Toxicological profile for

Lime oil terpeneless

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

Not applicable.

1.2. Synonyms

Citrus aurantifolia oil, terpeneless, FEMA No. 2632, Lime oil, expressed, terpeneless (Citrus aurantifolia (christman) Swingle), Lime oil, terpeneless, Oils, lime, terpene-free (ChemIDplus).

1.3. Molecular formula

Unspecified (ChemIDplus)

1.4. Structural Formula

No data available to us at this time.

1.5. Molecular weight (g/mol)

No data available to us at this time.

1.6. CAS registration number

68916-84-7

1.7. Properties

1.7.1. Melting point

(°C): -11.39 (estimated) or <25 (EPISuite, 2017).

1.7.2. Boiling point

(°C): 198 (EPISuite, 2017).

1.7.3. Solubility

1590 mg/L at 25°C (EPISuite, 2017).

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

No data available to us at this time.

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

(°C): No data available to us at this time.

1.7.8. Decomposition temperature

(°C): No data available to us at this time.

1.7.9. Stability

No data available to us at this time.

1.7.10. Vapor pressure

1.60E-01 mm Hg @ 25°C (EPISuite, 2017).

1.7.11. log Kow

2.97 (EPISuite, 2017).

2. General information

2.1. Exposure

Cosmetics: No data available to us at this time.

Food: Yes, individual consumption=0.01251 mg/kg/day (As taken from Fenaroli's Handbook of Flavor and Ingredients (2010). Sixth Edition. Burdock G.A. CRC Press. ISBN 978-1-4200-9077-2)

Environment: No data available to us at this time.

Pharmaceuticals: No data available to us at this time.

Lime oil, expressed (CAS RN 8008-26-2):

IFRA Critical Effect: Phototoxicity

IFRA fragrance material specification: Where the bergapten (5-methoxypsoralen) content of all relevant oils present in a compound has been determined, it is recommended that for applications on areas of skin exposed to sunshine, excluding bath preparations, soaps and other products which are washed off the skin, the total level of bergapten in the consumer products should not exceed 0.0015% (15 ppm). This is equivalent to 0.0075% (75 ppm) in a fragrance compound used at 20% in the consumer product. Where the level of bergapten has not been determined by appropriate methods, the limits specified in the guidelines on individual oils should apply. In those cases, where such oils are used in combination with other phototoxic ingredients, the additive effect has to be taken into consideration and the use levels have to be reduced accordingly. The sum of the concentrations of all phototoxic fragrance ingredients, expressed in % of their recommended maximum level in the consumer product, shall not exceed 100.

Limits in the finished product for - "leave on the skin contact": 0.7000 % Restriction.

As taken from IFRA, 2015a.

Reported uses (ppm): (FEMA, 1994)

Food Category	Usual	Max.	Food Category	Usual	Max.

Alcoholic beverages	97.65	122.30	Gelatins, puddings	76.91	91.97
Baked goods	75.69	100.10	Hard candy	52.64	52.64
Chewing gum	0.46	3.77	Meat products	8.97	10.00
Condiments, relishes	9.00	10.00	Nonalcoholic beverages	28.13	45.10
Frozen dairy	66.02	83.80	Soft candy	75.47	87.71

As taken from Fenaroli's Handbook of Flavor and Ingredients (2010). Sixth Edition. Burdock G.A. CRC Press. ISBN 978-1-4200-9077-2

Lime oil, terpeneless is reported as a fragrance ingredient by IFRA (2016).

2.2. Combustion products

No data available to us at this time.

2.3. Ingredient(s) from which it originates

Terpeneless oil is prepared from distilled lime oil by the conventional method of deterpenation. The terpoenless fraction constitutes a very small fraction of the lime oil. It has a powerful, sweet-fruity, grape-like odor (Burdock 2010).

Part II is an alphabetical list of other sources (complex synthetic materials or essential oils) for restricted materials having Standards and which must therfore be taken into account for determination of the maximum level.

NATURAL RAW MATERIALS						**CONSTITUENTS**		
Essential oil Category	**RIFM ID**	**Principle/ Main CAS RIFM DATABASE**	**ALTERNATIVE/EINECS CAS NO**	**Principle Name**	**Botanical/Binomial name**	**CAS No.**	**IFRA Name/Principle Name**	**Level (%)**
G2.29	170	68916-84-7	90063-52-8	Lime oil, terpeneless	Citrus latifolia Tanaka and Citrus aurantifolia (Christman) Swingle	5392-40-5	Citral	0.22
						2111-75-3	p-Mentha-1,8-dien-7-al (Perilla aldehyde)	0.3

As taken from IFRA, 2015b. Annex I to the IFRA standards (48th Amendment, June 2015).

3. Status in legislation and other official guidance

FEMA has given GRAS (generally recognised as safe) status to lime oil terpeneless for use as a food flavour (Cohen SM et al. 2019; Hall RL and Oser BL, 1965).

C of E no.: 141

FEMA no.: 2632

FDA:

21 CFR 182.20, 582.20,

As taken from Fenaroli's Handbook of Flavor and Ingredients (2010). Sixth Edition. Burdock G.A. CRC Press. ISBN 978-1-4200-9077-2

Lime, oil, terpeneless (citrus aurantifolia (christman) swingle) is included on the FDA's inventory of "Substances Added to Food (formerly EAFUS)" as a flavoring agent or adjuvant, and is generally recognized as safe for use in food under 21 CFR section 182.20 (Essential oils, oleoresins (solvent-free), and natural extractives (including distillates)) (US FDA, 2022a,b).

Neither registered nor pre-registered under REACH (ECHA).

Lime extract (CAS RN 68916-84-7) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2022).

Oils, lime, terpene-free are listed in the US EPA Toxic Substances Control Act (TSCA) inventory and 2020 CDR TSCA Inventory.

As taken from the TSCA and 2020 CDR TSCA inventories are available at http://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do

Oils, lime, terpene-free (CAS RN 68916-84-7) are included on the New Zealand Inventory of Chemicals and does not have an individual approval but may be used under an appropriate group standard (NZ EPA, 2006).

Lime oils, terpene free (CAS 68916-84-7) is listed on Australian Inentory of Industrial Chemicals (AICIS, formerly NICNAS). As taken from AICIS, undated.

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

No data available to us at this time.

4.2. Absorption, distribution and excretion

No data available to us at this time.

4.3. Interactions

No data available to us at this time.

5. Toxicity

5.1. Single dose toxicity

No data available to us at this time.

5.2. Repeated dose toxicity

No data available to us at this time.

5.3. Reproduction toxicity

No data available to us at this time.

5.4. Mutagenicity

No data available to us at this time.

5.5. Cytotoxicity

Lime oil distilled and lime oil terpeneless did not inhibit metabolic co-operation in Chinese hamster V79 cells up to a maximum non-toxic dose (Davidson, undated) (No specified CAS number).

5.6. Carcinogenicity

No data available to us at this time.

5.7. Irritation/immunotoxicity

No data available to us at this time.

5.8. All other relevant types of toxicity

No data available to us at this time.

6. Functional effects on

6.1. Broncho/pulmonary system

No data available to us at this time.

6.2. Cardiovascular system

No data available to us at this time.

6.3. Nervous system

No data available to us at this time.

6.4. Other organ systems, dependent on the properties of the substance

No data available to us at this time.

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. Burnt ingredient toxicity

Tobacco smoke condensates from cigarettes containing Lime oil (and other extractables) and an additive free, reference cigarettes were tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the condensate was not changed by the addition of Lime oil (and other extractables). Table below provides tested level(s) and specific endpoint(s)

Endpoint	**Tested level (ppm)**	**Reference**
Smoke chemistry	<1 (oil, terpeneless; 68916-84-7)	Carmines, 2002 & Rustemeier et al., 2002
In vitro genotoxicity	<1 (oil, terpeneless; 68916-84-7)	Carmines, 2002 & Roemer et al., 2002
In vitro cytotoxicity	<1 (oil, terpeneless; 68916-84-7)	Carmines, 2002 & Roemer et al., 2002
Inhalation study	<1 (oil, terpeneless; 68916-84-7)	Carmines, 2002 & Vanscheeuwijck et al., 2002

9. Heated/vapor emissions toxicity

Endpoint	**Tested level (ppm)**	**Reference**

Aerosol chemistry 100	**100**	**Crooks et al. 2018**
In vitro cytotoxicity	**100**	**Crooks et al. 2018**
In vitro genotoxicity	**100**	**Crooks et al. 2018**
In vitro carcinogenicity (Cell Transformation Assay)	**100**	**Crooks et al. 2018**

Aerosol from an electronic nicotine delivery system (ENDS) that creates a vapor by heating an e-liquid containing Lime oil (and other extractables) was tested in a battery of in vitro and/or in vivo test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) and no adverse findings from a 90-day in vivo repeat-dose inhalation toxicity study were observed after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to those observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic and adverse effects upon exposure. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	**Tested level (ppm)**	**Reference**
Aerosol chemistry	50	Logic (2019a) Labstat International Inc. (2021)
In vitro genotoxicity	50	Logic (2019a) Labstat International Inc. (2022)
In vitro cytotoxicity	50	Logic (2019a) Labstat International Inc. (2022)
In vivo genotoxicity	50	Logic (2019a)
Inhalation study	50	Logic (2019a)

Aerosol from an electronic nicotine delivery system (ENDS) product that creates a vapor by heating an e-liquid; the vapor then passes through a capsule containing tobacco granules, containing Lime oil (and other extractables) was tested in a battery of in vitro and/or in vivo test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) and no adverse findings from a 90-day in vivo repeat-dose inhalation toxicity study were observed after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to those observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic and adverse effects upon exposure. The table below provides tested level(s) and specific endpoint(s):

Endpoint	**Tested level**	**Reference**
Aerosol chemistry	0.0064 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vitro genotoxicity	0.0064 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vitro cytotoxicity	0.0064 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vivo genotoxicity	0.0064 mg/(tobacco portion; 310 mg)	Logic (2019b)
Inhalation study	0.0064 mg/(tobacco portion; 310 mg)	Logic (2019b)

10. Ecotoxicity

10.1. Environmental fate

No data available to us at this time.

10.2. Aquatic toxicity

No data available to us at this time.

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

No data available to us at this time.

10.5. All other relevant types of ecotoxicity

No data available to us at this time.

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- Labstat International Inc. (2022). Determination of Mutagenic Response (Ames), Cytotoxic Response (NRU) and Genotoxic Response (ivMN) of Mainstream Aerosol Collected Matter (ACM) and Mainstream Gas Vapor Phase (GVP) of Electronic Cigarette Products. Biological Activity Test Report.
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- Logic (2019b). G.5. Nonclinical Evaluation Summary - Logic Vapeleaf (PMTA)
- NZ EPA (2006). New Zealand Environmental Protection Authority. Inventory of Chemicals. Record for oils, lime, terpene-free (CAS RN 68916-84-7). Date added to inventory: 1 December 2006. Available at: https://www.epa.govt.nz/database-search/new-zealand-inventory-of-chemicals-nzioc/view/35DCBA2C-01C5-439C-9770-0A4B927C2D9C
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- Vanscheeuwijck PM et al., (2002). Evaluation of the potential effects of ingredients added to cigarettes.Part 4: subchronic inhalation toxicity. Food and Chemical Toxicology 40: 113-131.

12. References for reduced-risk products

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13. Other information

14. Last audited

August 2022

Toxicological profile for

Lime oil

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

Not applicable.

1.2. Synonyms

Citrus aurantifolia oil, distilled; Distilled lime oil; Lime oil, distilled (Citrus aurantifolia (christman) Swingle); Lime oil expressed; Oils, lime; Oil of lime; FEMA No. 2631; UNII-UZH29XGA8G; Lime oil; UNII-7937R189CB (ChemIDplus); Citrus aurantifolis swingle distilled oil; Lime oil distilled Haitian; Lime oil distilled Mexican; Lime oil distilled Peruvian; Lime oil distilled, West Indian; Citrus aurantifolia swingle oil distilled Mexico; Lime oil distilled (Citrus aurantifolia swingle) Mexico; Lime oil distilled Mexico.

1.3. Molecular formula

“Unspecified” (ChemIDplus)

1.4. Structural Formula

Not applicable.

1.5. Molecular weight (g/mol)

Not applicable.

1.6. CAS registration number

8008-26-2

1.7. Properties

1.7.1. Melting point

(°C): -14.83 (estimated) (EPISuite, 2017)

1.7.2. Boiling point

(°C): 225.35 (estimated) (EPISuite, 2017)

1.7.3. Solubility

47.5 mg/L at 25°C (estimated) (EPISuite, 2017)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): 52 (closed cup) (NZ EPA CCID)

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

(°C): No data available to us at this time.

1.7.8. Decomposition temperature

(°C): No data available to us at this time.

1.7.9. Stability

No data available to us at this time.

1.7.10. Vapor pressure

0.152 mm Hg at 25°C (estimated) (EPISuite, 2017)

1.7.11. log Kow

3.66 (estimated) (EPISuite, 2017)

2. General information

2.1. Exposure

Cosmetics: No evidence

Food: Yes (Burdock, 1995)

Environment: No evidence

Pharmaceuticals: No evidence

Oils, lime are listed as ingredients used in fragrance compounds (US EPA InertFinder Database, 2022) and Persian lime oil, expressed; lime oil, expressed; lime oil folded; lime oil and lime oil, expressed, rectified (all CAS RN 8008-26-2) are listed as fragrance ingredients by IFRA.

Reported uses (ppm): (FEMA, 1994)

Food Category	Usual	Max.	Food Category	Usual	Max.
Alcoholic beverages	394.10	452.10	Gelatins, puddings	337.20	477.30
Baked goods	356.70	557.40	Gravies	0.75	1.38
Breakfast cereals	122.40	199.70	Hard candy	338.10	948.20
Chewing gum	1878.00	2322.00	Meat products	69.18	84.01
Condiments, relishes	90.15	100.00	Nonalcoholic beverages	42.17	75.54
Frozen dairy	362.50	472.70	Soft candy	558.90	782.90

As taken from Fenaroli's Handbook of Flavor and Ingredients. Sixth Edition. Burdock G.A. CRC Press. ISBN 978-1-4200-9077-2

Typical Concentrations of Lime Oil in Finished Products (w/w%) (Opdyke, 1974a,b)

Ingredient	Soap	Detergent	Cream/Lotion	Perfume
	Usual/Maximum	Usual/Maximum	Usual/Maximum	Usual/Maximum
Lime oil (expressed)	0.05/ 0.25	0.005/ 0.025	0.03/ 0.1	0.5/ 1.5
Lime oil (distilled)	0.05/ 0.25	0.005/ 0.025	0.03/ 0.1	0.4/ 1.5

Citrus aurantifolia oil (CAS RN 8008-26-2) is listed as an ingredient (at given concentrations, where specified) in inside the home (<0.1-<1%), personal care (0.1-1.0%) and auto products by the CPID.

Citrus aurantifolia oil (CAS RN 8008-26-2/90063-52-8) is used as a perfuming, fragrance, cleansing, tonic, hair conditioning and skin conditioning ingredient; Citrus aurantifolia seed oil (CAS RN 8008-26-2/90063-52-8) as a flavouring, fragrance and skin conditioning ingredient; Citrus aurantifolia seed oil unsaponifiables (CAS RN 8008-26-2/90063-52-8) as a hair conditioning and skin conditioning ingredient; Citrus aurantifolia fruit oil (no CAS RN listed) as a fragrance and skin conditioning ingredient; and Citrus aurantifolia peel oil (no CAS RN listed) as a fragrance ingredient in cosmetics in the EU. As taken from CosIng, undated.

Citrus aurantifolia (lime) oil has been reported at up to 0.36% in a lipstick and at a maximum concentration of 0.12% in body and hand sprays, a majority of the uses for these ingredients

are in leave-on skin care preparations. (CIR, 2021)

Frequency and Concentration of Use According to Duration and Type of Exposure for Citrus Aurantifolia (lime) Oil.

	# of Uses	Max Conc of Use (%)
Totals	169	0.00000051-0.36
Duration of use		
Leave-on	90	0.00049-0.36
Rinse off	69	0.00015-0.17
Diluted for (bath)	10	0.00000051

Exposure type		
Eye area	NR	NR
Incidental ingestion	NR	0.0015-0.36
Incidental inhalation-spray	9; 34; 29	0.00049-0.12; 0.0015-0.1; 0.00067
Incidental inhalation powder	1; 29	0.022; 0.0075-0.023; 0.00067
Dermal contact	147	0.00000051-0.17
Hair-non-coloring	22	0.00049-0.02
Hair-coloring	NR	0.00015
Mucous membrane	35	0.00000051-0.36
Baby products	3	NR

As taken from (CIR, 2021).

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Results are given in Baker and Bishop (2005) J. Anal. Appl. Pyrolysis, 74, 2005, pp. 145-170.

Ingredients CAS Number Formulae or structure	Composition of pyrolysate (Compound %)	Max level in smoke (µg)
Lime oil, expressed CAS 8008-26-2	Limonene 48.4 Γ-Terpinene 13.4 B-Pinene 9.5 E-Citral 2.7 Cymen 2.3	2 0.7 0.5 0.1 0.1

2.3. Ingredient(s) from which it originates

Citrus aurantifolia (lime) oil is the volatile oil obtained from the whole plant, Citrus aurantifolia (CIR, 2021).

Citrus aurantifolia oil is the volatile oil obtained from the fruits of the lime, Citrus aurantifolia, Rutaceae

Citrus aurantifolia seed oil is the oil expressed from the seeds of the lime, Citrus aurantifolia, Rutaceae

Citrus aurantifolia seed oil unsaponifiables is the fraction of lime seed oil which is not saponified in the refining recovery of lime seed oil fatty acids of the lime, Citrus aurantifolia, Rutaceae

Citrus aurantifolia fruit oil is the volatile oil obtained from the fruit of Citrus aurantifolia, Rutaceae.

Citrus aurantifolia (lime) peel oil is the volatile oil obtained from the peel of Citrus aurantifolia, Rutaceae.

As taken from CosIng, undated.

“TSCA Definition 2019: Extractives and their physically modified derivatives. Citrus aurantiifolia, Citrus”.

As taken from ChemIDplus

3. Status in legislation and other official guidance

States approving use in tobacco: Belgium, France, Germany and UK

Food use approved in:

UK: Unknown

EU: Yes

USA: Yes FDA 182.20

ADI/TDI: Not identified.

FEMA has given GRAS (generally recognised as safe) status to lime oil, distilled (citrus aurantifolia (christman) swingle; CAS RN 8008-26-2 and 90063-52-8) (Hall RL and Oser BL, 1965; affirmed by Cohen et al. 2019), Persian lime oil, expressed (Citrus latifolia; CAS RN 8008-26-2) and Mexican lime oil, expressed (Citrus aurantifolia, Citrus medica var. acida; CAS RN 8008-26-2) (Marnett et al. 2013; affirmed by Cohen et al. 2019) for use as food flavours.

Codex Alim.: Not listed

C of E no.: 141

FEMA no.: 2631

TLV/OEL: Not listed

Cosmetics (UK): Not listed in Schedule 1

CoE: Lime distilled oil: Category 3 (with limits on eucalyptol and furocoumarins)

FDA: 21 CFR 182.20

As taken from Fenaroli's Handbook of Flavor and Ingredients. Sixth Edition. Burdock G.A. CRC Press. ISBN 978-1-4200-9077-2

Lime oil, cold pressed, Mexican CAS n° 8008-26-2; Lime oil, expressed CAS n° 8008-26-2; and Lime oil expressed rectified CAS n° 8008-26-2; "may be used in cosmetic products, provided that the total concentration of furocoumarin-like substances in the finished cosmetic product does not exceed 1 ppm."

As taken from SCCP, 2005.

