areas (each side of the back). Irradiation of the open application sites at 30 mins after application. Reading of the irradiated areas Day 2 (24 hrs): removal of patches and irradiation of these areas. Grading of skin signs. Day 3: (48 hrs): scoring. Day 4: scoring
GLP:	Unclear

Methods (taken from submission):
Site Preparation: Three areas on each side of the back were tape-stripped three times with cellophane tape to remove the stratum corneum layers. Areas I and 2 on each site were appropriate size to allow application of four test articles. Area 3 was sized to permit a single irradiation test area. In the two randomly selected test subjects who were treated with 8-MOP, a second site was tape-stripped in Area 3. Area 1 received application if the test articles and subsequent UVA and UVB irradiation. This area was covered so that it could not be irradiated and was a control for inherent irritation potential of test article. Area 3 received UVA and UVB irradiation only and was a control for irradiation- induced irritation. The 8-MOP site in two test subjects served as a positive control for photo toxicity.

Dosing Procedure: The test articles were tested as supplied or as directed. In half of the subjects, the left side and Test Articles E-H were applied by patching the left side of the back. In the other five subjects, the sides for the patched and open application methods were reversed. For the patched application, 0.2 ml of the test article was applied to a 1¼" Lintene™ disc (Filter Fabrics, Inc., Goshen, IN 46526) backed by Dermicel™ tape (Johnson and Johnson, New Brunswick, NJ 08903). The patches were applied immediately after tape-stripping and remained in place for 24 hours prior for irradiation. For the open application, 20µl of the test article was evenly applied with a micropipette to a 1.5 cm diameter test site and was allowed to air dry. 8-MOP was applied similarly to the selected subjects at concentrations of 0.0125 mg/ml in methanol for the closed patch (0.2 ml) and 0.1 mg/ml in methanol for the open patch (20 µl).

Irradiation Procedure: The patched sites in area 1 were irradiated 15 minutes after removal of the patches. The patches in area 2 were removed after completion of the irradiation. The open application sites in arts 1 were irradiated 30 minutes after test article application. The corresponding sites in Area 2 were covered by non-woven fabric during the irradiation period. One site in area 3 (either side) was irradiated similarly tot the test article site.
Sites in areas 1 and 3 were exposed to UVA light (solar simulator light source filtered with 1 mm WG360) for a time period calculated to deliver approximately 14 Joules/cm^2. Following the UVA irradiation, the filter was removed and the same sites were further exposed to 0.5 MED of UVA and UVB light based on the previous determined MED. After irradiation, all sites were uncovered and the subjects were instructed to wear loose fitting clothing and not expose the back to light.

Evaluation: An evaluation of the degree of skin reaction present at all sites was made approximately 5 minutes after the completion of irradiation. All sites were re-examined for reaction at 3, 24, 48 and 72 hours after irradiation.
The study was blinded through the 24-hour evaluation of all subjects, following which the identities of the test articles were known.

Data Treatment: Skin reactions were evaluated using the following scale.

0	=	no sign of irritation
0.5	=	barely perceptible erythema
1.0	=	slight erythema, no edema
2.0	=	moderate to marked erythema with slight edema
3.0	=	marked erythema with moderate to marked edema
4.0	=	erythema with blistering or ulceration

Results: Presented in tables
Conclusion: (taken from the submission) Both the 24-hour closed patch method and the 15-30-minute open patch method produced similar responses for all test articles. The vehicle control test article appeared to elicit approximately the same number and intensity of reactions as the various concentrations of the actual test articles. No concentration dependent graded response was observed in either the open or closed patched series for the range of concentrations tested.

SCCS comment:
The positive control with 8-MOP gave a clear skin reaction, however the 8-MOP dose (0.1mg/ml) and the UV dose (14 J/cm2) is higher than routinely used in pre-testing for topical phototherapy (normally approx 0.05 mg/ml gel and 0.5 J/cm2).
Because of the positive reactions to the vehicle, no conclusion can be drawn.

G.