IFRA standard for lime oil expressed (CAS RN 8008-26-2, 90063-52-8):

Implementation dates:	For new submissions*: February 10, 2021 For existing fragrance compounds*: February 10, 2022 *These dates apply to the supply of fragrance mixtures (formulas) only, not to the finished consumer products in the marketplace.		
RECOMMENDATION:		RESTRICTION	
RESTRICTION LIMITS IN THE FINISHED PRODUCT (%):			
Category 1	0.70%	Category 7A	No restriction
Category 2	0.70%	Category 7B	0.70%
Category 3	0.70%	Category 8	0.70%
Category 4	0.70%	Category 9	No restriction
Category 5A	0.70%	Category10A	No restriction
Category 5B	0.70%	Category 10B	0.70%
Category 5C	0.70%	Category 11A	No restriction
Category 5D	0.70%	Category 11B	0.70%
Category 6	0.70%	Category 12	No restriction
Fragrance ingredient restriction - Note box The Standard is set due to the phototoxic effects of Lime oil expressed. For more detailed information on the application of this Standard, please refer to the note on phototoxic ingredients in chapter 1 of the Guidance for the use of IFRA Standards. If the level of furocoumarins is unknown, the restriction level specified in this IFRA Standard applies. Combination effects of phototoxic ingredients are only taken into consideration for the furocoumarin-containing fragrance ingredients (extracts) listed in the IFRA Standard			

of Citrus oils and other furocoumarins containing essential oils. If combinations of furocoumarin-containing phototoxic fragrance ingredients (extracts) are used, the use levels must be reduced accordingly. The sum of the concentrations of all furocoumarin-containing phototoxic fragrance ingredients (extracts), expressed in % of their recommended upper concentration level in the consumer product shall not exceed 100. For qualities of the expressed oil in which the less volatile components have been concentrated by partial or total removal of the terpene fraction, this limit should be reduced in proportion to the degree of concentration.
FLAVOR REQUIREMENTS: Due to the possible ingestion of small amounts of fragrance ingredients from their use in products in Categories 1 and 6, materials must not only comply with IFRA Standards but must also be recognized as safe as a flavoring ingredient as defined by the IOFI Code of Practice (www.iofi.org). For more details see chapter 1 of the Guidance for the use of IFRA Standards.

INTRINSIC PROPERTY DRIVING RISK MANAGEMENT:	PHOTOTOXICITY

RIFM SUMMARIES: These recommendations are based on results of RIFM on the phototoxicity of Lime oil expressed (Fd. Cosm. Toxicol. 12, 731 (1974), its Bergapten content reported in J.A.O.A.C. 52, (4), 727 (1969) and the observed no-effect level of pooled samples in tests using the animal model.
EXPERT PANEL FOR FRAGRANCE SAFETY RATIONALE / CONCLUSION: The Expert Panel for Fragrance Safety reviewed all the available data for Lime oil expressed and recommends the limits for the 12 different product categories, which are the acceptable use levels of Lime oil expressed in the various product categories.

As taken from IFRA, 2020a

IFRA standard for citrus oils and other furocoumarins containing essential oils:

Implementation dates:	For new submissions*: February 10, 2021 For existing fragrance compounds*: February 10, 2022 *These dates apply to the supply of fragrance mixtures (formulas) only, not to the finished consumer products in the marketplace.		
RECOMMENDATION:		RESTRICTION	
RESTRICTION LIMITS IN THE FINISHED PRODUCT (%):			
Category 1	0.0015% (5-MOP)	Category 7A	No restriction
Category 2	0.0015% (5-MOP)	Category 7B	0.0015% (5-MOP)
Category 3	0.0015% (5-MOP)	Category 8	0.0015% (5-MOP)
Category 4	0.0015% (5-MOP)	Category 9	No restriction
Category 5A	0.0015% (5-MOP)	Category10A	No restriction
Category 5B	0.0015% (5-MOP)	Category 10B	0.0015% (5-MOP)
Category 5C	0.0015% (5-MOP)	Category 11A	No restriction
Category 5D	0.0015% (5-MOP)	Category 11B	0.0015% (5-MOP)
Category 6	0.0015% (5-MOP)	Category 12	No restriction

Fragrance ingredient restriction - Note box

The Standard is set due to the phototoxic effects of Citrus oils and other furocoumarins containing essential oils. For more detailed information on the application of this Standard, please refer to the note on phototoxic ingredients in chapter 1 of the Guidance for the use of IFRA Standards.

Where the Bergapten (5-Methoxypsoralen, (5-MOP)) content of all relevant oils present in a compound has been determined, it is recommended that for applications on areas of skin exposed to UV-light, the total level of Bergapten in the consumer products should not exceed 0.0015% (15 ppm). This upper concentration level only applies to applications on skin exposed to UV-light, excluding rinse-off products and incidental skin contact products as detailed in the Guidance for the use of IFRA Standards.

Where the level of Bergapten has not been determined by appropriate methods, the limits specified in the guidelines on individual oils should apply. In those cases, where such oils are used in combination with other furocoumarin-containing phototoxic fragrance ingredients (extracts), the additive effect has to be taken into consideration and the concentration levels have to be reduced accordingly.

The sum of the concentrations of all furocoumarin-containing phototoxic fragrance ingredients (extracts), expressed in % of their recommended upper concentration level in the finished consumer product, shall not

exceed 100. Restrictions for furocoumarin-containing fragrance ingredients (extracts) have been recommended for: • Angelica root oil, • Bergamot oil expressed, • Bitter orange oil expressed, • Cumin oil, • Grapefruit oil expressed, • Lemon oil cold pressed, • Lime oil expressed, • Rue oil. The following essential oils contain small amounts of phototoxic furocoumarins (typical levels are provided in brackets): • Petitgrain Mandarin oil (50 ppm), • Tangerine oil cold pressed (50 ppm), • Parsley leaf oil (20 ppm). These levels are not high enough to require special restrictions if used alone, but if used in combination with one or the other furocoumarin-containing phototoxic fragrance ingredients (extracts), attention should be paid that the total level of Bergapten in the consumer product does not exceed 15 ppm.	
FLAVOR REQUIREMENTS: Due to the possible ingestion of small amounts of fragrance ingredients from their use in products in Categories 1 and 6, materials must not only comply with IFRA Standards but must also be recognized as safe as a flavoring ingredient as defined by the IOFI Code of Practice (www.iofi.org). For more details see chapter 1 of the Guidance for the use of IFRA Standards.	
INTRINSIC PROPERTY DRIVING RISK MANAGEMENT:	PHOTOTOXICITY
RIFM SUMMARIES: These recommendations are based on the published phototoxic effects of Bergapten and the established dose-effect relationships (Young at al., J. Photochem. Photobiol. B,7, 231 (1990); Dubertret et al.ibid 7, 251 (1990), idem, ibid, 7, 362 (1990).	
EXPERT PANEL FOR FRAGRANCE SAFETY RATIONALE / CONCLUSION: The Expert Panel for Fragrance Safety reviewed all the available data for Citrus oils and other furocoumarins containing essential oils and recommends the limits for the 12 different product categories, which are the acceptable use levels of Citrus oils and other furocoumarins containing essential oils in the various product categories.	

As taken from IFRA, 2020b

Oils, lime (CAS RN 8008-26-2) are pre-registered under REACH (“envisaged registration deadline 31 May 2013”) (ECHA).

Citrus aurantifolia (lime) oil (CAS RN 8008-26-2), lime oil distilled and lime oil (both with no CAS RN) are not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2022).

Oils, lime (CAS RN 8008-26-2) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2020 CDR list (Chemical Data Reporting Rule).

The TSCA inventory and 2020 CDR list are available at https://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do

Oils, lime are listed in the US EPA InertFinder Database (2022) as approved for fragrance use and non-food use pesticide products.

Lime oil (CAS RN 8008-26-2) is included on the New Zealand Inventory of Chemicals and does not have an individual approval but may be used under an appropriate group standard (NZ EPA, 2006) and is classified by the Environmental Protection Authority of New Zealand (NZ EPA CCID).

The CIR Expert Panel concluded that the available data are insufficient to make a determination that Citrus aurantifolia (lime) oil is safe under the intended conditions of use in cosmetic formulations (CIR, 2021).

Lime oil, distilled (CAS RN 8008-26-2) is included on the FDA’s list of Substances Added to Food (formerly EAFUS) as a flavoring agent or adjuvant, and is included under 21 CFR section 172.230

(Microcapsules for flavoring substances) and 182.20 (Essential oils, oleoresins (solvent-free), and natural extractives (including distillates)) (FDA, 2022a,b).

Lime Oil (CAS RN 8008-26-2) is included on the US FDA's list of inactive ingredients for approved drug products.

LIME OIL, COLD PRESSED	ORAL	LOZENGE	8008262	UZH29XGA8G		10mg
LIME OIL, COLD PRESSED	SUBLINGUAL	TABLET	8008262	UZH29XGA8G	NA	

(FDA, 2022c).

IFRA has restricted the use of the lemon oil expressed (CAS 8008-26-2 and 90063-52-8) in the IFRA Standard 50th Amendment.
As taken from IFRA, 2022

Lime oils (CAS 80008-26-2) is listed on Australian Inentory of Industrial Chemicals (AICIS, formerly NICNAS). As taken from AICIS, undated.

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

No data available to us at this time.

4.2. Absorption, distribution and excretion

No data available to us at this time.

4.3. Interactions

No data available to us at this time.

5. Toxicity

5.1. Single dose toxicity

(No specified CAS number)

Lime oil distilled acute oral LD 50 rats: >5 g/kg bw (Hart, 1971).

Lime oil distilled acute dermal LD50 rabbits: >5 g/kg bw (Hart, 1971).

No phototoxic effects were reported for lime oil distilled (Urbach & Forbes, 1972).

Lime oil expressed was found to have phototoxic properties when tested on hairless mice, pigs and man (Urbach & Forbes, 1972).

(CAS 8008-26-2)

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
rabbit	LD50	skin	> 5gm/kg (5000mg/kg)		Food and Cosmetics Toxicology. Vol. 12, Pg. 729, 1974.

rat	LD50	oral	> 5gm/kg (5000mg/kg)		Food and Cosmetics Toxicology. Vol. 12, Pg. 729, 1974.

As taken from ChemIDplus available at https://chem.nlm.nih.gov/chemidplus/

"Citrus aurantifolia (Christm.) Swingle (syn. C. MEDICA var. ACIDA Brandis) (family: Rutaceae) essential oil is one of the cheapest oils found in local markets. Although, it is generally accepted as non-toxic to vital organs and cells, majority of people are cynical about it usage. Herein, the present study reports the chemical composition and in vivo oral toxicity study of unripe C. aurantifolia essential oil found in Ghana. The toxicity of C. aurantifolia essential oil extract was investigated via oral administration using two methods: The acute toxicity single dose study (SDS) and the repeated dose method. The oil exhibited no acute toxicity but in the sub-chronic studies, the effects was dose and time-dependent. Chemical profile investigation of the oil showed 9 constituent of phytochemicals (Germacrene isomers (61.2%), Pineen (14%), Linalool dimmer (2.9%), Bornane (11%), Citral (2.9%), Anethole (1.5%), Anisole (1.1%), Safrole (0.3%) and Demitol (0.6%)). Histopathological studies revealed conditions such as necrosis, edema and inflammatory reaction in the liver, spleen and kidneys. Marginal upsurge of biochemical parameters above normal and elevated levels of lymphocytes (35.20-46.40 g/dL) demonstrated mild toxicity among the 100 mg/kg and 500 mg/kg dose groups at the sub-chronic stage. Low levels of hemoglobin (13.60 to 12.70 g/dL), MCV (34.20-24.0 fL), MCH (40.20-36.40 g/dL) along with high levels of liver enzymes confirmed the mild toxicity of the oil at sub-chronic stage. These results demonstrate that, despite consideration of lime essential oil as safe, it can have mild hematotoxic, nephrotoxic and hepatotoxic effects." As taken from Adokoh CK et al. 2019. Toxicol. Rep. 6, 692-702. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/31372347

5.2. Repeated dose toxicity

No relevant published repeated dose toxicity studies on Citrus plant- and seed-derived ingredients were identified in a literature search for these ingredients, and no unpublished data were submitted. (CIR, 2021)

5.3. Reproduction toxicity

No relevant published reproductive and developmental studies on Citrus plant- and seed-derived ingredients were identified in a literature search for these ingredients, and no unpublished data were submitted. (CIR, 2021)

5.4. Mutagenicity

In vivo:

No data available to us at this time

In vitro: (No specified CAS number).

Test system	Test conditions	Endpoint	Activation	Result	References
Chinese hamster lung cells (fibroblasts)	Incubated for 24 hr at up to 0.04 mg/ml. Cells scored for chromosome aberrations and polyploidy.	Chromosome damage and changes in chromosome number	Without	-ve (limited study as activation was not used)	Ishidate et al., 1984

Salmonella typhimurium TA97, TA98, TA100, TA1535	Ames test with up to 10 mg/plate. Depending upon strain and activation status, toxicity was seen at doses of 33 ug/plate and above.	Mutation	With and without rat and hamster liver S9	-ve (a high quality study)	NTP, undated.
Salmonella typhimurium possibly TA98, TA100, TA1535, TA1537 and TA1538, and Escherichia coli WP2uvrA	Ames test with up to 20 mg/plate.	Mutation	Probably with and without S9	-ve (paper in Japanese, with minimal information in English)	Anon., 1985
Bacillus subtilis H17, M45	Tested at 20 mg/disc in an assay for differential toxicity.	DNA repair (indicative test)	With and without S9	+ve (paper in Japanese, with minimal information in English)	Anon., 1985.

5.5. Cytotoxicity

Lime oil distilled and lime oil terpeneless did not inhibit metabolic co-operation in Chinese hamster V79 cells up to a maximum non-toxic dose (Davidson, undated) (No specified CAS number).

In vitro antibacterial activity of some plant essential oils.

BACKGROUND: To evaluate the antibacterial activity of 21 plant essential oils against six bacterial species.

METHODS: The selected essential oils were screened against four gram-negative bacteria (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris) and two gram-positive bacteria Bacillus subtilis and Staphylococcus aureus at four different concentrations (1:1, 1:5, 1:10 and 1:20) using disc diffusion method. The MIC of the active essential oils were tested using two fold agar dilution method at concentrations ranging from 0.2 to 25.6 mg/ml.

RESULTS: Out of 21 essential oils tested, 19 oils showed antibacterial activity against one or more strains. Cinnamon, clove, geranium, lemon, lime, orange and rosemary oils exhibited significant inhibitory effect. Cinnamon oil showed promising inhibitory activity even at low concentration, whereas aniseed, eucalyptus and camphor oils were least active against the tested bacteria. In general, B. subtilis was the most susceptible. On the other hand, K. pneumoniae exhibited low degree of sensitivity.

CONCLUSION: Majority of the oils showed antibacterial activity against the tested strains. However Cinnamon, clove and lime oils were found to be inhibiting both gram-positive and gram-negative bacteria. Cinnamon oil can be a good source of antibacterial agents. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. BMC Complement Altern Med. 2006 Nov 30;6:39. As taken from PubMed at http://www.ncbi.nlm.nih.gov/pubmed/17134518 (No specified CAS number).

Evaluation of the antimicrobial properties of different parts of Citrus aurantifolia (lime fruit) as used locally.

We investigated the potency of Citrus aurantifolia (Lime fruit), against pathogens, in the different forms in which this fruit plant is used locally (juice of the fruit, burnt rind of the fruit commonly known

as "epa-ijebu" in the Yoruba dialect) and the oil obtained from steam distillation of the fruit. The antimicrobial activity of "epa-ijebu" in different solvents was also compared. The solvents include palm-wine (a local alcoholic drink tapped from palm trees), Seaman's Schnapps 40% alcoholic drink, water, ethanol and fermented water from 3 days soaked milled maize known as "ekan-ogi" or "omidun" in the Yoruba dialect. Antimicrobial activity was carried out by the agar well diffusion. The clinical isolates used included Anaerobic facultative bacteria, namely: Staphylococcus aureus ATCC 25213, Staphylococcus aureus, Salmonella paratyphi, Shigella flexnerii, Streptococcus faecalis, Citrobacter spp, Serratia spp, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli ATCC 25922, and Escherichia coli; Fungi such as Aspergillus niger and Candida albicans; and Anaerobes which includes Bacteroides spp, Porphyromonas spp, and Clostridium spp. Crude extracts of all solvents used varied in zones of inhibition. The anaerobes and the gram-positive bacteria were susceptible to all the extracts with minimum inhibitory concentration (MIC) ranging from 32 mg/ml-128 g/ml. The activity against the fungi showed only the oil extract potent for A. niger, while Candida albicans was susceptible to all the extracts with MIC ranging from 256 mg/ml-512 mg/ml. The gram-negatives have MIC ranging from 64 mg/ml-512 mg/ml. Minimum bactericidal concentration (MBC) ranged between 32 mg/ml to 512 mg/ml depending on isolates and extracting solvent. The oil and palm-wine extract of "epa-ijebu" showed greater activity than the other extracts. The killing rate of the schnapps extract on S. aureus and E. coli was 1 and 3.5 hours respectively. Aibinu I, Adenipekun T, Adelowotan T, Ogunsanya T, Odugbemi T. Afr J Tradit Complement Altern Med. 2006 Nov 13;4(2):185-90. As taken from PubMed at http://www.ncbi.nlm.nih.gov/pubmed/20162090 (No specified CAS number).

“The present study focused on using microencapsulation technique to develop an antioxidant and antibacterial active smart cotton fabric using encapsulated lime oil (LO). LO microcapsules were prepared via the complex coacervation method using chitosan and gum arabic wall materials. UV-Visible and FTIR spectrometry verified the successful encapsulation of LO. The synthesized LO microcapsules were irregular in shape and differed in size between 15-160 µm according to the optical and SEM images. The loading of the microcapsules was found to be 2943 ± 128 µL/g with a loading efficiency of 82 ± 4%. The antioxidant activity of the LO microcapsules was 1336 ± 17 µg PGE/g (Folin-Ciocalteu assay). Brine shrimp lethality assay indicated that the cytotoxicity of LO was minimized upon microencapsulation. Succinic acid was used as the binder to incorporate the LO microcapsules on to the cotton fabric. The SEM images confirmed the steady attachment of LO microcapsules to the cotton fibres. The cotton fabric containing LO microcapsules displayed significant antibacterial activity against four bacterial species prior to and after subjecting the fabric to wash conditions.” As taken from Wijesirigunawardana BP and K Perera BG. 2018. Acta Chim. Slov. 65(1), 150-159. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29562116

Record for Citrus aurantifolia Swingle, juice (no CAS RN listed):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
IC50 - Inhibitor Concentration 50	In vitro	Human - leukemia cells	>500 mg/L/72H	In Vitro Toxicity Studies - cell metabolic activity: Alamar Blue assay etc.	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 180,70,2016

As taken from RTECS, 2018

"The objective of this study was to characterize chemical composition and functional properties of essential oils extracted from leaves of Citrus sinensis Osbeck, Citrus grandis Osbeck and Citrus aurantifolia Swingle. The essential oil of the C. sinensis and C. grandis leaves contained 44 compounds, accounted for total percentages of 94.17% and 93.78% of the oils, respectively, whereas the essential oil of the C. aurantifolia leaves had 62 compounds, accounted for 97.19% of the oil. Limonene, β-pinene, sabinene, β-ocimene, β-cubebene, terpinen-4-ol, linalool and nerol were main volatile compounds of the essential oil of the C. sinensis leaves, while the essential oil of the C. grandis contained high percentages of limonene, α-phellandrene, β-ocimene, β-caryophyllene, nerol, citronellol, geraniol, geranial and neral. The main volatile compounds of the essential oils of the C. aurantifolia leaves were Limonene, β-pinene, β-ocimene, β-caryophyllene, α-terpineol, citronellol and (+)-citronellal. The essential oil of the C. aurantifolia leaves exhibited the strongest scavenging activities (IC50 = 1.21 mg/ml) as compared to those of the C. sinensis (IC50 = 1.49 mg/ml) and C. grandis leaves (IC50 = 2.18 mg/ml). The essential oil of C. grandis leaves exhibited stronger antimicrobial activities than the essential oils of C. sinensis and C. aurantifolia leaves." As taken from Chi PTL et al. 2019. Waste Biomass Valor. 11, 4849-4857. Available at https://link.springer.com/article/10.1007/s12649-019-00815-6

5.6. Carcinogenicity

No adequate lifetime carcinogenicity studies on lime oil available to us at this time. A few tumour promotion assays were identified, and some of these also gave limited information on carcinogenicity potential (see below) (No specified CAS number).