Reference:	RIFM 1987b (RIFM nr 5743)
Study period:	January 1987
Guideline/Method:	No specific method referenced.
Species:	Human
Group size:	10 subjects, all females
Test substances:	A: Tagetes absolute (T Minuta Egypt) 10% B: Vehicle control: 75% Ethanol, 25% Diethylphthalate
Batches:	No information. No certificate of analysis.
Vehicle:	Presumably 75% ethanol 25% Diethyl phthalate
Controls:	Within same test subjects. Vehicle, test substances without irradiation and 8-Methoxypsoralen as positive control. Irradiation of skin area without application of any test substance.
Route:	Test substances A and B open application (20 microliter) on the skin of the back, preceded by 3 times tape-stripping.
Patch:	n.a.
Scoring system:	Grading on signs of erythema, infiltration, edema, blistering/ulceration from 0 - 4.
Source of light:	Xenon arc solar simulator (Model 12S, Solar Light Company), with 1 mm WG360 filter.
UV dose:	10 MED time equivalents (presumably 8.34 – 17.84 J/cm2) UVA, followed by 0.5 MED of UVA plus UVB.
Testing schedule:	Pre-test determination of MED Day 1: tape-stripping (3 times) of all test areas. Open application (in duplicate) of test materials on designated skin areas. Irradiation of the open application sites at 30 mins after application. Covering of duplicate test areas to block irradiation. Day 2 (24 hrs): Scoring of skin signs. Day 3: (48 hrs): scoring One week: scoring
GLP:	Unclear

Methods (taken from the submission):
Test Article: The Test Articles were tested as supplied. Each Test Article was applied to two designated test sites, each approximately 1.5 cm in diameter. Subjects served as their own controls in this test procedure.
Control Article: Three of the ten test subjects were randomly selected and treated with 8-MOP in addition to the Test Article to provide a positive control photo toxicity reaction. The concentration of 8-MOP was 0.2 mg/ml in methanol.
Test Procedure: Three areas were tape-stripped with cellophane tape three times to remove the superficial stratum corneum layers. Areas I and 2 were employed for application of the Test Articles. Area 3 was employed as an irradiation control test site. In the three randomly selected test subjects, an 8-MOP site was also prepared in Area 3. Twenty micro liters of each Test Article was applied to its designated sites in Areas 1 and 2. After 30 minutes, Area 2 was covered to avoid irradiation and served as a control for inherent irritation potential of the Test Article. The sites in Area 1 were then irradiated. Area 3 received UVA and UVB irradiation only and served as a control for irradiation- induced irritation. The 8-

MOP site in three test subjects was irradiated approximately 30 minutes after application. This site served as a positive control for photo toxicity.

Based on the previous determined MED (UVA and UVB irradiation), sites in areas 1 and 3 were exposed to UVA light (solar simulator light source filtered with 1 mm WG360) for a time period of 10 MED time equivalents. Following irradiation with UVA, the filter was removed and the same sites were further exposed to 0.5 MED of UVA an UVB light.
An evaluation of the degree of skin reaction present at all sites was made at approximately 5 minutes after the completion of irradiation. They were then lightly covered with non-woven cotton cloth that was fastened with overlapping strips of non-allergenic tape. All sites were re-examined for reaction at 3, 24, 48 and 72 hours after irradiation. Sites were re-covered as above following the 3 hour reading through the 24 hour reading. The sites were uncovered subsequent to the 24-hour reading.

Data Treatment: Skin reactions were recorded using a 5 point scale as follows.

0	=	no sign of irritation
0.5	=	barely perceptible erythema
1+	=	slight erythema
2+	=	noticeable erythema with slight infiltration
3+	=	erythema with marked edema
4+	=	erythema with edema and blistering

Results: (presented in tables 1, 2 and 3) no reaction in all subjects on the sites where the test article was applied and irradiated.

Conclusion (taken from the submission): The results obtained from these studies indicate that Test Articles A, B, …and …are most likely not phototoxic. In three randomly selected subjects treated with 8-methoxypsoralen, all exhibited a definite phototoxic response.

SCCS comment:
The study was by an open application (i.e. no occlusion) of 30 minutes only, followed by irradiation. It had a more or less similar design, performed in the same institute as the one mentioned above (5752). In one of the tables in the report, the UVA dose that is listed is presumably the test substance exposure dose as mentioned above (from 8.34 to 17.84 J/cm2), and not the UVA dose for the MED.
No reactions were noted on the irradiated Tagetes exposed skin areas. In two subjects, barely perceptible erythema was noted on the vehicle exposed (and irradiated) skin area.
The positive control with 8-MOP gave a clear skin reaction, however the 8-MOP dose (0.1mg/ml) and the UV dose is higher than routinely used in pre-testing for phototherapy (normally 0.05 mg/ml and 0.5 J/cm2).

H.

Reference:	RIFM 1986 (RIFM nr 5748)
Study period:	December 1986
Guideline/Method:	In-house protocol, no specific method referenced.
Species:	Human
Group size:	10 subjects: 2 males, 8 females
Test substances:	A: Tagetes absolute 0.5% B: Vehicle: 75% ethanol, 25% Diethylphthalate
Batches:	Sample nrs 1235-84-0.5 and 2914-344. No certificates of analysis provided.
Vehicle:	Presumably 75% ethanol 25% diethylphthalate
Controls:	Within same test subjects. Vehicle. UVA irradiation outside the application area (volar forerarm).
Route:	24 hrs patch test with 0.2 ml on volar forearm.

Patch:	Readi-Bandage (Professional Medical Products).
Scoring system:	From 0 – 4 on erythema up to wheal-flare/blister, and on edema.
Source of light:	Four F40 BL fluorescent tubes with output at 360 nm of approx 1.23W per 10nm
UV dose:	Approx 0.22 J/cm2/min UVA. Total dose on patch-treated area not specified.
Testing schedule:	Day 1: duplicate patches on each forearm. Day 2: patch removal, irradiation and scoring Day 3 (24 hrs after irradiation): scoring Day 4 (72 hrs after irradiation): scoring

Methods (taken from the submission):
UV-A irradiation was from four F40BL fluorescent tubes with an output at 360nm of approximately 1.23W per 10 nm of wave-length. These lamps deliver a dose of approximately 0.22 J/cm2/min at a distance of 10 cm (the distance from the lamp to the skin sites) as measured with the International Ligth, Inc., IL443 Phototherapy System including IL443 radiometer S/N 1125, UV-A Filter S/N 704, W Diffuser S/N 1819 for cosine spatial response and IPIR calibration.
The Readi-Bandage (Professional Medical Products) patch was used occlusively. Approximately 0.2 ml was applied to each patch.
As per HRL Standard Operating Procedures (SOP) (HRL Form: SOP/PT/PA-1/82), the volar forearms were the patch sites. Patches were applied starting proximal (closest to the elbow fold) to distal (closest to the wrist).
One arm was patched with the test material; because the dosage of UV-A irradiation is not erythrogenic, no "control" site is delineated on the forearm - rather, the entire forearm serves as an irradiation "control" for the irradiated arm from exposure to sunlight throughout the test period.
The patch sites were recorded on the anatomical diagram of each subject's individual data sheet (HRL Form: PTDS/10-82).
Patching Schedule:
Day One: Subjects were patched with duplicate patches on each volar forearm. The test sites were marked with gentian violet. The subjects were then instructed to keep the patches dry. Patches were worn for 24 hr.
Day Two: Subjects returned to HRL. The HRL Project Manager removed the patches, read the sites and recorded the scores of the patch sites of both arms. The designated forearm was irradiated and scored immediately after irradiation. (The subject's non-irradiated arm was protected from the light source either by the subject's own long sleeve or the special HRL arm "mitten"). The test sites were re-marked with gentian violet. Subjects were reinstructed to protect both arms form ultraviolet irradiation throughout the test period.
Day Three: Subjects returned to HRL. The HRL Project Manager read and recorded the scores of the test sites of both arms. The gentian violet marks were renewed if necessary.
Day Four: Subjects returned to HRL. The HRL Project Manager read and recorded the scores of the test sites of both arms. If a 2-level or greater reaction was not observed at any site, the Phototoxicity Test was deemed complete, except that each subject was instructed to report to HRL any delayed reaction experienced.

Results and Conclusions (taken from the submission):
Three subjects exhibited a 2-level reaction with oedema on the irradiated (test material) sites and one subject exhibited a 2-level reaction (without oedema) on the irradiated (test material) site. Five subjects exhibited + or 1 level reactions on the irradiated (test material) sites. No reactions were exhibited on the non-irradiated (test material) sites.
In this Phototoxicity study performed according to the Experimental Design, aforementioned, test material 1235-84-0.5 did induce contact dermal phototoxic response in human subjects.

SCCS comment:
The total UV dose on the irradiated areas was not specified. At 24 hrs after irradiation, there were clear signs of photoxicity in 3 out of the 10 subjects (two with mild erythema and oedema, one with mild erythema).