Species	Test conditions	Evidence of carcinogenicity	Reference
Mouse (12-17 female mice per group)	Experiment 1: Animals given a single oral dose of known tumour initiator (DMBA or B(a)P) (some animals received solvent (polyethylene glycol; PEG) only or no initiation treatment) followed by weekly oral dosing with "undiluted" lime oil (or no promotion treatment) for 40 weeks. Animals kept for "natural lifespan".	No clear evidence of carcinogenicity. Benign forestomach tumours were seen in 1/15 non-initiated, lime oil treated mice, compared with 0/17 mice that had no initiation or promotion treatment. Interestingly, 5/13 mice given PEG in the initiation phase and lime oil as promoter developed benign forestomach tumours. Some evidence of tumour promotion. Increase in number of malignant forestomach tumours after lime oil treatment (2 in each group that received known initiator plus lime oil; 0 in other groups). No obvious lime oil-treatment effect on the incidence of tumours at other sites.	Field & Roe, 1965
Mouse (17-25 animals (approx. 50% female) per group)	Experiment 2: Animals given a single oral dose of known tumour initiator (DMBA or B(a)P) (some animals received solvent [presumably PEG] only or no initiation treatment) followed by weekly oral	Some evidence of carcinogenicity. Increase in number of animals with benign forestomach tumours after lime oil treatment without initiation (2/21 with solvent only as initiation treatment, 2/22 with no	Field & Roe, 1965

	dosing with "undiluted" lime oil (or no promotion treatment) for 40 weeks. Animals kept for "natural lifespan".	initiation treatment, 0/18 with no initiation or promotion treatment). Some evidence of tumour promotion. Increase in number of malignant forestomach tumours after lime oil treatment (0, 1 & 4 in each group that received known initiator plus lime oil; 0 in other groups). No obvious lime oil-treatment effect on the incidence of tumours at other sites.	
Mouse (31-37 animals (approx. 25% female) per group)	Experiment 3: Animals given a single oral dose of known tumour initiator (urethan) (some animals received distilled water only or no initiation treatment) followed by weekly oral dosing with "undiluted" lime oil (or no promotion treatment) for 40 weeks.	No convincing evidence of carcinogenicity. Benign forestomach papillomas occurred in 1/34 untreated mice and 2/32 mice given lime oil but no initiator. Evidence of tumour promotion. Increase in number of animals with benign forestomach papillomas in animals initiated with urethane (7/31 with lime oil; 1/37 without).	Field & Roe, 1965
Mouse (29-39 animals (approx. 50% female) per group)	Experiment 4: Animals given a single dose of known tumour initiator (B(a)P) and 3 weeks later fed lime oil in the diet at up to 32 ml/kg diet [equivalent to approx. 4.2 g/kg bw per day]. Animals killed for necropsy 42 weeks after start of study.	Evidence of carcinogenicity. Increase in number of animals with benign forestomach tumours in animals that received lime oil alone (control: 0/36; 0.26 g/kg bw: 1/36; 1 g/kg bw: 2/29; 4.2 g/kg bw: 3/39). Some evidence of tumour promotion. Increase in number of animals with benign forestomach tumours in animals initiated with urethane (control: 13/37; 0.26 g/kg bw: 16/35; 1 g/kg bw: 17/30; 4.2 g/kg bw: 18/38). There was also 1 carcinoma in each of these 4 groups.	Field & Roe, 1965
Mouse (20 females per group)	Animals given a single oral dose of known tumour initiator (DMBA or 3,4-BP) (some animals received solvent (PEG) only or no initiation treatment) followed by weekly oral dosing with 0.05 ml undiluted lime oil for 40	Some evidence of carcinogenicity. Increase in number of animals with tumours after lime oil treatment without initiation (3/11 with solvent only as initiation treatment, 2/8 with no initiation treatment, 0/9 with no initiation or lime oil treatment).	Peirce, 1961

	weeks. Half the surviving animals killed on day 280. (The remainder were kept for their "natural lifespan" [results not reported].)	Some evidence of tumour promotion. Increase in number of animals with forestomach tumours in animals initiated with DMBA (control: 8/10; lime oil: 9/9), 200 µg 3,4-BP (control: 5/11; lime oil: 10/11), 50 µg 3,4-BP (control: 0/9; lime oil: 8/9) or 12.5 µg 3,4-BP (control: 2/10; lime oil: 4/9). In the group treated with 200 µg 3,4-BP and lime oil, there was also 1 squamous carcinoma.	
Mouse (20 males & females per group)	Following treatment with a known tumour initiator, animals were treated weekly for 12 weeks with 0.25 ml lime oil (expressed from fruit peel) applied to the skin.	Evidence of tumour promotion. Papillomas developed in 10 of 19 survivors (the first one appeared in the 5th week) compared with 1 in control animals.	Roe & Peirce, 1960

Route/Organism	Dose	Effect	Reference
oral, mouse	TDLo: 67 gm/kg/39W-I	TUMORIGENIC: Equivocal tumorigenic agent by RTECS criteria; GASTROINTESTINAL: Tumors;	Journal of the National Cancer Institute. (Washington, DC) V.1-60, 1940-78. For publisher information, see JJIND8. 35,771,1965

As taken from RTECS, 1997

5.7. Irritation/immunotoxicity

Applied full strength to rabbit skin the material was slightly irritating (Hart, 1971). (No specified CAS number).

Tested at 15% it was not irritating to humans (Kligman, 1971). (No specified CAS number).

Skin and respiratory tract sensitization

In a maximization test, a 15% concentration did not sensitise any of the volunteers (Kligman, 1971) (No specified CAS number).

Route/Organism	Dose	Effect	Reference
administration onto the skin, rabbit	500 mg/24H	mild irritant effect	Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. 12,729,1974

As taken from RTECS, 1997

5.8. All other relevant types of toxicity

Database searches indicate that phototoxicity might be an issue with expressed lime oil. Internet searches identified several references, from flavour companies, to an IFRA recommended restriction on the content of lime oil (possibly a maximum content of 0.7%) in certain products intended for skin

application. It is understood that an EC Committee (the SCCPNFP) has considered this issue and might have made a similar recommendation, but this could not be confirmed via official websites. It may be worth following up in order to establish the exact position (No specified CAS number).

“Lime peels are mainly obtained from the byproducts of the juice manufacturing industry, which we obtained and used to extract essential oil (2.3%) in order to examine the antioxidant and hypolipidaemic effects. We identified 60 volatile compounds of lime essential oil (LEO) with GC/MS, of which the predominant constituents were limonene, γ-terpinene, and β-pinene. Lime essential oil was measured according to the DPPH assay and ABTS assay, with IC50 values of 2.36 mg/mL and 0.26 mg/mL, respectively. This study also explored the protective effects of LEO against lipid-induced hyperlipidemia in a rat model. Two groups of rats received oral LEO in doses of 0.74 g/100 g and 2.23 g/100 g with their diets. Eight weeks later, we found that the administration of LEO improved the serum total cholesterol, triglyceride, low-density lipoprotein cholesterol, alanine aminotransferase, and aspartate transaminase levels in the hyperlipidemic rats ($p < 0.05$). Simultaneously, the LEO improved the health of the rats in terms of obesity, atherogenic index, and fatty liver.” As taken from Lin LY et al. 2019. Foods 8(9), E398. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/31500259

6. Functional effects on

6.1. Broncho/pulmonary system

No data available to us at this time.

6.2. Cardiovascular system

“Citrus aurantifolia leaf essential oil was extracted via hydrodistillation, chemical composition of the oil was analyzed using gas chromatography-mass spectrometry and its antidiabetic potentials was assessed in alloxan-induced hyperglycaemic rats using metformin as the reference drug for comparison. Chemical analysis showed that D-limonene (57.84%) was the major constituent of the oil. Other notable compounds identified were neral (7.81%), linalool (4.75%), sulcatone (3.48%) and isogeraniol (3.48%). Intraperitoneal administration of C. aurantifolia oil (100 mg/Kg b.wt.) to hyperglycaemic rats for 14 days caused significant reduction in fasting blood and hepatic glucose, whereas hepatic concentration of glycogen was significantly increased. Also, improvement in dyslipidaemia was observed in C. aurantifolia essential oil-treated hyperglycaemic rats; serum concentration of total cholesterol, triacylglycerol and low density lipoprotein-cholesterol were significantly reduced and high density lipoprotein-cholesterol was increased, resulting in decreased predisposition of rats to cardiac risks. Antihyperglycaemic potential of administration of the oil was lower but compared favourably with the oral antihyperglycaemic agent used as reference antidiabetic drug. Overall, data from this study showed that essential oil from the leaf of C. aurantifolia grown in North-Central Nigeria is a D-limonene chemotype. The oil showed considerable glucose lowering effect as well as the potential to ameliorate hyperglycaemia-induced dyslipidaemic complications in alloxanized rats.” As taken from Ibrahim FA et al. 2019. Drug Res. (Stuttg.) 69(4), 201-206. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30273946

6.3. Nervous system

No data available to us at this time.

6.4. Other organ systems, dependent on the properties of the substance

No data available to us at this time.

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. Burnt ingredient toxicity

This ingredient was considered as part of an overall safety assessment of ingredients added to tobacco in the manufacture of cigarettes. An expert panel of toxicologists reviewed the open literature and internal toxicology data of 5 tobacco companies to evaluate a composite list of ingredients used in the manufacture of cigarettes. The conclusion of this report was that these ingredients did not increase the inherent biological activity of tobacco cigarettes, and are considered to be acceptable under conditions of intended use (Doull et al., 1994 & 1998).

Tobacco smoke condensates from cigarettes containing Lime oil (and other extractables) and an additive free, reference cigarettes were tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the condensate was not changed by the addition of Lime oil (and other extractables). Table below provides tested level(s) and specific endpoint(s)

Endpoint	**Tested level (ppm)**	**Reference**
Smoke chemistry	10 (oil; 8008-26-2)	Carmines, 2002 & Rustemeier et al., 2002
	12 (8008-26-2)	Baker et al., 2004a
	0.13 (8008-26-2) 39 (oil expressed; 8008-26-2)	JTI KB Study Report(s)
	1,180 (8008-26-2)	Gaworski et al., 2011 & Coggins et al., 2011b
In vitro genotoxicity	10 (oil; 8008-26-2)	Carmines, 2002 & Roemer et al., 2002
	12 (8008-26-2)	Baker et al., 2004b
	0.13 (8008-26-2)	Renne et al., 2006
	0.13 (8008-26-2) 39 (oil expressed; 8008-26-2)	JTI KB Study Report(s)
	1 (90063-52-8)	fGLH Study Report (2010)
	1,180 (8008-26-2)	Gaworski et al., 2011 & Coggins et al., 2011b
In vitro cytotoxicity	10 (oil; 8008-26-2)	Carmines, 2002 & Roemer et al., 2002
	12 (8008-26-2)	Baker et al., 2004b
	0.13 (8008-26-2) 39 (oil expressed; 8008-26-2)	JTI KB Study Report(s)
	1 (90063-52-8)	fGLH Study Report (2010)
	1,180 (8008-26-2)	Gaworski et al., 2011 & Coggins et al., 2011b

Inhalation study	10 (oil; 8008-26-2)	Carmines, 2002 & Vanscheeuwijck et al., 2002
	12 (8008-26-2)	Baker et al., 2004b
	0.13 (8008-26-2)	Renne et al., 2006
	0.13 (8008-26-2) 39 (oil expressed; 8008-26-2)	JTI KB Study Report(s)
Skin painting	0.13 (8008-26-2) 39 (oil expressed; 8008-26-2)	JTI KB Study Report(s)

9. Heated/vapor emissions toxicity

Aerosol from an electronic nicotine delivery system (ENDS) that creates a vapor by heating an e-liquid containing Lime oil (and other extractables) was tested in a battery of in vitro and/or in vivo test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) and no adverse findings from a 90-day in vivo repeat-dose inhalation toxicity study were observed after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to those observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic and adverse effects upon exposure. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	**Tested level (ppm)**	**Reference**
Aerosol chemistry	1,050	Logic (2019) Labstat International Inc. (2021)
In vitro genotoxicity	1,050	Logic (2019) Labstat International Inc. (2022)
In vitro cytotoxicity	1,050	Logic (2019) Labstat International Inc. (2022)
In vivo genotoxicity	50	Logic (2019)
Inhalation study	50	Logic (2019)

“The thermal decomposition of three essential oils has been studied at 300 °C, using a 9% oxygen in nitrogen atmosphere, to mimic the thermal environment of flavours under low-temperature tobacco heating conditions. The starting compositions of the lime, bergamot and cardamom oils were determined by gas chromatography/mass spectrometry (GC/MS). The thermo-oxidative decomposition was evaluated by applying on-line pyrolysis-GC/MS. The main constituents of the oils studied were cyclic and linear monoterpenoids; however, the relative intensities of these components were characteristically different between oils. Lime oil was dominated by monoterpene hydrocarbons, while the other citrus oil, bergamot oil contained in addition a significant number of esters and alcohols. Oxygen-containing monoterpenoids were the dominant constituents of cardamom oil. The relative proportion of the constituents of all three essential oil samples significantly altered during oxidative pyrolysis at 300 °C. The strained rings of bicyclic monoterpenes (pinenes, sabinene, and thujene) underwent scission, resulting in the formation of monocyclic monoterpenes (limonene etc.). Both linear and cyclic terpene acetates decomposed via elimination of acetic acid, so linalyl acetate produced myrcene and ocimene, while terpinyl acetate formed mostly limonene and terpinolene. The relative intensities of linalool and eucalyptol were reduced during pyrolysis, which can be explained by dehydration reactions resulting in the formation of myrcene and ocimene, or limonene and terpinolene, respectively. The chemical reactions that occurred were explained by bond splitting and

intramolecular rearrangement mechanisms, with oxygen playing a role in the initiation processes." As taken from Jakab E et al. 2018. Journal of Analytical and Applied Pyrolysis 134, 552-561. Available at https://www.sciencedirect.com/science/article/pii/S0165237018304194

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oils, lime are not persistent in the environment.

Data accessed January 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List state that oils, lime are not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	5.132
Comment iT	Group: individual; Subgroup: Other oils (individual)
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	9.392
Toxicity to fish (LC50 in mg/l) as predicted by Oasis Forecast M v1.10	5.132
Toxicity to fish (LC50 in mg/l) as predicted by PNN	17.03306
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	7.997
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	3.39E-002

Data accessed January 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

Record for lime oil:

Flowers, Trees, Shrubs, Ferns								
Abutilon theophrasti Butter Print	SD 10 d	CUL LAB	FU	SL		REP GERM		NC 1 ml
Elymus repens Quackgrass	SD 10 d	CUL LAB	FU	SL		REP GERM		NC 0.5 ml
Amaranthus retroflexus Green Amaranth	SD 10 d	CUL LAB	FU	SL		REP GERM		NC 1 ml
Ambrosia artemisiifolia Common Ragweed	SD 10 d	CUL LAB	FU	SL		REP GERM		NC 0.5 ml

Chenopodium album Lamb's-Quarters	SD 10 d	CUL LAB	FU	SL		REP GERM		NC 0.5 ml
Datura stramonium Jimsonweed	SD 10 d	CUL LAB	FU	SL		REP GERM		NC 1 ml
Ipomoea purpurea Common Morning-Glory	SD 10 d	CUL LAB	FU	SL		REP GERM		NC 0.5 ml
Plantago lanceolata Narrowleaf Plantain	SD 10 d	CUL LAB	FU	SL		REP GERM		NC 0.5 ml
Polygonum pensylvanicum Smartweed	SD 10 d	CUL LAB	FU	SL		REP GERM		NC 1 ml
Rumex acetosella Field Sorrel	SD 10 d	CUL LAB	FU	SL		REP GERM		NC 0.5 ml
Rumex crispus Curley Dock	SD 10 d	CUL LAB	FU	SL		REP GERM		NC 1
Setaria faberi Giant Foxtail	SD 10 d	CUL LAB	FU	SL		REP GERM		NC 1 ml
Setaria viridis Green Foxtail	SD 10 d	CUL LAB	FU	SL		REP GERM		NC 0.5 ml
Sorghum halepense Johnson Grass	SD 10 d	CUL LAB	FU	SL		REP GERM		NC 1 ml

10.5. All other relevant types of ecotoxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oils, lime are not bioaccumulative in the environment.

Data accessed Janaury 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

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Cosmetic Ingredient Review

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ABSTRACT

The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) assessed the safety of 30 *Citrus* plant- and seed-derived ingredients, which are most frequently reported to function in cosmetics as fragrances and/or skin conditioning agents. Because final product formulations may contain multiple botanicals, each containing similar constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. With *Citrus* plant- and seed-derived ingredients, the Panel was concerned about the presence of the hydroperoxides of limonene and linalool in cosmetics. Industry should use good manufacturing practices to limit impurities. The Panel reviewed the available data presented and concluded that 18 of these ingredients are safe in the present practices of use and concentration when formulated to be non-irritating and non-sensitizing. The data for the remaining 12 ingredients are insufficient to determine safety.

INTRODUCTION

This report assesses the safety of the 30 *Citrus* plant- and seed-derived ingredients listed below, which are reported in the *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*) to mainly function as skin conditioning agents-miscellaneous in cosmetic products (Table 1).[1] Citrus Aurantium (Bitter Orange) Oil is not currently listed in the *Dictionary*, but has been included in this report because of its high reported number of uses in the Food and Drug Administration (FDA) Voluntary Cosmetic Registration Program (VCRP) database and presumed similarities to the other ingredients in this report. Two ingredients (Citrus Sunki Seed Extract and Citrus Sunki Seed Oil) are reported to function as skin bleaching agents; use as a skin bleaching agent is classified as a drug use and, as such, does not fall under the purview of CIR.

Citrus Aurantifolia (Lime) Oil
Citrus Aurantium (Bitter Orange) Oil
Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Extract
Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil
Citrus Aurantium Dulcis (Orange) Flower/Leaf/Stem Powder
Citrus Aurantium Dulcis (Orange) Oil
Citrus Aurantium Dulcis (Orange) Seed Extract
Citrus Aurantium Sinensis Powder
Citrus Australasica Seed Oil
Citrus Depressa Seed Oil
Citrus Glauca Seed Oil
Citrus Grandis (Grapefruit)
Citrus Grandis (Grapefruit) Extract
Citrus Grandis Peel/Seed Extract
Citrus Grandis (Grapefruit) Seed Extract
Citrus Iyo Oil
Citrus Junos Extract
Citrus Junos Seed Extract
Citrus Junos Seed Oil
Citrus Limon (Lemon) Flower/Leaf/Stem Extract
Citrus Limon (Lemon) Flower/Leaf/Stem Oil
Citrus Limon (Lemon) Leaf/Peel/Stem Oil
Citrus Nobilis (Mandarin Orange)
Citrus Nobilis (Mandarin Orange) Oil
Citrus Nobilis (Mandarin Orange) Water
Citrus Paradisi (Grapefruit) Seed Extract
Citrus Sunki Seed Extract
Citrus Sunki Seed Oil
Citrus Reticulata (Tangerine) Extract
Citrus Unshiu Extract

The Panel previously reviewed the safety of *Citrus*-derived peel oils, *Citrus* peel-derived ingredients, and *Citrus* fruit-derived ingredients in separate assessments and concluded that 14 *Citrus*-derived peel oil, 47 *Citrus* peel-derived ingredients, and 80 *Citrus* fruit-derived ingredients are safe for use in both rinse-off and leave-on cosmetic products when formulated to be non-sensitizing and non-irritating, provided that leave-on products do not contain more than 0.0015% (15 ppm) 5-methoxypsoralen (5-MOP).[2-4] The Panel also determined that 33 *Citrus* flower- and leaf-derived ingredients are safe in the present practices of use and concentration when formulated to be non-irritating and non-sensitizing.[6] The Panel has also reviewed the safety of Citrus Aurantifolia (Lime) Seed Oil, Citrus Aurantifolia (Lime) Seed Oil Unsaponifiables, Citrus Aurantium Dulcis (Orange) Seed Oil, Citrus Aurantium Dulcis (Orange) Seed Oil Unsaponifiables, Citrus Grandis (Grapefruit) Seed Oil, Citrus Grandis (Grapefruit) Seed Oil Unsaponifiables, Citrus Limon (Lemon) Seed Oil, and Citrus Paradisi (Grapefruit) Seed Oil, and concluded that these ingredients are safe in the present practices of use and concentration as described in the safety assessment of plant-derived fatty acid oils.[5]

To avoid redundancy of effort, CIR generally excludes from review ingredients that are known to function exclusively as fragrance ingredients when the ingredient has been or will be evaluated by the Research Institute for Fragrance Materials (RIFM). According to the *Dictionary*, three of the *Citrus* plant- and seed-derived ingredients in this report are reported to function exclusively as fragrance ingredients (see Table 2).[1] However, personal communications with RIFM in March 2015 revealed that these ingredients have neither been assessed for safety by

the RIFM Expert Panel, nor are these ingredients on RIFM's prioritized agenda to be reviewed in the foreseeable future. Thus CIR is reviewing the safety of these ingredients as part of this current assessment.