Studies from the 2004 submission which report testing with Tagetes 0.01% (taken from the SCCP 2005 Opinion)

Reference: RIFM 1986f, RIFM 1986g (RIFM# 4348, 1690
Material tested: Tagetes minuta Flower Extract
Sample: Tagetes minuta absolute (Marigold abs. - Egypt)A-1235-84-0.01/0.05, B -91235-84-0.25
Subjects : male and female volunteers
Concentration : 0.01 %, 0.05% and 0.25% in 75% ethanol/25% diethyl phthalate.

0.3 ml aliquots of the test material were applied to a pair of contact Parker-Davis Webril patches, which were then applied under occlusion to naive skin sites on the back under occlusion for 24-hours, together with a vehicle control patch. After 24 hours one of each pair of patches was removed, any excess test material was wiped off with a moistened towel and the site was irradiated with 16-20 J/cm2 of UVA (1000 W Xenon Arc Solar Simulator) within 10 minutes following patch removal. The duplicate patch was then removed. The irradiated site was used for evaluation of phototoxicity potential and the other to evaluate existing contact sensitisation or primary irritation. Reactions were scored at 1, 24, 48 and 72 hours after patch removal.

Results:
No reactions at 0.01 % (0/25).
No reactions at 0.05% (0/25).
2/25 reactions at 0.25%. According to authors, the test material is phototoxic at this concentration.

SCCS comment
The original report could not be accessed. Information on the TTP content is not available.

3.3.3 Safety evaluation (including calculation of the MoS)

n.a.

3.3.4 Discussion

Physico-chemical properties

Tagetes spp extracts are widely used fragrance ingredients of many fragrance compounds used in perfumery and perfumed cosmetics. In leave-on cosmetics, tagetes extracts and tagetes oils are used at a maximum concentration up to 0.01%.
Tagetes minuta flower extract, Tagetes minuta flower oil, Tagetes patula flower extract and Tagetes patula flower oil are mixtures of many substances. Major constituents of these extracts/oils are limonene, (E)-β-ocimine, β-phelandrene, p-cymene, β –caryophyllene, α-muurolene, terpinolene, α-terpineol, (Z-)-tagetone, (Z)-tagetenone, (E)-tagetenone, dihydrotagtenone, (E)-ocimenone, verbenone, piperitone, pepritenone.
Chemical composition of these extracts/oils vary depending upon the harvesting location, growth stage of the plant, and harvesting time of the budding.

No information was found on the levels of alpha-terthienyl, a phototoxic thiophene compound responsible for the phototoxicty of tagetes extracts/oils in the tagetes extracts/oils used in perfumes and cosmetic products. However, the tagetes extracts/oil used for phototoxicity testing are reported to contain from below detection limit to 2.45% alpha-terthienyl (see 3.3.1)

Phototoxicity

Tagetes species belong to the family of Compositae. Several members of this plant family are known to be phototoxic. The component of Tagetes for which phototoxicity in humans has clearly been demonstrated is alpha-terthienyl, also called terthiophene (TTP) (Chan 1977, Rampone 1986). The available literature does not provide a dose-response relationship for the phototoxicity of TTP. Chemical analyses of extracts of different tagetes species have been published and show considerable variability in composition, (Chamorro 2008, Moghaddam 2007, Romagnoli 2005, Ramaroson 2009). While the presence or absence of terthiophene (TTP) is stated on the various submitted certificates of analysis, the composition of Tagetes extracts was not noted in these publications.
Phototoxic properties of different fragrances have been demonstrated (Placzek 2007), but these molecules are not listed in the published Tagetes extract analyses.

In general, it is only to a certain extent possible to apply the results from *in vitro* phototoxicity studies to humans (EVCAM 2014, Kejlova 2010). The results from the submitted *in vitro* studies on Tagetes cannot be used for this Opinion (see 3.3.1.1).

In the human photoxicity studies there are reactions to the DEP/EtOH vehicle. While this material is not considered to be phototoxic (Api 2001), an explanation could be that the stratum corneum is made more "translucent", upon which UV erythema could occur at doses below the MED. For a topically applied substance to increase the transmission of UV into the skin it needs to have a refractive index close to that of stratum corneum (approximately 1.55). Diethylphthalate - ethanol solutions are close to this (Diffey 2014, Miller 2006). Although it is unknown whether sufficient amounts of DEP/EtOH are still present on the patch-tested and irradiated skin, the reported erythema on the vehicle patch-tested skin creates problems in the interpretation of the studies in humans, especially where positive reactions on the test article are observed (see 3.3.2: Human data, studies A-D, F and H). Therefore, no firm conclusion regarding a safe level of Tagetes can be drawn from the human studies.

Some reports base their conclusions on a 72 hrs reading; for phototoxicity studies it is essential to read and report the skin reactions at 24 hrs and 48 hrs (and preferably also 4 hrs) after irradiation.

Overviewing the results of the submitted study reports, there is no clear pattern indicative of fewer phototoxic reactions to the low terthiophene (TTP) test articles, except in the 2008 study in hairless mice (3.3.1.2). In that study, all test articles with a concentration of 0.01% Tagetes elicited no phototoxic reactions, while the 0.1% Tagetes concentrations showed phototoxic reactions when the TTP content was 0.35% and above. In the older hairless mice studies, the TTP content is unknown. Hairless mice are more sensitive in photoxicity testing than humans (Forbes 1977).
The study in Guinea pigs cannot be relied upon because of the high number of reactions to the non-irradiated test material. Moreover, guinea pigs are considered to be more sensitive than humans, at least in tests with bergamot oil (Marzulli 1970).

Thus, based on the study in hairless mice (RIFM 2008), and in view of the inconclusive, albeit mild, reactions in humans, a maximum concentration of 0.01% Tagets extracts and oils which contain less than 0.35% TTP can be considered as safe for humans. A maximum concentration of 0.01% Tagetes extracts and oils would then amount to 0.35 ppm of the

phototoxic agent terthiophene (TTP) in the final product. This TTP content can be considered as a sufficiently safe margin when compared with the well-documented strongly phototoxic agent 8-MOP (8-methoxypsoralene) which gives phototoxic reactions in humans above 6 ppm (Grundmann-Kollmann 2001).

Sunscreens tend to be used to prolong exposure to UV-light. Because the non-physical sunscreens do not shield from UV-exposure to the skin, the effect of a phototoxic chemical such as TTP as ingredient of such a product is unpredictable. Therefore, the Tagetes extracts and oils should not be used as component of sunscreen products.

Table: Overview of phototoxicity studies

In vitro	Tested from 0.1% upwards	No reaction on low TTP. Phototox at 0.1% absolute EG (% TTP not known). Not reliable.
Hairless mice	Phototoxic at 0.1 % in the higher TTP content. Not phototoxic at 0.01%	Well conducted study. Hairless mice tend to be more sensitive than humans.
A	From 2004 dossier/opinion	2/6 mild reactions at 0.01%. TTP content unknown.
B	From 2004 dossier/opinion	No reaction at 0.01%. TTP content unknown.
Guinea pigs	From 2004 dossier/opinion	Slight reactions in 6/10 or 7/10 at 0.01%. Four animals also reacted on application without irradiation. And all animals reacted to 3% non-irradiated application. TTP content unknown.
Humans		
A	Also positive reactions on skin area exposed to vehicle	Study quality higher than those mentioned below. Nevertheless difficult to interpret.*) Low TTP seems to give no reactions.
B	Also positive reactions to vehicle	Difficult to interpret results *)
C	Also reactions to vehicle	Difficult to interpret results *)
D	Also reactions to vehicle	Difficult to interpret results *
E		Study report inadequate
F	Tested at 0.1% and higher	Difficult to interpret results *)
G	Tested at 10%. Open applic, irradiation after 30 mins exposure. No photox reactions. Faint erythema	Exposure time may be too short.

	reaction to vehicle.	
H	Phototoxic at 0.5%. Also reactions to vehicle	
	From 2004 dossier/opinion	No reactions at 0.01%. TTP content unknown.

*) because of the reactions to the vehicle

4. CONCLUSION

1. On the basis of data submitted, does the SCCS consider Tagetes minuta and T. patula extracts and essential oils safe for use as fragrance ingredients in cosmetic leave-on products with a maximum concentration limit of 0.01%?

The SCCS considers a maximum level of 0.01% Tagetes minuta and T. patula extracts and essential oils in leave-on products (except sunscreen cosmetic products and products marketed for exposure to natural/artificial UV light) as safe, provided that the alpha terthienyl (terthiophene) content of the Tagetes extracts and oils does not exceed 0.35%.

2. Does the SCCS have any further scientific concerns with regard to the use of Tagetes minuta and T. patula extracts and essential oils as fragrance ingredients in cosmetic products?

The Tagetes extracts and oils should not be used as ingredients of sunscreen products and of products marketed for exposure to natural/artificial UV light.

5. MINORITY OPINION

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