Botanical ingredients are composed of numerous constituents, some of which have the potential to cause toxic effects. In this assessment, CIR is reviewing the potential toxicity of each *Citrus* plant- or seed-derived ingredient as a whole, complex substance. Except for specific constituents of concern that the Panel has identified, CIR is not reviewing the potential toxicity of the individual constituents of the *Citrus* plants and seeds from which the ingredients in this report are derived.

Note: In many of the published studies included in this assessment, the information provided is not sufficient to determine how well the substance being tested represents the cosmetic ingredient. In this safety assessment, if a substance tested in a study is not clearly a cosmetic ingredient, because of lack of information on the genus and species from which the substance was derived and/or the method of extraction used, the test substance will be referred to by a common name (e.g. lemon extract). If the substance is clearly a cosmetic ingredient, the International Nomenclature of Cosmetic Ingredients (INCI) name will be used (e.g. "Citrus Limon (Lemon) Extract"). Additionally, some inconsistencies were noted in both taxonomic and INCI naming conventions. For example, this report includes the sweet orange ingredient described as Citrus Aurantium Dulcis (Orange) in the *Dictionary*.[1] In contrast, most of the published literature and the FDA VCRP refer to this ingredient as Citrus Sinensis (Sweet Orange). Another example of a naming inconsistency is Citrus Grandis (Grapefruit); *Citrus grandis* is generally considered a name for a pomelo, which may also be referred to as *Citrus maxima*. *Citrus paradisi* appears to be the more widely accepted nomenclature for grapefruit. Finally, Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil is also known as petitgrain bigarade oil. The INCI Committee of the Personal Care Products Council (Council) is working to correct some of these inconsistencies. The genus and species names associated with the ingredient names designated by the INCI Committee are listed in Table 3.[7]

CHEMISTRY

Definition and General Characterization

The definitions and functions of the *Citrus* plant- and seed-derived ingredients included in this report are provided in Table 1. The definition indicates what part(s) of the plant from which an ingredient is obtained. In some cases, the definition provides insight on the method(s) of manufacture.

According to the *Dictionary*, essential oils and waters are prepared from leaves, stems, flowers, bark, roots, or other parts of a plant or the whole plant.[1] Essential oils are prepared by a number of processes including, but not limited to, steam or dry distillation, flash pasteurization and mechanical processes such as cold-pressing; however, the most widely used method for preparing essential oils from plants is steam distillation. The condensate from steam distillation produces two distinct fractions that contain the volatile ingredients from the plant. The water insoluble fraction contains the "oil." The water soluble fraction contains constituents of the plant that are dissolved in water. The name assigned to the water insoluble fraction from steam distilled plant materials includes the term "oil" in the INCI name. The water soluble fraction from the steam distilled plant material includes the term "water" in the INCI name.

Essential oils are the hydrophobic, liquid, volatile aromatic compounds in the insoluble condensate fraction, and typically are small molecules, but their chemical structures can vary widely. Fixed oils, on the other hand, are hydrophobic, nonvolatile, fatty compounds from plants (including *Citrus* seeds), animals or algae. These are primarily composed of glycerides and, to some extent, free fatty acids. Constituents of these *Citrus*-derived ingredients may include both oil types. The volatile nature of essential oils makes them more likely to be useful as fragrances, but use as fragrances is not their only reported function.

Physical and Chemical Properties

Citrus Australasica Seed Oil

Citrus Australasica Seed Oil is reported to be a straw/yellow colored liquid with a refractive index of 1.476 (specification range 1.450-1.490 at 20º C) and a specific gravity of 0.917 (specification range 0.900-0.940 at 20º C).[8]

Citrus Glauca Seed Oil

According to a supplier, Citrus Glauca Seed Oil is a light brown to dark brown liquid.[9] At 20º C, the refractive index is 1.472 (specification range 1.450-1.490) and the specific gravity is 0.921 (specification range 0.900-0.940).

Method of Manufacturing

Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil

According to the *Food Chemicals Codex*, "petitgrain oil, Paraguay type" is a volatile oil obtained by steam distillation from the leaves and small twigs of the bitter orange tree, *Citrus aurantium* L. subspecies *amara*.[10]

Citrus Junos Seed Extract

A supplier has reported that Citrus Junos Seed Extract is produced by extracting dried seeds with 90% ethanolic solution, which is then filtered.[11] The material then undergoes sedimentation, filtration, and adjustment before packaging.

Citrus Paradisi (Grapefruit) Seed Extract

A supplier reported that Citrus Paradisi (Grapefruit) Seed Extract is manufactured by first grinding grapefruit seeds and then extracting in a mix of water and glycerin.[12] The mixture is then clarified and decontaminated by heat.

Constituents/Composition

The *Citrus* ingredients are complex botanicals composed of numerous constituents. Table 4 lists the fatty acid profiles for Citrus seed-derived oils that were previously reviewed in the safety assessment of plant-derived fatty acid oils.[5] The major fatty acid components in Citrus seed-derived oils are palmitic acid, oleic acid, and linoleic acids.

The International Fragrance Association (IFRA) has issued standards for limonene and linalool in natural products, stating that these constituents "should only be used when the level of peroxides is kept to the lowest practical level, for instance by adding antioxidants at the time of production."[13,14]

Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil

According to the Food Chemicals Codex, "petitgrain oil, Paraguay type" contains not less than 45.0% and not more than 60% esters calculated as linalyl acetate.[10] A fragrance raw materials monograph lists the components of petitgrain bigarade oil as α-pinene, β-pinene, sabinene, myrcene, limonene, cis-β-ocimene, trans-β-ocimene, linalool, linalyl acetate, terpineol-4, β-caryophyllene, α-terpineol, neryl acetate, geranyl acetate, nerol, geraniol, and nerolidol.[15] A breakdown of the key constituents of Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil is detailed in Table 5.

Citrus Australasica Seed Oil and Citrus Glauca Seed Oil

The fatty acid profiles for Citrus Australasica Seed Oil and Citrus Glauca Seed Oil are listed in Table 6.

Citrus Junos Seed Extract

A supplier reports that Citrus Junos Seed Extract is composed of saponin and sugar.[11] The concentrations of heavy metal impurities are not more than 20 ppm and the concentration of arsenic is not more than 2 ppm.

The fatty acid profile for *Citrus junos* Sieb. ex Tanaka is also listed in Table 6.

Citrus Paradisi (Grapefruit) Seed Extract

A supplier reported that a trade name material contains 67.0% to 73.0% glycerin, 26.0% to 32.8% water, and 0.2% to 1.0% Citrus Paradisi (Grapefruit) Seed Extract.[12]

Citrus Sinensis

In gas chromatography and gas chromatography-mass spectroscopy analysis of the essential oils from the leaves and green branches of Egyptian navel orange trees (*Citrus sinensis* (L.) Osbeck var. Malesy), 33 and 24 compounds were identified for the leaves and branches, respectively.[16] These compounds made up 96.0% and 97.9%, respectively, of the total detected constituents. The major constituents were sabinene (36.5% leaves, 33.0% branches), terpinen-4-ol (8.2% leaves, 6.2% branches), δ-3-carene (7.0% leaves, 9.4% branches), limonene (6.8% leaves, 18.7% branches), trans-ocimene (6.7% leaves, 6.1% branches), and β-myrcene (4.5% leaves, 9.4% branches).

The composition of samples of dehulled sweet orange (Citrus *sinensis*) seed flour (dry weight) was reported to be 54.2% fat, 28.5% carbohydrate, 5.5% crude fiber, 3.1% crude protein, and 2.5% ash.[17] Mineral analysis showed high levels of calcium and potassium.

USE

Cosmetic

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the FDA and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's VCRP database. Use concentration data are submitted by Industry in response to surveys, conducted by the Council, of maximum reported use concentrations by product category.

According to 2016 VCRP data, Citrus Aurantium (Bitter Orange) Oil has the most reported uses of the cosmetic ingredients in this report, with a total of 295; more than half are in leave-on skin care preparations (Table 7).[18] This ingredient is not currently in the *Dictionary*, but has been included in this report because of the number of uses and presumed similarities to the other ingredients in this report. The ingredients with the next highest frequency of use are Citrus Aurantifolia (Lime) Oil (169 total uses) and Citrus Grandis (Grapefruit) Seed Extract (144 total uses); a majority of the uses for these ingredients are in leave-on skin care preparations. The results of the concentration of use survey indicate Citrus Aurantium Dulcis (Orange) Oil has the highest reported maximum concentration of use; it is used at up to 1% in a body and hand formulation.[19] Citrus Aurantifolia (Lime) Oil had the second highest reported maximum concentration of use; it is used at up to 0.36% in a lipstick.

In some cases, reports of uses were received from the VCRP, but no concentration of use data were provided. For example, Citrus Limon (Lemon) Flower/Leaf/Stem Extract is reported to be used in 8 formulations, but no use concentration data were available. In other cases, no uses were reported to the VCRP, but a maximum use concentration was provided in the industry survey. For example, Citrus Junos Seed Oil was not reported in the VCRP database, but the industry survey indicated that it is used in face and neck and body and hand formulations at up to 0.1%. It is presumed that Citrus Junos Seed Oil is used in at least one cosmetic formulation.

Table 8 lists all *Citrus* plant- and seed-derived ingredients not currently in use based on the VCRP data or the results of the Council concentration of use survey.

Some of these ingredients may be used in products that can come into contact with the eye or mucous membranes. For example, Citrus Aurantifolia (Lime) Oil is used in a lipstick at up to 0.36%. Additionally, some of these ingredients were reported to be used in hair sprays, fragrance preparations, face powder and body powders, spray deodorants, and spray skin care preparations and could possibly be inhaled. For example, Citrus Aurantifolia (Lime) Oil was reported to be used in body and hand sprays at a maximum concentration of 0.12% and Citrus Junos Seed Oil was reported to be used in face powders at up to 0.1%. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 μm, with propellant sprays yielding a greater fraction of droplets/particles below 10 μm compared with pump sprays.[20-23] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[21,22] There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.[22] However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[24-26]

The *Citrus* ingredients described in this safety assessment are not restricted from use in any way under the rules governing cosmetic products in the European Union (EU). However, furocoumarins are prohibited from use in cosmetics, except for normal content in natural essences and in sun protection and bronzing products where the content shall be below 1 mg/kg.[27]

Non-Cosmetic

Petitgrain bigarade oil (Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil) is generally recognized as safe (GRAS) for intended use in foods for human consumption (21CFR182.20) and in animal drugs, feeds, and related products (21CFR582.20).

TOXICOKINETICS

No relevant published toxicokinetics studies on *Citrus* plant- and seed-derived ingredients were identified in a literature search for these ingredients, and no unpublished data were submitted; toxicokinetics data are not expected to be found because botanical ingredients are mixtures of hundreds of constituents.

TOXICOLOGICAL STUDIES

Acute Toxicity

Dermal – Animal

Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil

The dermal LD_{50} of Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil (described as petitgrain bigarade oil) was reported to be greater than 2 g/kg in rabbits; however, only 2 rabbits were used in the study.[15] An occlusive patch of undiluted test material was applied for 24 h.

Repeated Dose Toxicity

No relevant published repeated dose toxicity studies on *Citrus* plant- and seed-derived ingredients were identified in a literature search for these ingredients, and no unpublished data were submitted.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No relevant published reproductive and developmental studies on *Citrus* plant- and seed-derived ingredients were identified in a literature search for these ingredients, and no unpublished data were submitted.

GENOTOXICITY

No relevant published genotoxicity studies on *Citrus* plant- and seed-derived ingredients were identified in a literature search for these ingredients, and no unpublished data were submitted.

CARCINOGENICITY

No relevant published carcinogenicity studies on *Citrus* plant- and seed-derived ingredients were identified in a literature search for these ingredients, and no unpublished data were submitted.

IRRITATION AND SENSITIZATION

Dermal Irritation

Dermal irritation studies are summarized in Table 9.[15,28,29] In rabbit studies, slight erythema was observed after exposure to 2g/kg Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil (described as petitgrain bigarade oil). In human subjects, no irritation was observed after topical exposure to petitgrain bigarade oil (up to 8% in petrolatum) or Citrus Grandis (Grapefruit) Seed Extract (0.15% in formulation).

Dermal Sensitization

Dermal sensitization studies are presented in Table 10.[15,30] Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil (described as petitgrain bigarade oil) at up to 8% in petrolatum was not sensitizing in humans.

Photosensitization

Photosensitization studies are presented in Table 11.[31] Undiluted Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil (described as petitgrain bigarade oil) was not photosensitizing in tests with hairless mice or miniature swine.

CLINICAL STUDIES

No relevant published clinical studies on *Citrus* plant- and seed-derived ingredients were identified in a literature search for these ingredients, and no unpublished data were submitted.

SUMMARY

The 30 *Citrus* plant- and seed-derived ingredients described in this report function primarily as skin conditioning agents-miscellaneous. Botanical ingredients are composed of hundreds of constituents, some of which

have the potential to cause toxic effects. Presently, CIR reviewed the information available on the potential toxicity of each *Citrus* plant- and seed-derived ingredient as a whole, complex substance; CIR does not review the potential toxicity information on the individual constituents of which the *Citrus* plant- and seed-derived ingredients are comprised.

Citrus seed oils are fixed oils that are composed primarily of glycerides and, to some extent, free fatty acids, while the other *Citrus* oils in this safety assessment are essential oils that primarily contain volatile compounds. No composition information was found for ingredients defined as being derived from the whole plant.

Citrus Aurantium (Bitter Orange) Oil has the most reported uses of the ingredients in this report in cosmetic products, with a total of 295; more than half of the uses are in leave-on skin care preparations. This ingredient is not currently in the *Dictionary* but has been included in this report because of its high reported number of uses and presumed similarities to the other ingredients in this report. The ingredients with the next highest frequency of use are Citrus Aurantifolia (Lime) Oil (169 total uses) and Citrus Grandis (Grapefruit) Seed Extract (144 total uses); a majority of the uses for these ingredients are in leave-on skin care preparations. The results of the concentration of use survey indicate Citrus Aurantium Dulcis (Orange) Oil has the highest reported maximum concentration of use; it is used at up to 1% in a body and hand formulation. Citrus Aurantifolia (Lime) Oil had the second highest reported maximum concentration of use; it is used at up to 0.36% in a lipstick.

The *Citrus* ingredients described in this safety assessment are not restricted from use in any way under the rules governing cosmetic products in the European Union (EU); however, furocoumarins are prohibited from use in cosmetics except for normal content in natural essences and in sun protection and bronzing products where the content shall be < 1 mg/kg.

Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil is considered GRAS in foods for human consumption and in animal drugs, feed, and related products.

The dermal LD_{50} of Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil (described as petitgrain bigarade oil) was reported as greater than 2 g/kg in rabbits.

In rabbit dermal irritation studies, slight erythema was observed after exposure to unreported concentrations of Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil (described as petitgrain bigarade oil). In human subjects, no irritation was observed after topical exposure to petitgrain bigarade oil (up to 8%).

Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil (described as petitgrain bigarade oil) at up to 8% was not sensitizing in humans and undiluted petitgrain bigarade oil was not photosensitizing in tests with hairless mice or miniature swine.

No relevant published studies on the toxicokinetics, repeated dose toxicity, reproductive and development toxicity, carcinogenicity, genotoxicity, or clinical assessments of *Citrus* plant- and seed-derived ingredients were discovered and no unpublished data were submitted to address these topics.

DISCUSSION

During its review of *Citrus* plant- and seed-derived ingredients, the Panel noted that, because botanical ingredients are complex mixtures, there is concern that multiple botanical ingredients may each contribute to the final concentration of a single constituent. Therefore, when formulating products, manufacturers should avoid reaching levels in final formulation of botanical constituents that may cause sensitization or other adverse effects. Specific examples of constituents that could induce adverse effects include the hydroperoxides of limonene and linalool.

The issue of incidental inhalation exposure from hair sprays, fragrance preparations, face powder and body powders, spray deodorants, and spray skin care preparations was discussed by the Panel. There were no inhalation toxicity data available. The Panel noted that droplets/particles from cosmetic products would not be respirable to any appreciable amount. The potential for inhalation toxicity is not limited to respirable droplets/particles deposited in the lungs. In principle, inhaled droplets/particles deposited in the nasopharyngeal and thoracic regions of the respiratory tract may cause toxic effects depending on their chemical and other properties. However, coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at http://www.cir-safety.org/cir-findings.

The Panel also expressed concern about pesticide residues, heavy metals, and other plant species that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit impurities.

The Panel determined that the composition data on the *Citrus* seed-derived ingredients found in this report were sufficient and no individual component of the seeds yielded any toxicological concern. The Panel also considered the composition, GRAS status, and safety test data on Citrus Aurantium Amara (Bitter Orange) Twig/Leaf Oil to be sufficient to support the safety of the use of this ingredient and the extract in cosmetics. The Panel determined that the conclusion of safe with the listed qualifications could be extended to Citrus Grandis (Grapefruit) Extract, Citrus Junos Extract, Citrus Nobilis (Mandarin Orange), Citrus Nobilis (Mandarin Orange) Oil, and Citrus Reticualta (Tangerine) Extract because these ingredients are largely used in rinse-off formulations at very low concentrations. However, the Panel concluded that the data are insufficient to make a conclusion on the safety of 12 *Citrus* plant-derived ingredients found in this safety assessment. The data that are needed to properly evaluate the safety of these ingredients are:

- Method of manufacturing
- Chemical composition and impurities
- Irritation and sensitization data
- If the composition data for these *Citrus* plant-derived ingredients are substantially different from that of the *Citrus* peel-, flower-, leaf- and seed-derived ingredients, then studies of systemic endpoints such as a 28-day dermal toxicity, reproductive and developmental toxicity, and genotoxicity are needed, as well as UV absorption spectra.

CONCLUSION

The CIR Expert Panel concluded that the following 18 ingredients are safe in the present practices of use and concentration when formulated to be non-irritating and non-sensitizing.

Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Extract*
Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil
Citrus Aurantium Dulcis (Orange) Seed Extract
Citrus Australasica Seed Oil*
Citrus Depressa Seed Oil*
Citrus Glauca Seed Oil*
Citrus Grandis (Grapefruit) Extract
Citrus Grandis Peel/Seed Extract*
Citrus Grandis (Grapefruit) Seed Extract
Citrus Junos Extract
Citrus Junos Seed Extract
Citrus Junos Seed Oil
Citrus Nobilis (Mandarin Orange)
Citrus Nobilis (Mandarin Orange) Oil
Citrus Paradisi (Grapefruit) Seed Extract
Citrus Reticulata (Tangerine) Extract
Citrus Sunki Seed Extract*
Citrus Sunki Seed Oil*

The Panel concluded the data on the remaining 12 ingredients listed below are insufficient to determine safety.

Citrus Aurantifolia (Lime) Oil
Citrus Aurantium (Bitter Orange) Oil
Citrus Aurantium Dulcis (Orange) Flower/Leaf/Stem Powder*
Citrus Aurantium Dulcis (Orange) Oil
Citrus Aurantium Sinensis Powder
Citrus Grandis (Grapefruit)*
Citrus Iyo Oil*
Citrus Limon (Lemon) Flower/Leaf/Stem Extract
Citrus Limon (Lemon) Flower/Leaf/Stem Oil*
Citrus Limon (Lemon) Leaf/Peel/Stem Oil*
Citrus Nobilis (Mandarin Orange) Water*
Citrus Unshiu Extract*

*Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.

TABLES

Table 1. Definitions and functions of *Citrus* plant- and seed-derived ingredients.[1]

Ingredient	Definition*	Function
Citrus Aurantifolia (Lime) Oil CAS No. 8008-26-2	Citrus Aurantifolia (Lime) Oil is the volatile oil obtained from the whole plant, *Citrus aurantifolia*.	Fragrance Ingredients; Skin-Conditioning Agents - Miscellaneous
Citrus Aurantium (Bitter Orange) Oil	Not in *Dictionary*.	Not in *Dictionary*.
Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Extract CAS No. 72968-50-4; 8016-38-4	Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Extract is the extract of the leaves and twigs of *Citrus aurantium amara.*	Skin-Conditioning Agents - Miscellaneous
Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil	Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil is the volatile oil obtained from the leaves and twigs of *Citrus aurantium amara*.	Flavoring Agents; Fragrance Ingredients
Citrus Aurantium Dulcis (Orange) Flower/Leaf/Stem Powder	Citrus Aurantium Dulcis (Orange) Flower/Leaf/Stem Powder is the powder obtained from the dried, ground flowers, leaves and stems of *Citrus aurantium dulcis.*	Exfoliants
Citrus Aurantium Dulcis (Orange) Oil	Citrus Aurantium Dulcis (Orange) Oil is the volatile oil obtained from the whole plant, *Citrus aurantium dulcis*.	Fragrance Ingredients
Citrus Aurantium Dulcis (Orange) Seed Extract	Citrus Aurantium Dulcis (Orange) Seed Extract is the extract of the seeds of *Citrus aurantium dulcis.*	Skin-Conditioning Agents - Miscellaneous
Citrus Aurantium Sinensis Powder	Citrus Aurantium Sinensis Powder is the powder obtained the dried ground plant, *Citrus aurantium sinensis.*	Exfoliants
Citrus Australasica Seed Oil CAS No. 1174331-57-7 (generic)	Citrus Australasica Seed Oil is the fixed oil expressed from the seeds of *Citrus australasica*.	Antioxidants; Hair Conditioning Agents; Humectants; Skin-Conditioning Agents - Miscellaneous
Citrus Depressa Seed Oil	Citrus Depressa Seed Oil is the oil expressed from the seeds of *Citrus depressa.*	Skin-Conditioning Agents - Emollient
Citrus Glauca Seed Oil	Citrus Glauca Seed Oil is the oil expressed from the seeds of *Citrus glauca.*	Antioxidants; Humectants; Skin Protectants; Skin-Conditioning Agents - Emollient; Skin-Conditioning Agents - Humectant
Citrus Grandis (Grapefruit)	Citrus Grandis (Grapefruit) is a plant material derived from the whole plant, *Citrus grandis.*	Not reported
Citrus Grandis (Grapefruit) Extract	Citrus Grandis (Grapefruit) Extract is the extract of the whole plant, *Citrus grandis.*	Skin-Conditioning Agents - Miscellaneous
Citrus Grandis Peel/Seed Extract	Citrus Grandis Peel/Seed Extract is the extract of the peel and seeds of *Citrus grandis.*	Antifungal Agents; Antimicrobial Agents; Preservatives
Citrus Grandis (Grapefruit) Seed Extract	Citrus Grandis (Grapefruit) Seed Extract is the extract of the seeds of Citrus grandis.	Preservatives; Skin-Conditioning Agents - Miscellaneous
Citrus Iyo Oil	Citrus Iyo Oil is the oil expressed from the whole plant, *Citrus iyo*.	Skin-Conditioning Agents - Emollient
Citrus Junos Extract	Citrus Junos Extract is the extract of the whole plant, *Citrus junos.*	Antioxidants
Citrus Junos Seed Extract	Citrus Junos Seed Extract is the extract of the seeds of *Citrus junos*.	Antioxidants
Citrus Junos Seed Oil	Citrus Junos Seed Oil is the oil expressed from the seeds of *Citrus junos.*	Skin-Conditioning Agents - Emollient
Citrus Limon (Lemon) Flower/Leaf/Stem Extract CAS No. 84929-31-7; 85085-28-5	Citrus Limon (Lemon) Flower/Leaf/Stem Extract is the extract of the flowers, leaves and stems of *Citrus limon*.	Fragrance Ingredients; Skin-Conditioning Agents - Miscellaneous
Citrus Limon (Lemon) Flower/Leaf/Stem Oil CAS No. 84929-31-7; 85085-28-5	Citrus Limon (Lemon) Flower/Leaf/Stem Oil is the volatile oil obtained from the flowers, leaves and stems of *Citrus limon*.	Fragrance Ingredients
Citrus Limon (Lemon) Leaf/Peel/Stem Oil CAS No. 84929-31-7; 85085-28-5	Citrus Limon (Lemon) Leaf/Peel/Stem Oil is the volatile oil obtained from the leaves, peels, and stems of *Citrus limon.*	Skin-Conditioning Agents - Miscellaneous
Citrus Nobilis (Mandarin Orange)	Citrus Nobilis (Mandarin Orange) is a plant material derived from the whole plant, *Citrus nobilis.*	Not reported
Citrus Nobilis (Mandarin Orange) Oil	Citrus Nobilis (Mandarin Orange) Oil is the volatile oil obtained from the whole plant, *Citrus nobilis.*	Fragrance Ingredients
Citrus Nobilis (Mandarin Orange) Water	Citrus Nobilis (Mandarin Orange) Water is an aqueous solution of the steam distillate obtained from *Citrus nobilis.*	Skin-Conditioning Agents - Miscellaneous
Citrus Paradisi (Grapefruit) Seed Extract CAS No. 90045-43-5 (generic)	Citrus Paradisi (Grapefruit) Seed Extract is the extract of the seeds of *Citrus paradisi*.	Skin-Conditioning Agents - Miscellaneous
Citrus Reticulata (Tangerine) Extract	Citrus Reticulata (Tangerine) Extract is the extract of the whole plant, *Citrus reticulata.*	Skin-Conditioning Agents - Miscellaneous
Citrus Sunki Seed Extract	Citrus Sunki Seed Extract is the extract of the seeds of *Citrus sunki.*	Antioxidants; Skin Bleaching Agents; Skin-Conditioning Agents - Miscellaneous
Citrus Sunki Seed Oil	Citrus Sunki Seed Oil is the oil expressed from the seeds of *Citrus sunki.*	Antioxidants; Skin Bleaching Agents; Skin-Conditioning Agents - Miscellaneous
Citrus Unshiu Extract CAS No. 98106-71-9	Citrus Unshiu Extract is the extract of the whole plant, *Citrus unshiu.*	Skin-Conditioning Agents - Miscellaneous

*Accepted or alternate scientific names for these Citrus ingredients are found in Table 3.

Table 2. *Citrus* plant- and seed-derived ingredients that potentially function solely as fragrance ingredients.

Citrus Aurantium Dulcis (Orange) Oil
Citrus Limon (Lemon) Flower/Leaf/Stem Oil
Citrus Nobilis (Mandarin Orange) Oil

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Table 3.Review of *Citrus* species names[7]

Species Name Used in INCI Names (common name)	Accepted Species Name
Citrus aurantifolia (lime)	*Citrus x aurantifolia*
Citrus aurantium amara (bitter orange)	*Citrus x aurantium*
Citrus aurantium bergamia (bergamot)	*Citrus x limon*
Citrus aurantium dulcis (orange) ALSO *Citrus sinensis* (orange)	*Citrus x aurantium*
Citrus clementina (clementine)	*Citrus x aurantium*
Citrus depressa	*Citrus reticulata*
Citrus glauca	*Citrus glauca*
Citrus grandis (grapefruit or pomelo)	*Citrus maxima* or *Citrus x aurantium*
Citrus hassaku	*Citrus medica x Citrus x aurantium*
Citrus iyo	*Citrus x aurantium*
Citrus jabara	Not known
Citrus japonica (kumquat)	*Citrus japonica*
Citrus junos	*Citrus x junos*
Citrus limon (lemon)	*Citrus x limon*
Citrus madurensis	*Citrus x microcarpa*
Citrus medica vulgaris	*Citrus reticulata*
Citrus natsudaidai	*Citrus x aurantium*
Citrus nobilis (mandarin orange)	*Citrus reticulata*
Citrus paradisi (grapefruit)	*Citrus x aurantium*
Citrus reticulata (tangerine)	*Citrus reticulata*
Citrus shunkokan	Cultivated hybrid
Citrus sinensis (orange) ALSO *Citrus aurantium dulcis* (orange)	*Citrus x aurantium*
Citrus sphaerocarpa	Cultivated hybrid
Citrus sudachi	*Citrus reticulata*
Citrus tachibana	Not listed
Citrus tamurana	Cultivated hybrid
Citrus tangelo (tangelo)	*Citrus x aurantium*
Citrus tangerine (tangerine)	*Citrus reticulata*
Citrus tankan	*Citrus reticulata*
Citrus unshiu	*Citrus reticulata*

Table 4. Total fatty acid composition of Citrus seed oils, as previously reported (%).[5]

Fatty Acids	Citrus Aurantifolia (Lime) Seed Oil	Citrus Aurantium Dulcis (Orange) Seed Oil	Citrus Grandis (Grapefruit) Seed Oil	Citrus Limon (Lemon) Seed Oil	Citrus Paradisi (Seed) Oil
Lauric (C12)	NR	NR	1.5	NR	2.95
Myristic (C14)	1	NR	1	NR	1.01
Palmitic (C16)	20-30	14-22	18-30	18.8	36.25
Heptadecanoic (C17:0)	NR	NR	NR	0.08	NR
Stearic (C18)	3-8	2-6	2-8	3.5	5.95
Oleic (C18:1)	20-38	26-35	20-38	30.1	18.34
Linoleic (C18:2)	30-45	35-45	30-48	33.4	29.26
Linolenic (C18:3)	5-15	2-6	2-6	13.5	3.58
Arachidic (C20)	2	0.5	NR	0.3	0.38
Eicosenoic (C20:1)	NR	NR	NR	0.03	0.84
Behenic (C22)	NR	NR	NR	0.08	NR
Lignoceric (C24)	NR	NR	NR	0.2	NR
Others	NR	NR	NR	C23:0 = <0.01; C26:0 = 0.01	C12:1=1.44

NR = Not Reported

Table 5. Key constituents (%) of Citrus Aurantium Amara (Bitter Orange) Leaf /Twig Oil*[32]

	Bigarade Type	Paraguayan Type
linalyl acetate	51.0-71.0	47.4-58.0
linalool	12.3-24.2	20.8-25.2
(+)-limonene	0.4-8.0	0.3-1.1
α-terpineol	2.1-5.2	4.4-6.8
geranyl acetate	1.9-3.4	2.9-4.5
β -pinene	0.3-2.7	0.3-1.2
neryl acetate	0-2.6	2.1-3.0
geraniol	1.4-2.3	2.1-3.0
(E)-β-ocimene	0.2-2.2	0-2.0
β-myrcene	0-2.0	0-2.0
nerol	0.4-1.1	NR

NR = Not reported

*Composition reported down to the level of 1%, or lower for known toxic constituents.

Table 6. Fatty acid profiles (area %) by gas chromatography.[8,9,33]

Fatty acid	**Citrus Australasica Seed Oil**	**Citrus Glauca Seed Oil**	***Citrus junos* Sieb. ex Takana seed oil***
undecanoic acid	NR	NR	3.27
myristic acid	0.07	NR	NR
palmitic acid	10.50	8.07	19.16
palmitoleic acid	0.24	0.17	0.62
margaric acid	0.08	0.05	NR
heptadecanconic acid	0.07	NR	NR
stearic acid	3.36	2.52	3.76
elaidic acid	0.11	NR	NR
oleic acid	36.55	47.39	32.01
cis-vaccenic acid	1.67	2.00	NR
linolelaidic acid	0.05	NR	NR
linoleic acid	41.01	36.28	33.99
α-linolenic acid	4.70	1.28	2.05
arachadic acid	0.40	0.31	0.26
11-eicosenoic acid	0.28	0.42	NR
behenic acid	0.42	0.61	NR
lignoceric acid	0.15	0.32	NR
unknown	NR	NR	4.48

*Reported as acid methyl esters.

Table 7. Frequency and concentration of use according to duration and type of exposure for *Citrus* plant- and seed-derived ingredients.[18,19]

	# of Uses	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*
	Citrus Aurantifolia (Lime) Oil		**Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil[d]**		**Citrus Aurantium (Bitter Orange) Oil[e]**		**Citrus Aurantium Dulcis (Orange) Oil[f]**	
Totals[†]	**169**	**0.00000051-0.36**	**43**	**0.0000000026-0.0032**	**295**	**NR**	**4**	**0.0000032-1**
Duration of Use								
Leave-On	90	0.00049-0.36	25	0.00000014-0.0032	178	NR	3	0.000094-1
Rinse Off	69	0.00015-0.17	14	0.00000015-0.000013	96	NR	NR	0.000026-0.81
Diluted for (Bath) Use	10	0.00000051	4	0.0000000026	21	NR	1	0.0000032-0.94
Exposure Type								
Eye Area	NR	NR	NR	NR	NR	NR	NR	0.00078-0.034
Incidental Ingestion	NR	0.0015-0.36	NR	NR	2	NR	NR	0.034-0.95
Incidental Inhalation-Spray	9; 34[a]; 29[b]	0.00049-0.12; 0.0015-0.1[a]; 0.00067[b]	11[a]; 11[b]	0.00000014-0.00000043; 0.0000025[a]	31; 37[a]; 49[b]	NR	1; 2[a]	0.0022-0.12; 0.0053-0.26[a]; 0.000094[b]
Incidental Inhalation-Powder	1[c]; 29[b]	0.022; 0.0075-0.023[c];0.00067	11[b]	0.000011[c]	1; 3[c]; 49[b]	NR	NR	0.0012-0.0014; 0.18-1[c]; 0.000094[b]
Dermal Contact	147	0.00000051-0.17	40	0.0000000026-0.0032	250	NR	3	0.0000032-1
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	0.039[a]; 0.038-0.06[g]
Hair - Non-Coloring	22	0.00049-0.02	3	0.00000014-0.0000025	32	NR	1	0.0022-0.81
Hair-Coloring	NR	0.00015	NR	NR	11	NR	NR	0.000026-0.042
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	35	0.00000051-0.36	11	0.0000000026-0.000013	53	NR	1	0.0000032-0.95
Baby Products	3	NR	NR	NR	9	NR	NR	NR

	Citrus Aurantium Dulcis (Orange) Seed Extract[h]		**Citrus Aurantium Sinensis Powder[i]**		**Citrus Grandis (Grapefruit) Extract**		**Citrus Grandis (Grapefruit) Seed Extract**	
Totals[†]	**2**	**NR**	**1**	**NR**	**NR**	**0.0017-0.0059**	**144**	**0.0004-0.15**
Duration of Use								
Leave-On	2	NR	1	NR	NR	0.0017-0.003	85	0.076-0.15
Rinse Off	NR	NR	NR	NR	NR	0.0022-0.0059	51	0.0004-0.12
Diluted for (Bath) Use	NR	NR	NR	NR	NR	NR	8	NR
Exposure Type								
Eye Area	NR	NR	NR	NR	NR	NR	5	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	12	0.076
Incidental Inhalation-Spray	2[b]	NR	1[b]	NR	NR	0.0017-0.003	2; 28[a]; 17[b]	NR
Incidental Inhalation-Powder	2[b]	**NR**	1[b]	NR	NR	NR	17[b]; 2[c]	0.1[c]
Dermal Contact	2	NR	1	NR	NR	0.0022-0.0059	106	0.02-0.15
Deodorant (underarm)	NR	NR	NR	NR	NR	0.0024[g]	14[a]	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	0.0017-0.005	23	0.0004
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	0.0022-0.0059	26	0.076
Baby Products	NR	NR	NR	NR	NR	NR	7	NR

Table 7. Frequency and concentration of use according to duration and type of exposure for *Citrus* plant- and seed-derived ingredients.[18,19]

	# of Uses	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*
	Citrus Junos Extract		**Citrus Junos Seed Extract[j]**		**Citrus Junos Seed Oil**		**Citrus Limon (Lemon) Flower/Leaf/Stem Extract**	
Totals[†]	**NR**	**0.0001**	**7**	**0.001-0.0045**	**NR**	**0.001-0.1**	**8**	**NR**
Duration of Use								
Leave-On	NR	NR	7	0.001-0.0045	NR	0.01-0.1	8	NR
Rinse Off	NR	0.0001	NR	0.001	NR	0.001	NR	NR
Diluted for (Bath) Use	NR	NR	NR	0.001	NR	NR	NR	NR
Exposure Type								
Eye Area	NR	NR	1	0.001	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	4[a]; 2[b]	NR	NR	NR	6[a]; 1[b]	NR
Incidental Inhalation-Powder	NR	NR	2[b]	0.0045[c]	NR	0.1; 0.1[c]	1[b]	NR
Dermal Contact	NR	NR	7	0.001-0.0045	NR	0.001-0.1	8	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	0.01	NR	NR
Hair-Coloring	NR	0.0001	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	0.001	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

	Citrus Nobilis (Mandarin Orange)		**Citrus Nobilis (Mandarin Orange) Oil**		**Citrus Paradisi (Grapefruit) Seed Extract**		**Citrus Reticulata (Tangerine) Extract**	
Totals[†]	**NR**	**0.0005**	**36**	**0.0009-0.035**	**52**	**NR**	**NR**	**0.0001-0.0051**
Duration of Use								
Leave-On	NR	NR	28	NR	39	NR	NR	0.0002-0.0051
Rinse Off	NR	0.0005	4	0.0009-0.035	13	NR	NR	0.0001-0.005
Diluted for (Bath) Use	NR	NR	4	NR	NR	NR	NR	NR
Exposure Type								
Eye Area	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	1	0.035	1	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	8; 2[a]; 4[b]	NR	16[a]; 17[b]	NR	NR	0.0051[a]
Incidental Inhalation-Powder	NR	NR	4[b]; 1[c]	NR	17[b]; 1[c]	NR	NR	0.0002[c]
Dermal Contact	NR	0.0005	35	0.0009-0.0017	50	NR	NR	0.0002
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	0.0005	NR	NR	1	NR	NR	0.0001-0.0051
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	0.0005	7	0.0009-0.035	8	NR	NR	NR
Baby Products	NR	NR	2	NR	2	NR	NR	NR

NR = Not reported.

† Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

[a.] It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.

[b.] Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.

[c.] It is possible these products may be powders, but it is not specified whether the reported uses are powders.

[d] Listed as Citrus Aurantium (Bitter Orange) Leaf/Twig Oil in the VCRP database.

[e] Only listed in the VCRP database, not in the INCI dictionary. Included because of assumed similarity.

[f] Listed as Citrus Sinensis (Sweet Orange) Plant Oil in the VCRP database.

[g]Not a spray deodorant.

[h] Listed as Citrus Sinensis (Sweet Orange) Seed Extract in the VCRP database.

[i] Listed as Citrus Sinensis (Orange) Powder in the VCRP database.

[j] Listed as Citrus Junos (Xiang Cheng) Seed Extract in the VCRP database.

Table 8. Ingredients that are not reported to be in use[18,19]

Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Extract
Citrus Aurantium Dulcis (Orange) Flower/Leaf/Stem Powder
Citrus Australasica Seed Oil
Citrus Depressa Seed Oil
Citrus Glauca Seed Oil
Citrus Grandis (Grapefruit)
Citrus Grandis Peel/Seed Extract
Citrus Iyo Oil
Citrus Limon (Lemon) Flower/Leaf/Stem Oil
Citrus Limon (Lemon) Leaf/Peel/Stem Oil
Citrus Nobilis (Mandarin Orange) Water
Citrus Sunki Seed Extract
Citrus Sunki Seed Oil
Citrus Unshiu Extract

Table 9. Dermal irritation studies for *Citrus* plant- and seed-derived ingredients.

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
			ANIMAL		
Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil (described as "petitgrain bigarade oil")	2g/kg; undiluted	2 rabbits	24-h occlusive, single dose study	slight erythema	[15]
			HUMAN		
Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil (described as "petitgrain bigarade oil")	0.1%, 2% or 5%; multiple vehicles	48 subjects at 0.1%, 30 subjects at 2%, and 30 subjects at 5%	24-72 h occlusive patch tests	no irritation	[28]
Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil (described as "petitgrain bigarade oil")	8% in petrolatum	25 subjects	48 h occlusive patch applied to the forearm or back	no irritation	[15]
Citrus Grandis (Grapefruit) Seed Extract	0.15% in a foot gel	12 subjects with normal, lesion-free skin	48 h occlusive patch (Finn chambers) on external arm, single application of 0.02 ml	no irritation (mean irritation index = 0.13)	[29]

Table 10. Sensitization studies for *Citrus* plant- and seed-derived ingredients.

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
			HUMAN		
Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil (described as "petitgrain bigarade oil")	2% in paraffin	200 patients with dermatitis tested with 35 essential oils plus an additional 50 patients with balsam sensitivity	sensitization patch study, details not provided	3 positive reactions, details not provided	[30]
Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil (described as "petitgrain bigarade oil")	8% in petrolatum	25 subjects	maximization study, details not provided	not sensitizing	[15]

Table 11. Photosensitization studies.

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
			NON-HUMAN		
Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil (described as "petitgrain bigarade oil")	undiluted	hairless mice (#/group not stated)	test material was applied, and the test sites were irradiated with UVA irradiation by blacklight or xenon lamp	not photosensitizing	[31]
Citrus Aurantium Amara (Bitter Orange) Leaf/Twig Oil (described as "petitgrain bigarade oil")	undiluted	miniature swine (#/group not stated)	test material was applied, and the test sites were irradiated with UVA irradiation by blacklight or xenon lamp	not photosensitizing	[31]

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Article

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Safety Assessment of Citrus-Derived Peel Oils as Used in Cosmetics

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Abstract

The Cosmetic Ingredient Review Expert Panel assessed the safety of 14 citrus-derived peel oil ingredients and concluded that these ingredients are safe for use in cosmetic products when finished products, excluding rinse-off products, do not contain more than 0.0015% (15 ppm) 5-methoxypsoralen, and when formulated to be nonsensitizing and nonirritating. The citrus-derived peel oil ingredients are most frequently reported to function in cosmetics as fragrances and/or skin conditioning agents. The Panel reviewed the available animal and clinical data to determine the safety of these ingredients. Because final product formulations may contain multiple botanicals, each containing the same constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. Industry should use good manufacturing practices to limit impurities that could be present in botanical ingredients.

Keywords

cosmetics, safety, citrus peel oils

Introduction

Citrus-derived peel oils are widely used as cosmetic ingredients and are most frequently reported to function in cosmetics as fragrances and/or skin conditioning agents (Table 1). This report assesses the safety of the following 14 citrus-derived peel oils:

Citrus aurantifolia (lime) peel oil
Citrus aurantium amara (bitter orange) peel oil
Citrus aurantium currassuviensis peel oil
Citrus aurantium dulcis (orange) peel oil
Citrus clementina peel oil
Citrus grandis (grapefruit) peel oil
Citrus iyo peel oil
Citrus junos peel oil
Citrus limon (lemon) peel oil
Citrus medica vulgaris peel oil
Citrus nobilis (mandarin orange) peel oil
Citrus reticulata (tangerine) peel oil
Citrus tachibana/reticulata peel oil
Citrus tangerina (tangerine) peel oil

The citrus ingredients in this assessment are found in foods, and daily exposure from food use would result in much larger systemic exposures than those from use in cosmetic products. Additionally, essential oils, oleoresins (solvent-free), and natural extracts (including distillates) derived from some citrus fruits are generally recognized as safe (GRAS) for their intended use in foods for human and animal consumption according to the US Food and Drug Administration (FDA). Volatile oils of limes, lemons, grapefruits, bitter oranges, oranges, and tangerines are described as flavoring agents in the US Pharmacopeia (USP) Food Chemicals Codex.[1] Thus, the systemic toxicity potential of citrus-derived peel oils via oral exposure is not addressed further in this report. The primary focus of this safety assessment of these citrus ingredients as used in cosmetics is on the potential for irritation and sensitization from dermal exposure.

The Cosmetic Ingredient Review (CIR) does not review ingredients that function only as fragrance ingredients because, as fragrances, the safety of these ingredients is evaluated by the Research Institute for Fragrance Materials (RIFM). Three of the citrus-derived peel oils in this report function only as

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Table 1. Definitions and Functions of Citrus-Derived Ingredients.

Ingredient	Definition[2]	Function[2]
Citrus aurantifolia (lime) peel oil	*C aurantifolia* (lime) peel oil is the volatile oil obtained from the peel of *C aurantifolia*	Fragrance ingredients
Citrus aurantium amara (bitter orange) peel oil CAS No. 68916-04-1	*C aurantium* amara (bitter orange) peel oil is the volatile oil obtained from the peel of *C aurantium amara*	Fragrance ingredients; skin conditioning agents-miscellaneous
Citrus aurantium currassuviensis peel oil	*C aurantium currassuviensis* peel oil is the volatile oil derived from the peel of the laraha orange, *C aurantium currassuviensis*	Fragrance ingredients
Citrus aurantium dulcis (orange) peel oil CAS No. 8008-57-9	*C aurantium dulcis* (orange) peel oil is the volatile oil obtained by expression from the peel of *Citrus sinensis*	Fragrance ingredients; skin conditioning agents-miscellaneous
Citrus clementina peel oil	*C clementina* peel oil is the volatile oil obtained from the peel of *C clementina*	Fragrance ingredients; skin conditioning agents-miscellaneous
Citrus grandis (grapefruit) peel oil CAS No. 8016-20-4	*C grandis* (grapefruit) peel oil is the volatile oil obtained from the peel of the grapefruit, *C grandis*	Fragrance ingredients; skin conditioning agents-miscellaneous
Citrus iyo peel oil	*C iyo* peel oil is the volatile oil obtained from the peel of *C iyo*	Skin conditioning agents-emollient
Citrus junos peel oil	*C junos* peel oil is the volatile oil obtained from the peel of *C junos*	Cosmetic astringents
Citrus limon (lemon) peel oil CAS Nos. 8008-56-8; 8020-19-7; 84929-31-7; 85085-28-5	*C limon* (lemon) peel oil is the volatile oil obtained from the peel of *C limon*	Fragrance ingredients; skin conditioning agents-miscellaneous
Citrus medica vulgaris peel oil	*C medica vulgaris* peel oil is the volatile oil obtained from the peel of *C medica vulgaris*	Fragrance ingredients
Citrus nobilis (mandarin orange) peel oil CAS No. 8008-31-9; 84696-35-5	*C nobilis* (mandarin orange) peel oil is the oil obtained from the peel of the mandarin orange, *C nobilis*	Fragrance ingredients; skin conditioning agents-miscellaneous
Citrus reticulata (tangerine) peel oil CAS No. 8008-31-9	*C reticulata* (tangerine) peel oil is the volatile oil obtained from the peel of *C reticulata*	Deodorant agents; flavoring agents; fragrance ingredients
Citrus tachibana/reticulata peel oil	*C tachibana/reticulata* peel oil is the volatile oil obtained from the peel of the hybrid of *C tachibana* and *C reticulata*	Skin conditioning agents-emollient
Citrus tangerina (tangerine) peel oil	*C tangerina* (tangerine) peel oil is the volatile oil obtained from the peel of *C tangerina*	Fragrance ingredients; skin conditioning agents-miscellaneous

Table 2. Citrus Ingredients That Potentially Function Solely as Fragrance Ingredients.

Citrus aurantifolia (lime) peel oil
Citrus aurantium currassuviensis peel oil
Citrus medica vulgaris peel oil

fragrance ingredients, according to the *International Cosmetic Ingredient Dictionary and Handbook* (see Table 2).[2] However, RIFM has not confirmed that these 3 ingredients function only as fragrances and therefore within its purview; thus, CIR is reviewing the safety of these ingredients.

Botanicals such as those derived from citrus contain hundreds of constituents, some of which have the potential to cause toxic effects. For example, bergapten (also known as 5-methoxypsoralenor [5-MOP]) is a naturally occurring phototoxic furanocoumarin (psoralen) in citrus peel oils. In this assessment, CIR is reviewing information available to evaluate the potential toxicity of each of the citrus-derived peel oils as a whole, complex substance. Except for specific constituents of concern, CIR is not reviewing information that may be available to assess the potential toxicity of the individual constituents of which the citrus-derived ingredients are composed. Cosmetic Ingredient Review requested information on the concentrations (including ranges, means, upper 95%confidence limits, detection limits, etc) of individual constituents in citrus-derived peel oils used in cosmetics to facilitate the safety assessment of these ingredients. Such information on constituents that have been identified as constituents of concern by the Panel in previous safety assessments, or by other recognized scientific expert review bodies, is especially important.

Some toxicological data on lemon oil and sweet orange oil (synonyms: lemon, ext. and orange, sweet, extract, respectively) reviewed in this safety assessment were obtained from robust summaries of data submitted to the European Chemical Agency (ECHA) by companies as part of the REACH chemical registration process. These data are available on the ECHA website.[3,4]

Note that, in many of the published studies included in this assessment, the information provided is not sufficient to determine how well the substance being tested represents the cosmetic ingredient. In this safety assessment, if a substance tested in a study is not clearly a cosmetic ingredient, because of lack of information on the genus and species from which the substance was derived and/or the method of extraction used, the test substance will be referred to by a common name (eg, lime oil). If the substance is clearly a cosmetic ingredient, the International Nomenclature of Cosmetic Ingredients (INCI) name

will be used (eg, "*C aurantifolia* [lime] peel oil"). In some instances, the part of the plant from which the oil was expressed is not known but, based on the method of manufacture, the oil could have been expressed from the peels of the citrus fruit. Additionally, some inconsistencies were noted in both taxonomic and INCI naming conventions. For example, this report includes the sweet orange ingredient described as *C aurantium dulcis* (orange) peel oil in the *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*).[2] In contrast, most of the published literature and the FDA refer to this ingredient as *Citrus sinensis* (sweet orange) peel oil. Another example of a naming inconsistency is *C grandis* (grapefruit) peel oil; *C grandis* is generally considered a name for a pummelo and is more commonly called *Citrus maxima*. The INCI Committee of the Personal Care Products Council (Council) is working to correct some of these errors.

Chemistry

Definition and General Characterization

The definitions and functions of the citrus-derived peel oils included in this report are provided in Table 1. In some cases, the definition provides insight on the method(s) of manufacture. It should be noted that essential oils are hydrophobic, liquid, and volatile aroma compounds from plants. These are typically mixtures of small molecules, but their chemical structures can vary widely. The volatile nature of essential oils makes them likely to be useful as fragrances but does not preclude other functions for them in cosmetics.

There are numerous *Citrus* varieties distributed throughout the world; most citrus fruits are grown in the temperate and tropical zones in both the northern and southern hemispheres.[5] Citrus can be propagated and new varieties can be produced by cultivating asexual nuclear or chance seedlings or by crossing or mutation.

Physical and Chemical Properties

Physical and chemical properties of the citrus-derived peel oils are provided in Table 3.

Method of Manufacturing

A suitable antioxidant can be added to citrus oil during preparation.[1]

Bitter orange oil and orange oil are obtained using a cold-pressed extraction method from the fresh peel of the fruit of *Citrus aurantium* and *C sinensis*, respectively.[1] *Citrus grandis* oil[6] and *C junos* oil[7] are also extracted using a cold-press method. Tangerine oil (cold-pressed) is obtained from the peels of the ripe fruit of the Dancy tangerine, *C nobilis* or *C reticulata*, and from some other closely related varieties.[1]

Lemon oil and lime oil, expressed, are produced by pressing the outer rind of the ripe fruit manually or by machine.[8] More economical processes involve an integrated juice-oil procedure. These oils can also be produced by distillation of expressed oils or direct distillation of fruit; distilling (rectifying) removes terpenes. Steam distillation removes nonvolatile furocoumarins.

Citrus iyo oil is produced by removing the peel from the fruit, homogenization of the peel with distilled water, and lyophilization.[9] The oil is purified using vacuum distillation.

Mandarin peel oil, expressed (identified in the literature as *C reticulata*), is prepared by expression of the peels of the ripe fruit of the mandarin orange.[10]

Constituents/Composition

The citrus-derived peel oils are complex botanicals composed of numerous constituents; there is great variation among citrus species and cultivars because of frequent bud mutations, interspecific and intergeneric hybridization, and apomixis (ie, 1 or more of several types of asexual reproduction).[11] The composition of citrus oils will vary based on the location where the plant is grown, the maturity of the plant, and storage conditions.[5] The method of extraction will also affect the composition.

Citrus oils contain large amounts of monoterpene hydrocarbons. Limonene is the constituent present in the greatest amount, often comprising greater than 90% of the oil, and the amount present can vary within the oil; for example, limonene is reported to compose 38.1% to 95.8% *C limon* (lemon) peel essential oils, cold-pressed.[12,13] Citrus oils also contain sesquiterpene hydrocarbons, which are responsible for the characteristic flavors of these oils.[12] Table 4 lists the chemical compositions of cold-pressed,[6,7,11-16] hydrodistilled,[17-21] lyophilization/vacuum distillation,[9] and steam-distilled[22] peel oils sorted by citrus plant species; Table 5 presents the typical levels of 5-MOP found in some of the oils; and Table 6 provides the levels of major coumarins and furocoumarins in lemon and lime oil. Table 7 lists citrus constituents that are established contact allergens, according the European Commission's Scientific Committee on Consumer Safety.

Use

Cosmetic

Table 8 presents the current product-formulation use data for citrus-derived peel oils. These ingredients are most frequently reported to function as fragrances and/or skin conditioning agents-miscellaneous.[2]

According to information supplied to the FDA by industry as part of the Voluntary Cosmetic Registration Program (VCRP), *C limon* (lemon) peel oil has the most reported uses in cosmetic and personal care products, with a total of 490; more than half of the uses are in leave-on skin care preparations.[23] *Citrus aurantium dulcis* (orange) peel oil (reported as *C sinensis* [sweet orange] peel oil by the VCRP) has the second greatest number of overall uses reported, with a total of 289; about half of those uses are in leave-on skin care preparations.

In a use concentration survey conducted by the Council, *C limon* (lemon) peel oil had a maximum use concentration range

Table 3. Physical and Chemical Properties of Citrus-Derived Peel Oils.

Property	Description	Reference
Citrus aurantifolia (lime) oil		
Color	Colorless to greenish yellow	8
Odor	Fresh citrus, intense	8
Optical rotation	+34° to +47°	8
Solubility	Insoluble in water, soluble in ethanol and propylene glycol	8
Refractive index	1.4477-1.4745	8
Specific gravity	0.855-0.863	8
C aurantifolia or *Citrus latifolia* (lime) oil (distilled)		
Color	Colorless to greenish yellow	1
Odor	Mild citrus, floral	1
Optical rotation/angular rotation	+34° to +47°	1
Solubility	Soluble in most fixed oils and mineral oil; insoluble in glycerin and propylene glycol	1
Solubility in alcohol	1-mL sample dissolves in 5 mL of 90% alcohol	1
Aldehydes	Between 0.5% and 2.5% of aldehydes, calculated as citral	1
Refractive index	1.474-1.477 at 20°C	1
Specific gravity	0.855-0.863	1
C aurantifolia (lime) oil (cold-pressed)		
Color	Yellow to brown-green to green liquid	1
Odor	Fresh lime peel	1
Optical rotation/angular rotation	Mexican type: +35° to +41°; Tahitian type: +38° to +53°	1
Solubility	Soluble in most fixed oils and mineral oil; insoluble in glycerin and propylene glycol	1
Aldehydes	Mexican type: no less than 4.5% and no more than 8.5% of aldehydes, calculated as citral; Tahitian type: no less than 3.2% and no more than 7.5% of aldehydes, calculated as citral	1
Refractive index	Mexican type: 1.482-1.486; Tahitian type: 1.476-1.486	1
Residue of evaporation	Mexican type: 10.0%-14.5%; Tahitian type: 5.0%-12.0%	1
Specific gravity	Mexican type: 0.872-0.881; Tahitian type: 0.858-0.876	1
UV absorbance	Max. at 315 nm; Mexican type: no less than 0.45; Tahitian type: no less than 0.24	1
Citrus aurantium (bitter orange) oil (cold-pressed)		
Color	Pale yellow or yellow-brown liquid	1
Odor	Characteristic aromatic odor of the Seville orange	1
Optical rotation/angular rotation	+88° to +98°	1
Solubility	Miscible with absolute alcohol and with an equal volume of glacial acetic acid; soluble in fixed oils and mineral oil; slightly soluble in propylene glycol; relatively insoluble in glycerin	1
Aldehydes	No less than 0.5% and no more than 1.0% of aldehydes, calculated as decyl aldehyde	1
Refractive index	1.472-1.476 at 20°C	1
Residue of evaporation	2.0%-5.0%	1
Specific gravity	0.845-0.851	1
Citrus clementina		
Color	Orange to brownish liquid	43
Odor	Characteristic	43
Optical rotation	+89 to +97° (15°C)	43
Solubility	Insoluble in water; soluble in ethyl alcohol	43
Refractive index	1.5640-1.4820 (20°C)	43
Specific gravity	0.840-0.875 (15°C)	43
Citrus limon (lemon) oil		
Color	Pale to deep yellow or greenish yellow	8
Odor	Fresh citrus, intense	8
Optical rotation	+57 to +65.6	8
Solubility	Insoluble in water, soluble in ethanol and propylene glycol	8
Refractive index	1.474-1.467	8
Specific gravity	0.849-0.855	8
C limon (lemon) oil (distilled)		
Color	Colorless to pale yellow liquid	1
Odor	Fresh lemon peel	1
Optical rotation/angular rotation	+55° to +75°	1
Solubility	Soluble in most fixed oil, mineral oil, and alcohol (with haze); insoluble in glycerin and propylene glycol	1
Solubility in alcohol	1-mL sample dissolves in 5 mL of 90% alcohol	1

(continued)

Table 3. (continued)

Property	Description	Reference
Aldehydes	Between 1.0% and 3.5% of aldehydes, calculated as citral	1
Refractive index	1.470-1.475 at 20°C	1
Specific gravity	0.842-0.856	1
UV absorbance	Maximum at 315 nm, no less than 0.01	1
C limon (lemon) oil (cold-pressed)		
Color	Pale to deep yellow or green-yellow	1
Odor	Fresh lemon peel	1
Optical rotation/angular rotation	California and Italian types: +57° to +65.6°; desert type: +67° to +78°	1
Solubility	Miscible with dehydrated alcohol and glacial acetic acid	1
Solubility in alcohol	1 mL of sample dissolves in 3 mL of 95% alcohol, slight haze possible	1
Aldehydes	Desert type: no less than 1.7% of aldehydes, calculated as citral; California type: no less than 2.2% and no more than 3.8% of aldehydes, calculated as citral; Italian type: no less than 3.0% and no more than 5.5% of aldehydes, calculated as citral	1
Refractive index	1.473-1.476 at 20°C	1
Residue of evaporation	Between 5.0% and 14.5%	1
Specific gravity	Desert type: 0.846-0.851; California and Italian types: 0.849-0.855	1
UV absorbance	Maximum at 315 nm; desert and California types: no less than 0.2; Italian type: no less than 0.49	1
Citrus nobilis or Citrus reticulata (tangerine) oil (cold-pressed)		
Color	Red-orange to brown-orange	1
Odor	Pleasant, orange	1
Optical rotation/angular rotation	+88° to +96°	1
Solubility	Soluble in most fixed oils and mineral oil; slightly soluble in propylene glycol; relatively insoluble in glycerin	1
Aldehydes	0.8% to 1.9% of aldehydes, calculated as decyl aldehyde	1
Refractive index	1.473-1.476 at 20°C	1
Residue of evaporation	2.3%-5.8%	1
Specific gravity	0.844-0.854	1
Citrus reticulata peel oil		
Physical state and appearance	Clear, mobile, dark orange to reddish-orange or Brownish-orange liquid	10
Odor	Orange-like	10
Optical rotation	+63° to +78°	10
Refractive index	1.4730-1.4770 (20°C)	10
Specific gravity	0.847-0.853 (25/25°C)	10
Citrus sinensis (orange) oil (distilled)		
Color	Colorless to pale yellow liquid	1
Odor	Mild citrus floral	1
Optical rotation/angular rotation	+94° to +99°	1
Solubility	Soluble in most fixed oil, mineral oil, and alcohol (with haze); insoluble in glycerin and propylene glycol	1
Solubility in alcohol	1-mL sample dissolves in 5 mL of 90% alcohol	1
Refractive index	1.471-1.474 at 20°C	1
Specific gravity	0.840-0.844	1
UV absorbance	Maximum at 330 nm, no less than 0.01	1
Citrus sinensis (orange) oil (cold-pressed)		
Color	Intensely yellow, orange, or deep orange liquid	1
Odor	Characteristic of fresh, sweet orange peel	1
Optical rotation/angular rotation	+94° to +99°	1
Solubility	Miscible with dehydrated alcohol and carbon disulfide; soluble in glacial acetic acid	1
Aldehydes	No less than 1.2% and no more than 2.5% of aldehydes, calculated as decyl aldehyde	1
Refractive index	1.472-1.474 at 20°C	1
Specific gravity	0.842-0.846	1
UV absorbance	Maximum at 330 nm; California type: no less than 0.130; Florida type: no less than 0.240	1

Abbreviation: UV, ultraviolet.

of 0.0001% to 0.5%, with 0.5% reported in "other" skin care preparations.[24] *Citrus aurantium dulcis* (orange) peel oil had a maximum use concentration range of 0.00002% to 29%, with 29% reported in noncoloring hair conditioners.

In some cases, reports of uses were received from the VCRP, but no concentration of use data were provided. For example, *C junos* peel oil is reported to be used in 6 formulations, but no use concentration data were available. In other

Table 4. Chemical Composition (%) of Citrus Peel Oils.[a]

	Citrus aurantifolia (lime) peel oil		*Citrus aurantium amara* (bitter orange) essential oil				*Citrus aurantium dulcis* (orange) essential oil		*Citrus clementina* Peel essential oil		*Citrus grandis* (grapefruit) peel essential oil		*Citrus iyo* peel essential oil		*Citrus junos* peel essential oil	
	Cold-pressed[13]		Cold-pressed[14]		Hydrodistilled[18,20]		Cold-pressed[14]		Hydrodistillation[17]		Cold-pressed[6]		Lyophilization/vacuum distillation[9]		Cold-pressed[7] or not specified[16]	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Essential oil content (%)					0.12[18]-1.67[20]							0.12[6]		1.5[9]		
Constituent																
Limonene	39.9	94.4			67.9	94.67			93.77	96.19		94.8	79	89	63.1	77.44
(E)-α-Bergamotene															Trace	
(E)-β-farnesene	Trace	0.1													Trace	
(E)-β-ocimene	Trace	0.5			0.02	0.5									ND	Trace
(E)-decenal															ND	Trace
(E)-carvone												0.005-0.05				
(E)-caryophyllene	0.1	0.5														
(E)-caryophyllene oxide												Trace				
(E)-linalool oxide															ND	Trace
(E)-nerolidol															Trace	
(E)-p-mentha-2,8-dienol												0.1				
(E)-p-2,8-menthadien-1-ol												Trace				
(E, E)-α-farnesene	0.3	1.9										0.1				
(E, E)-farnesyl acetate															ND	Trace
(E, Z)-2,6-nonadien-1-ol											ND					
(Z)-β-ocimene	Trace	0.7													Trace	
Z-carveol												0.005-0.05				
(Z)-carvone												0.1				
(Z)-β-farnesene															0.9	1.3
(Z)-limonene oxide															ND	Trace
(Z)-piperitol												0.005-0.05				
1-Octanol																
1,3,8-p-Menthatriene															Trace	
1,8-Cineole	0.5	0.6			Trace	14.72										
2Z.6E-farnesol					Trace											
3-Carene	Trace	0.3														
3,3',4',5,6,7-Heptamethoxyflavone			X				X									
3,3',4',5,6,7-Hexamethoxyflavone							X									
3',4',5,6,7,8-Hexamethoxyflavone			X													
3',4',5,6,7-Pentamethoxyflavone							X									
4',5,6,7-Tetramethoxyflavone			X				X									
4',5,6,7,8-Pentamethoxyflavone			X				X									
5-Methoxypsoralen			X													
(5-(6',7'-Dihydroxy-geranyloxy)psolaren)			X													
(5-(6',7'-Epoxy-geranyloxy)psolaren)			X													
5,7-Dimethoxycoumarin			X													
6-Methylhept-5-en-2-one		Trace														
7-Methoxy-8(2',3'-dihydroxy)isopentyloxycoumarin			X													
7-Methyoxy-8(2',3'-epoxy)isopentenylcoumarin			X													
7-Methyoxy-8-isopentenylcoumarin			X													
7-Methoxy-8-(2'-one-isopentyl)coumarin)			X													
30 carvacrol					Trace	0.69										
Allo-ocimene		Trace														
Aromadendrene															ND	Trace
Bicycloelemene															Trace	
Bicyclogermacrene	Trace	0.4													0.7	2.0
Borneol					0.08	0.16										

(continued)

Table 4. (continued)

	Citrus aurantifolia (lime) peel oil		*Citrus aurantium amara* (bitter orange) essential oil				*Citrus aurantium dulcis* (orange) essential oil		*Citrus clementina* Peel essential oil		*Citrus grandis* (grapefruit) peel essential oil		*Citrus iyo* peel essential oil		*Citrus junos* peel essential oil	
	Cold-pressed[13]		Cold-pressed[14]		Hydrodistilled[18,20]		Cold-pressed[14]		Hydrodistillation[17]		Cold-pressed[6]		Lyophilization/vacuum distillation[9]		Cold-pressed[7] or not specified[16]	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Bornyl acetate	Trace	Trace			Trace											
Camphene	Trace	0.2			Trace	0.24									Trace	
Camphor					0.17	6.37										
Caryophyllene															ND	Trace
Caryophyllene oxide	Trace	0.1			0.08	0.21						Trace				
Cedrol											Trace					
cis-Dihydrocarvone							0.03	0.28								
cis-Limonene-1,2-oxide	Trace	0.1														
cis- Linalool oxide THF		Trace			Trace	3.4										
cis-β-Ocimene						0.30										
cis-Sabinene hydrate					Trace	0.18									Trace	0.1
Citronellal	Trace	1.6							0.03	0.08		0.1				
Citronellol	Trace	0.6					Trace	0.07							ND	Trace
Citronellyl acetate	0.1	0.2														
Cumin aldehyde												0.005-0.05				
Decanal	Trace	0.3				0.16						0.3			Trace	
Dihydrocarveol											Trace					
Dodecanol		Trace										0.1			ND	Trace
Elemol											Trace				Trace	
Eugenol															ND	Trace
Geranial	0.5	6.1										0.1				
Geraniol	Trace	0.3														
Geranyl acetate	0.1	2.4			Trace	0.11										
Geranyl propionate											Trace					
Germacrene B															0.1	0.2
Germacrene D	Trace	1.0			0.04	0.25									0.3	0.4
Germacrene D-4-ol															0.3	0.4
Globulol		0.1														
Guaiene															ND	Trace
Heptyl acetate												0.3				
Isothyujol												0.005-0.05				
Limonen-10-ol											Trace					
Linalool	0.1	1.4			Trace	0.76			0.03	0.08		0.1	1.3	7.3	1.6	2.8
Linalool oxide																
Linalyl acetate	Trace	Trace			0.01	0.18						0.1				
Methyl-N-methyl anthranilate												0.005-0.05				
Myrcene	0.8	2.0			0.07	2.0			0.73	0.78			X		0.4	3.2
Neral	Trace	3.1									Trace					
Nerol	Trace	0.1			0.03	0.12					Trace				ND	Trace
Nerolidyl acetate											Trace					
Neryl acetate	Trace	2.0				0.10										
Nonanal	Trace	0.1			Trace	0.18						0.005-0.05			Trace	
Nonanoic acid												Trace				
Nootkatone												0.1.				
Octanal	Trace	0.1			0.02	1.59						0.4			Trace	
Octyl acetate	Trace	Trace														
p-Cymene	Trace	5.3			Trace	0.09									0.4	0.6
p-Cymenene		0.1														
p-Mentha-1-en-9-ol											Trace					

(continued)

Table 4. (continued)

	Citrus aurantifolia (lime) peel oil		*Citrus aurantium amara* (bitter orange) essential oil				*Citrus aurantium dulcis* (orange) essential oil		*Citrus clementina* Peel essential oil		*Citrus grandis* (grapefruit) peel essential oil		*Citrus iyo* peel essential oil		*Citrus junos* peel essential oil	
	Cold-pressed[13]		Cold-pressed[14]		Hydrodistilled[18,20]		Cold-pressed[14]		Hydrodistillation[17]		Cold-pressed[6]		Lyophilization/vacuum distillation[9]		Cold-pressed[7] or not specified[16]	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Perillaldehyde											Trace				Trace	
Perillyl alcohol															Trace	
Perillyl aldehyde												0.005-0.05				
Piperitenone		Trace													Trace	
Pseudolimonene															Trace	
Sabina ketone												0.1				
Sabinene	0.1	19.6			Trace	0.200.18						0.4			0.4	0.5
Santal-10-en-2-ol	Trace	Trace														
Spathulenol					Trace	0.21									ND	Trace
Terpinen-4-ol	Trace	1.7			0.01	0.54									Trace	0.1
Terpinolene	Trace	1.1			0.02	0.22					ND				0.6	0.7
Terpinyl acetate	Trace	Trace														
Tetradecane															ND	Trace
Tetradecenal											Trace					
Thymol															0.2	0.3
Thymyl methyl oxide	1.2	1.6														
trans-α-Bergamotene	0.2	1.6														
trans-Limonene-1,2-oxide	Trace	0.2														
trans-Sabinene hydrate	Trace	0.1													Trace	0.1
Tri-Cyclen					0	0.75										
Undecanal	Trace	0.1														
Valencene					0.04	0.2										
(−)-α-Copaene															Trace	0.1
α-Cadinene																0.1
α-Cadinol															ND	Trace
α-Cedrene												Trace				
α-Copaene												0.005-0.05				
α-Cubebene												0.1			Trace	
α-Farnesene															ND	Trace
α-Humulene	Trace	0.2			0.03	0.13										0.1
α-Muurolene															Trace	
α-p-Dimethyl styrene															Trace	
α-Phellandrene	Trace	0.1													0.7	0.9
α-Pinene	0.2	2.7			0.03	0.53			0.15	0.31		0.5			2.0	2.7
α-Sinensal																
α-Terpinene	Trace	0.4			0.91	1.66						1.8	X			0.3
α-Terpineol	Trace	4.7			0.07	0.35			0.04	0.09		0.1			0.2	0.3
α-Terpinyl acetate					Trace	1.4										
α-Thujene	Trace	0.7			0.14	0.44					ND					
α-Thujone					Trace	0.35										
α-ylangene															ND	Trace
β-Bisabolene	0.3	2.4														
β-Caryophyllene												0.1				0.3
β-Cubebene															Trace	0.1
β-Eleme	Trace	0.8														0.1
β-Eudesmol															Trace	
β-Ionone															ND	Trace
β-Phellandrene	0.2	0.8													2.16	5.4
β-Pinene	Trace	19.2			0.07	0.62			0.06	0.36		0.005-0.05			0.9	1.1

(continued)

Table 4. (continued)

	Citrus aurantifolia (lime) peel oil		*Citrus aurantium amara* (bitter orange) essential oil				*Citrus aurantium dulcis* (orange) essential oil		*Citrus clementina* Peel essential oil		*Citrus grandis* (grapefruit) peel essential oil		*Citrus iyo* peel essential oil		*Citrus junos* peel essential oil	
	Cold-pressed[13]		Cold-pressed[14]		Hydrodistilled[18,20]		Cold-pressed[14]		Hydrodistillation[17]		Cold-pressed[6]		Lyophilization/vacuum distillation[9]		Cold-pressed[7] or not specified[16]	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
β-Sesquiphellandrene															0.1	
β-Sinensal	Trace	0.1									Trace					
γ-Terpinene	Trace	21.5			0.14	0.31						0.005-0.05			11.4	12.3
δ-Carene															ND	Trace
δ-Elemene											Trace				0.1	0.2
δ-3-Carene						0.17										
Δ3-Carene					Trace	0.01										

	Citrus limon (lemon) peel essential oils				*Citrus nobilis* (mandarin orange) essential oil		*Citrus sinensis* (orange) peel oil						*Citrus reticulata* (tangerine) peel oil	
	Cold-pressed[12,13]		Hydrodistilled[18]		Cold-pressed[14]		Cold-pressed[11,12,15,16]		Hydrodistilled[44]		Steam-distilled[22]		Hydrodistilled[21,44]	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Essential oil content (%)	1.1212		1.318				0.98-1.2112		0.24-1.0744		2.4922		0.30-0.5044	
Constituent														
Limonene	38.1	95.8	37.631	69.71			61.08	96.57	66.8	80.9		77.49	64.1	92.4
(*E*)-β-Farnesene	Trace	0.2												
(*E*)-β-Ocimene	Trace	0.4						0.1						
(*E*)-Caryophyllene	Trace	0.7												
(*E*)-Nerolidol	Trace	Trace												
(Z)-β-Ocimene	Trace	0.3	Trace	0.5						0.34				0.28
Z-Carveol									0.68	4.53				1.29
1-Octanol													0.55	0.95
1,3,8-*p*-Menthatriene									1.21	1.69				
1,8-Cineole			Trace	0.82										
2Z.6E-farnesol			Trace											
3-Carene	Trace	Trace												
3-Furanacetic acid										3.0				
3,3′,4′,5,6,7-Heptamethoxyflavone					X									
3′,4′,5,6,7,8-Hexamethoxyflavone					X									
4-Carene									0.61	0.85				
4-Carvon methanol														0.95
4-Terpineol												0.43		
4-Vinyl guaiacol									1.21	1.27			0.87	2.32
4′,5,6,7-tetramethoxyflavone					X									
4′,5,6,7,8-Pentamethoxyflavone					X									
6-Methylhept-5-en-2-one	Trace	Trace												
30 Carvacrol			Trace	0.69										
Allyl isovalerate														0.36
Benzaldehyde		0.11												
Bicyclogermacrene								Trace						
Borneol		0.16	0.33	2.54				7.63						
Bornyl acetate	Trace	Trace	Trace	0.01										
Camphene	Trace	0.13	Trace	0.24				0.32						
Camphor			Trace	0.58										
Capraldehyde							0.35	5.62						
Caprinaldehyde		0.26						2.10						

(continued)

Table 4. (continued)

	Citrus limon (lemon) peel essential oils				*Citrus nobilis* (mandarin orange) essential oil		*Citrus sinensis* (orange) peel oil						*Citrus reticulata* (tangerine) peel oil	
	Cold-pressed[12,13]		Hydrodistilled[18]		Cold-pressed[14]		Cold-pressed[11,12,15,16]		Hydrodistilled[44]		Steam-distilled[22]		Hydrodistilled[21,44]	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Caryophyllene oxide	Trace	0.3	Trace											
Carvone												0.17	0.47	0.62
d-Carvone										0.39				
Cephrol												0.15		
cis-Carveol												0.22		
cis-Dihydrocarvone			Trace	0.29										
cis-Limonene-1,2-oxide	Trace	0.5												
cis-Linalool oxide THF			0	0.49										
cis-Sabinene hydrate			Trace	0.05										
cis-β-Farnesene														0.1
cis-β-Ocimene								0.26						
Citral		0.27					1.74	7.74						
Citronellyl acetate							0.01	0.22						
Citronellal	Trace	0.4					0.06	0.2					0.45	0.78
Citronellol	Trace	0.15	Trace	0.04				4.18	0.71	1.6			0.8	1.2
Citronellyl acetate	Trace	0.3										0.12		
Copaene														0.89
Decanal	Trace	Trace						0.21	0.71	1.02		0.11	2.33	7.71
Decanol								0.35						
Dehydrolinalool												0.40		
Dodecanol								0.03					0.42	0.65
Elemol													0.35	0.89
Eugenol										0.53				
Farnesol														1.14
Geranial	0.1	2.9					Trace	0.11						
Geraniol	Trace	0.2												
Geranyl acetate	Trace	3.2	Trace	0.56										
Germacarene													0.30	1.07
Germacrene D	Trace	0.8	0.019	1.35				0.08				0.27		
n-Heptane														0.4
Hexadecane								0.2						
Isophorone									1.09	2.92				
Isopropyl cresol														1.36
Lemonol												0.28		
Limonene oxide										0.76				
Linalool	0.1	25.1	0	1.59			0.12	2.32	1.52	2.1		0.22	0.7	2.56
Linalool oxide									0.48	1.12				0.6
Linalyl acetate	Trace	31.2	Trace	2.61			Trace					0.28		0.4
Longipinene														0.4
Menthadien-1-ol														0.42
Myrcene	0.7	10.16	Trace	1.54				2.5				6.27		
Neral	Trace	1.5						0.06						
Nerol	Trace	0.1	Trace	0.24										
Neryl acetate	0.1	3.9						0.04						
Nikkol									5.7					
Nonanal	Trace	0.3	Trace					0.08						
n-Nonane								0.2						
Nootkatone								0.01					0.30	3.95
Octanal	Trace	0.1	0.02	1.59			0.34	0.8					1.06	1.15

(continued)

Table 4. (continued)

	Citrus limon (lemon) peel essential oils				*Citrus nobilis* (mandarin orange) essential oil		*Citrus sinensis* (orange) peel oil						*Citrus reticulata* (tangerine) peel oil	
	Cold-pressed[12,13]		Hydrodistilled[18]		Cold-pressed[14]		Cold-pressed[11,12,15,16]		Hydrodistilled[44]		Steam-distilled[22]		Hydrodistilled[21,44]	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Octyl acetate	Trace	0.1												
p-Cymene	0.1	7.8	0.23	9.84										
p-Cymenene	Trace	0.3												
Pentadecane								0.2						
Perillaldehyde								0.03					1.64	1.65
Piperitenone										0.42				
Sabinene	Trace	6.3	3.8	6.48			0.21	0.5	0.37	0.62		1.29		0.49
Spathulenol	Trace	0.1	Trace											
Terpineol-4	Trace	0.1												
Terpinen-4-ol			Trace	1.28										0.1
Terpinolene	Trace	0.8	0.02	0.22				0.08						
Terpinyl acetate		0.5	Trace	0.15										
Tetradecane								0.2						
Thymol	Trace	1.1						Trace						
trans-α-Bergamotene	0.2	1.3												
trans-Carveol												0.44		
trans-Limonene oxide								0.01				0.20		
trans-Limonene-1,2-oxide	Trace	0.6												
trans-Linalool oxide THF	Trace	Trace												
trans-p-1,8-Dienol														0.52
trans-*p*-menth-2-en-1-ol	Trace	0.1												
trans-p-2,8-Menthadien-1-ol									0.79	1.07				
trans-Sabinene hydrate	Trace	0.1												
Tri-Cyclen			0.02	0.16										
Valencene			Trace	0.37				0.05	0.41	1.2				
α-Bisabolene		0.2												
α-Cadinol														0.46
α-Copaene								0.04						
α-Cubebene										0.7				0.48
α-Farnesene													0.36	0.48
α-Humulene	Trace	0.2	Trace	0.04										
α-Phellandrene	Trace	0.6								0.46				
α-Pinene	0.1	2.63	1.14	5.9			0.35	1.4	1.65	2.48		1.49	0.6	1.67
α-Sinensal									0.35	0.49			0.93	5.0
α-Terpinene	Trace	0.4	Trace	1.05										
α-Terpineol			0.93	1.7										1.51
α-Terpineol	Trace	0.4						0.07	2.34	3.1		0.14	0.2	1.1
α-Terpinolene		0.25					1.56	2.06				0.38		0.1
α-Thujene	Trace	1.7	Trace	0.38			0.04	0.11				0.11		
α-Thujone			Trace	0.35										
β-Bisabolene	0.2	2.0												
β-Caryophyllene												0.21		1.39
β-Cubebene								0.03						0.37
β-Eleme	Trace	0.5						0.1						
β-Elemene												0.17		
β-Farnesene												3.64		
β-gurjurene								0.0						
β-Myrcene								1.88	3.76	4.41			3.27	4.05
β-Ocimene												0.27		
β-Phellandrene	0.1	4.2												1.8

(continued)

Table 4. (continued)

	Citrus limon (lemon) peel essential oils				*Citrus nobilis* (mandarin orange) essential oil		*Citrus sinensis* (orange) peel oil						*Citrus reticulata* (tangerine) peel oil	
	Cold-pressed[12,13]		Hydrodistilled[18]		Cold-pressed[14]		Cold-pressed[11,12,15,16]		Hydrodistilled[44]		Steam-distilled[22]		Hydrodistilled[21,44]	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
β-Pinene	0.1	15.8	0.63	31.49			Trace	5.45				0.41	0.2	0.4
β-Sinensal									0.33	0.83				0.67
γ-Eudesmol														1.08
γ-Munrolene														1.16
γ-Terpinene	Trace	18.57	0.04	9.96			0.2	1.21				3.34	0.23	2.6
δ-Amorphene								0.05						
δ-Cadinene										0.32				0.88
δ-3-Carene								0.14				0.69		
Δ3-Carene	0.1	4.2	Trace						0.78	1.03				

Abbreviation: ND, none detected.

[a]Information on various cultivars and/or harvest times. X denotes these compounds were present; however, the amount present was not specified.

Table 5. Typical Levels of 5-Methoxypsoralen (5-MOP).

Ingredient	5-MOP level[26]
Tangerine oil cold-pressed	50 ppm
Mandarin oil cold-pressed	250 ppm

Table 6. Levels of Major Coumarins and Furocoumarins in Lemon Oil and Lime Oil.

Compound	% in lemon oil[8]	% in lime oil[8]	Photosensitizing activity[8]
5-Geranoxypsoralen	0.0387	2.2-2.5	0
5-Geranoxy-7-methoxycoumrin	0.0603	2.2-5.2	0
5-Geranoxy-8-methoxypsoralen	Not analyzed	0.945	0
5,7-Dimethoxycoumarin	0.0295	0.464	0
5,8-Dimethoxypsoralen	Not analyzed	0.508	0
Oxypeucedanin	0.005-0.073	0.0025	+
5-Methoxypsoralen	0.0001-0.0087	0.17-0.33	++++

Table 7. Constituents That Are Established Contact Allergens in Humans, According to the SCCS.

Constituent	Categorized according to number of patients reacting positively and to the number of patients tested (>1000 patients tested, unless indicated as rt, ie, rarely tested)[45]
β-Caryophyllene	≤10 (oxidized and nonoxidized)
Carvone	≤10 (rt)
Citral	101-1000
Citronellol	11-100
Coumarin	101-1000
Farnesol	101-1000
Geraniol	101-1000
Linalyl acetate	≤10
α- and β-pinene	11-100
(DL)-Limonene	11-100 (nonoxidized); 101-1000 (oxidized)
Terpineol (mixture of isomers)/ α-terpineol	≤10
Terpinolene	11-100

Abbreviations: rt, room temperature; SCCS, Scientific Committee on Consumer Safety.

cases, no reported uses were identified by the VCRP, but a maximum use concentration was provided in the industry survey. For example, *C grandis* (grapefruit) peel oil was not reported in the VCRP database to be in use, but the industry survey indicated that it is used at concentrations up to 0.05%. It should be presumed that *C grandis* (grapefruit) peel oil is used in at least 1 cosmetic formulation.

Table 9 lists the 7 citrus-derived peel oils not indicated to be in use based on the VCRP data and the results of the Council concentration of use survey.

Under the rules governing cosmetic products in the European Union, citrus-derived ingredients must have furocoumarin content below 1 mg/kg in sun protection products and in bronzing products.[25] The International Fragrance Association (IFRA) has issued standards for citrus oils and other furocoumarin-containing essential oils.[26] Thus, finished products that are applied to the skin, excluding rinse-off products like bath preparations and soaps, must not contain more than 0.0015%, or 15 ppm, 5-MOP. This equates to 0.0075%, or 75 ppm, in a fragrance compound used at 20% in a consumer product that is applied to the skin. If the level of 5-MOP has not been determined, limits specified for individual oils should be observed, and when such oils are used in combination with other phototoxic ingredients, the potential for an additive effect should be considered and use levels should be reduced accordingly. Restrictions for furocoumarin-containing essential oils have been recommended for bitter orange oil expressed, grapefruit oil expressed, lemon oil cold-pressed, and lime oil expressed.

An IFRA standard also has been issued for 7-methoxycoumarin, which is prohibited for use in fragrance compounds.[27] Based on established maximum levels of this substance from commercially available natural sources (like essential oils, extracts, and absolutes), exposure to 7-methoxycoumarin from the use of these oils and extracts is regarded to be acceptable if the level of 7-methoxycoumarin in the finished product does not exceed 100 ppm. An example of a maximum concentration based on this standard is 0.1% for lime cold-pressed oil.

Additionally, IFRA has set a standard stating that D-, L-, and DL-limonene and natural products containing substantial amounts of it should only be used when the level of peroxides is kept to the lowest practical level; such products should have a peroxide value of <20 mmol/L.[28] This standard is also cited by the European Commission.[29] The European Union also states that limonene must be included in the list of ingredients when its concentration exceeds 0.001% in leave-on products and 0.01% in rinse-off products.

The IFRA has also set limits on the amounts of some citrus-derived oils in finished products. For leave-on products applied to skin areas exposed to direct sunlight, these limits include 1.25% bitter orange peel expressed,[30] 4% grapefruit oil expressed,[31] 2% lemon oil cold-pressed,[32] and 0.7% lime oil expressed.[33] There are no restrictions for any of these oils in rinse-off products and products that are not applied to the skin. International Fragrance Association specified that if combinations of phototoxic fragrance ingredients are used, the use levels must be reduced accordingly, so that the sum of the concentrations of all phototoxic fragrance ingredients, expressed as a percentage of their respective recommended maximum levels, shall not exceed 100% in the consumer product. Additionally, the IFRA general standard described above for "Citrus oils and other furocoumarins-containing essential oils" must be considered.

Table 8. Frequency (2014) and Concentration of Use (2013) According to Duration and Type of Exposure for Citrus-Derived Ingredients.[23,24]

	Number of use[46]	Maximum concentration of use (%)	Number of uses	Maximum concentration of use (%)	Number of uses	Maximum concentration of use (%)	Number of uses	Maximum concentration of use (%)
	Citrus aurantium amara (bitter orange) peel oil		*Citrus aurantium dulcis* (orange) peel oil (Listed as *Citrus sinensis* (sweet orange) peel oil in VCRP)		*Citrus grandis* (grapefruit) peel oil		*Citrus junos* peel oil	
Totals[a]	127	0.05-2	289	0.00002-29	NR	0.00004-0.05	6	NR
Duration of use								
Leave-on	74	0.2-2	156	0.00038-0.54	NR	0.0004-0.0008	4	NR
Rinse-off	45	0.05-0.25	110	0.00002-29	NR	0.00004-0.05	2	NR
Diluted for (bath) use	8	NR	23	0.33	NR	0.0014	NR	NR
Exposure type								
Eye area	2	NR	3	0.1	NR	NR	NR	NR
Incidental ingestion	1	0.75	4	NR	NR	NR	NR	NR
Incidental inhalation—Spray	27[b]; 28[c]	NR	56[b]; 55[c]	0.00038[b]	NR	NR	2	NR
Reported spray	NR	NR	NR	NR	NR	NR	NR	NR
Incidental inhalation—Powder	28[c]	0.9-2[d]	3[d]; 55[c]	0.03-0.4[d]	NR	0.0004[d]	1	NR
Reported powder	NR	NR	NR	NR	NR	NR	NR	NR
Dermal contact	115	0.05-2	218	0.001-0.4	NR	0.00004-0.05	6	NR
Deodorant (underarm)	NR	NR	1[b]	NR	NR	NR	NR	NR
Reported spray	NR	NR	NR	NR	NR	NR	NR	NR
Reported as not spray	NR	NR	NR	NR	NR	NR	NR	NR
Hair—Noncoloring	11	NR	65	0.00002-29	NR	0.005	NR	NR
Hair—Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	2	0.5-0.54	NR	NR	NR	NR
Mucous membrane	21	0.25-0.75	56	0.1-0.33	NR	0.0014	1	NR
Baby products	6	NR	8	NR	NR	NR	NR	NR

	Number of use[46]	Maximum concentration of use (%)	Number of uses	Maximum concentration of use (%)	Number of uses	Maximum concentration of use (%)	Number of uses	Maximum concentration of use (%)
	Citrus limon (lemon) peel oil		*Citrus nobilis* (mandarin orange) peel oil		*Citrus reticulata* (mandarin orange) peel oil[e]		*Citrus sinensis sanguinello* (blood orange) peel oil[e]	
Totals[a]	490	0.0001-0.5	152	0.00005-0.1	2	NR	8	NR
Duration of use								
Leave-on	297	0.0001-0.5	86	0.00005-0.1	1	NR	4	NR
Rinse-off	165	0.0006-0.001	58	0.00005-0.03	1	NR	4	NR
Diluted for (bath) use	28	0.012	8	NR	NR	NR	NR	NR
Exposure type								
Eye area	9	NR	NR	NR	NR	NR	NR	NR
Incidental ingestion	8	NR	1	0.0099	NR	NR	NR	NR
Incidental inhalation—Spray	84[b]; 96[c]	0.06[c]	45[b]; 26[c]	NR	1[c]	NR	1[b]; 2	NR
Reported spray	NR	NR	NR	NR	NR	NR	1	NR
Incidental inhalation—Powder	1[d]; 96[c]	0.001-0.14[d]; 0.06[c]	26[c]	0.00005-0.14	1[c]	NR	2[c]	NR
Reported powder	NR	NR	NR	NR	NR	NR	NR	NR

(continued)

Table 8. (continued)

	Number of use[46]	Maximum concentration of use (%)	Number of uses	Maximum concentration of use (%)	Number of uses	Maximum concentration of use (%)	Number of uses	Maximum concentration of use (%)
	Citrus limon (lemon) peel oil		*Citrus nobilis* (mandarin orange) peel oil		*Citrus reticulata* (mandarin orange) peel oil[e]		*Citrus sinensis sanguinello* (blood orange) peel oil[e]	
Dermal contact	411	0.0001-0.5	136	0.00005-0.1	2	NR	8	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Reported spray	NR	NR	NR	NR	NR	NR	NR	NR
Reported as not spray	NR	0.002	NR	NR	NR	NR	NR	NR
Hair—Noncoloring	67	NR	15	0.00005-0.03	NR	NR	NR	NR
Hair—Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	3	0.0001-0.14	NR	0.012	NR	NR	NR	NR
Mucous membrane	96	0.001-0.012	40	0.0099	NR	NR	4	NR
Baby products	8	NR	NR	NR	NR	NR	NR	NR

	Number of use[46]	Maximum concentration of use (%)	Number of uses	Maximum concentration of use (%)	Number of uses	Maximum concentration of use (%)	Number of uses	Maximum concentration of use (%)
	Citrus tangerina (tangerine) peel oil							
Totals[a]	34	0.0000013						
Duration of use								
Leave-on	18	0.0000013						
Rinse-off	15	0.0000013						
Diluted for (bath) use	1	NR						
Exposure type								
Eye area	NR	NR						
Incidental ingestion	1	NR						
Incidental inhalation—Spray	5[b]; 8[c]	0.0000013b						
Reported spray	1	NR						
Incidental inhalation—Powder	8[c]	NR						
Reported powder	NR	NR						
Dermal contact	26	NR						
Deodorant (underarm)	NR	NR						
Reported spray	NR	NR						
Reported as not spray	NR	NR						
Hair—Noncoloring	7	0.0000013						
Hair—Coloring	NR	NR						
Nail	NR	NR						
Mucous membrane	7	NR						
Baby products	NR	NR						

Abbreviations: INCI, International Nomenclature of Cosmetic Ingredient; NR, not reported; VCRP, Voluntary Cosmetic Registration Program.
[a]Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
[b]It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.
[c]Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.
[d]It is possible these products may be powders, but it is not specified whether the reported uses are powders.
[e]Use in a spray product has been reported in response to a survey conducted by the Council.

Table 9. Ingredients That Are Not Reported to Be in Use.

Citrus aurantifolia (lime) peel oil
Citrus aurantium currassuviensis peel oil
Citrus clementina peel oil
Citrus iyo peel oil
Citrus medica vulgaris peel oil
Citrus reticulata (Tangerine) peel oil
Citrus tachibana/reticulata peel oil

Noncosmetic

The essential oils, oleoresins (solvent-free), and natural extractives (including distillates) derived from the following citrus fruits are GRAS for their intended use in foods for human consumption: *C aurantifolia* (lime), *C aurantium* (bitter orange; the flowers and peel), *C limon* (lemon), *C reticulata* (tangerine), *Citrus reticulata blanco* (mandarin), *C sinensis* (orange; the leaf, flowers, and peel), and citrus peels (species not specified; 21CFR182.20). These essential oils, oleoresins (solvent-free), and natural extractives (including distillates) of these citrus fruits are GRAS for their intended use in animal drugs, feeds, and related products (21CFR582.20).

Citrus essential oils are used in the pharmaceutical industry as flavoring agents to mask the unpleasant taste of drugs.[18] *Citrus aurantium amara* (bitter orange) and extracts of its dried fruit and peel have been used in traditional Western medicines and in Chinese and Japanese herbal medicines.[34]

Toxicological Studies

As noted earlier, the citrus ingredients in this assessment are found in foods, and daily exposures from food use would result in a much larger systemic exposure than those from use in cosmetic products. Essential oils, oleoresins (solvent-free), and natural extracts (including distillates) derived from some citrus fruits are GRAS for their intended use in foods for human and animal consumption according to the FDA. Volatile oils of limes, lemons, grapefruits, bitter oranges, oranges, and tangerines are described as flavoring agents in the USP Food Chemicals Codex.[1] Therefore, the systemic toxicity potential of these ingredients is not addressed further in this report. The primary focus of this safety assessment is on the potential for irritation and sensitization from dermal exposure to these citrus ingredients as used in cosmetic products.

Acute Toxicity

Dermal—Nonhuman

Lemon oil. The dermal LD_{50} of lemon oil was greater than 10 g/kg in rabbits.[3] An occlusive patch of undiluted oil was applied to the skin of 6 animals for 24 hours. One animal died during the observation period.

Mandarin oil—Expressed (C reticulata). The dermal LD_{50} of mandarin peel oil (C reticulata) was greater than 5 g/kg in rabbits.[10] An occlusive patch of undiluted oil was applied to the skin of seven animals for 24 hours.

Orange oil. The dermal LD_{50} of sweet orange oil was greater than 5 g/kg in female New Zealand White rabbits.[4] An occlusive patch of undiluted oil was applied to the skin of 10 animals for 24 hours. Signs of skin irritation were reported, including moderate redness in 10 of 10 animals, slight edema in 3 of 10 animals, and moderate edema in 5 of10 animals.

Reproductive and Developmental Toxicity

No published reproductive and developmental studies on citrus-derived peel oils were identified in a literature search for these ingredients, and no unpublished data were submitted.

Genotoxicity

Genotoxicity studies for in vitro assays are summarized in Table 10. No genotoxic effects were observed for lemon oil or sweet orange oil in bacterial reverse mutation assays, mouse lymphoma cell mutation assays, and Chinese hamster chromosome aberration assays.[3,4]

Carcinogenicity

Orange Oil, Lemon Oil, Grapefruit Oil, and Lime Oil

Tumor-promoting activity was observed in mouse skin exposed to essential oils of orange (sweet), lemon, grapefruit, or lime.[35] Chemical constituents of these oils were not fully identified in this study, although the terpene and nonterpene fractions were separated. Groups of 10 male and 10 female strain 101 mice received a single application of 9,10-dimethyl-1,2-benzanthracene (DMBA) in acetone (300 μg in 0.2 mL in 4 groups, 225 μg in 0.15 mL in a fifth group). Group 1 was a control group that received no further treatments. Groups 2 through 5 received weekly applications of 0.25 mL of the test substances 3 weeks after the application of DMBA.

By the fifth week, papillomas were observed in group 3 (lemon oil), group 4 (grapefruit oil), and group 5 (lime oil). Papillomas were observed in group 2 (orange oil) by the 12th week. After 33 weeks, 10 of 20 mice in the lemon oil and lime oil treatment groups and 13 of 20 mice in the grapefruit oil and orange oil groups had papillomas. Only 1 mouse in the control group had papillomas after 33 weeks, and the affected site was not the treated skin. Additionally, one female mouse of the lemon oil group developed a sebaceous-gland tumor of the nipple. No malignant skin tumors were observed in the orange oil group: Treatment for this group was stopped after 42 weeks. Squamous cell carcinomas of the skin were observed in 2 mice from the lemon oil group and2 mice of the grapefruit oil group between weeks 36 and 55. One malignant skin tumor was observed in the lime oil group at week 34; however, the mouse was found dead and a proper histological examination was not possible. No malignant skin tumors were observed in the control group. Nondermal tumors during the treatment period were observed in one mouse of the orange oil group (a hemangioma

Table 10. Genotoxicity.

Test article	Concentration/ dose	Procedure	Results	Reference
In vitro				
Lemon oil in ethanol	10-5000 μg/plate	Reverse mutation assay using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2uvrA, with and without S9 metabolic activation	Toxicity was observed in all test strains except WP2uvrA with and without S9; no significant dose-related increases in the number of revertant colonies in any test strain at any dose level, with or without metabolic activation; controls yielded expected results; lemon oil was not mutagenic in this assay	[3]
Lemon oil in ethanol	40-100 μg/mL	Cell mutation assay in mouse lymphoma L5178Y TK ± cells in accordance with OECD guideline 476 in 2 independent experiments; with and without S9 metabolic activation	No significant increases in the mutation frequency were observed in either experiment, with or without S9 activation; controls yielded the expected results; lemon oil did not induce gene mutations in mouse lymphoma cells	[3]
Lemon oil in ethanol	Up to 0.125 mg/mL	Chromosome aberration study using Chinese hamster lung fibroblasts (CHL) in accordance with OECD guideline 473, without metabolic activation, 100 metaphases examined	The incidence of polyploidy cells at 24 hours post-treatment was 1.0%, and the incidence of cells with structural chromosome aberrations at 24 hours after treatment was 2.0%; the test material did not significantly induce chromosomal aberrations in CHL cells; lemon oil was not considered clastogenic	[3]
Sweet orange oil in ethanol	1-5000 μg/plate	Reverse mutation assay using *S typhimurium* strains TA98, TA100, TA1535, and TA1537 and *E coli* strain WP2uvrA, with and without S9 metabolic activation	Cytotoxicity was observed in all test strains except WP2uvrA with and without S9; no significant dose-related increases in the number of revertant colonies in any test strain at any dose level, with or without metabolic activation; controls yielded expected results; sweet orange oil was not mutagenic in this assay	[4]
Sweet orange oil in ethanol	40-100 μg/mL	Cell mutation assay in mouse lymphoma L5178Y TK ± cells in accordance with OECD guideline 476 in 2 independent experiments; with and without S9 metabolic activation	The test material did not induce a significant increase in the mutation frequency in both experiments, with or without metabolic activation; sweet orange oil did not induce gene mutations in mouse lymphoma cells	[4]
Sweet orange oil in ethanol	Up to 0.125 mg/mL	Chromosome aberration study using CHL fibroblasts in accordance with OECD guideline 473, without metabolic activation, 100 metaphases examined	The incidence of polyploidy cells at 48 hours post-treatment was 1.0%, and the incidence of cells with structural chromosome aberrations at 48 hours after treatment was 1.0%; the test material did not significantly induce chromosomal aberrations in CHL cells; sweet orange oil was not considered clastogenic	[4]

Abbreviation: OECD, Organisation for Economic Co-operation and Development.

of the subcutaneous tissue starting at week 7) and in one mouse of the grapefruit oil group (a spindle cell sarcoma of the subcutaneous tissues). No tumors of the internal organs were observed. The survival of all the mice, including the controls, in this experiment was poor because of a very high incidence of renal disease.[35]

Orange Oil

Tumor-promoting activity was observed in mouse skin exposed to orange (sweet) oil.[35] In the study, groups of 10 male and 10 female strain 101 mice received a single application of DMBA in acetone (300 μg in 0.15 mL). One group (15 mice of each

Table 11. Dermal Irritation Studies for Citrus-Derived Ingredients.

Test article	Concentration/ dose	Test population	Procedure	Results	Reference
Nonhuman					
Lemon oil	5 mL/kg	6 New Zealand White rabbits	Acute dermal toxicity limit test scored under the Draize method; 24-hour occlusive patches on intact and abraded skin	Irritating	3
Orange oil, cold-pressed	0.5 mL of undiluted material	3 male albino rabbits	Skin irritation study conducted according to OECD guideline 404; semi-occluded patch for 4 hours	Mean erythema/eschar scores were 2.0, 1.7, and 2.0; mean edema scores were 2.0, 1.3, and 1.3; irritating to skin	4
Mandarin peel oil, expressed (described as *Citrus reticulata*)	5 mg/kg	7 rabbits	24-hour occlusive, single dose study	Slight erythema and edema	10
Mandarin peel oil, expressed (described as *C reticulata*)	Not reported	Hairless mice and miniature swine; details not provided	Open patch tests; details not provided	2 of 3 samples were irritating	10
Human					
Lemon oil	0.3%, 2%, or 20%; multiple vehicles	34 patients at 0.3%, 30 subjects at 2%, and 35 patients at 20%	24-72 hours occlusive patch tests	No irritation at 0.3% and 20%, 1 $\pm$ reaction at 2%	36
Mandarin peel oil, expressed (described as *C reticulata*)	8% in petrolatum	5 patients	48 hours closed patch test; details not provided	No irritation	10

Abbreviation: OECD, Organisation for Economic Co-operation and Development.

sex) was a control group that received no further treatments. Two groups received weekly applications of 0.25 mL of 40% orange oil in acetone or 80% orange oil in acetone 3 weeks after the initial application of DMBA. The applications continued for 37 weeks.

Papillomas were observed in both groups treated with orange oil starting on the 12th week. After 33 weeks, 5 of 10 mice treated with 40% orange oil and 10 of 10 mice treated with 80% orange oil had papillomas, and at study end, only 1 tumor of each group was found outside the treated area. Four mice in the control group had papillomas by week 33, but these tumors were outside the treated area of the skin. Malignant tumors were observed in one mouse of each treatment group, arising from the preexisting papilloma. Both tumors were squamous cell carcinomas, infiltrating the panniculus muscle. Additionally, tumors of the urethral orifice were observed in 4 female mice of the 40% orange oil group. The survival of all the mice in this experiment was poor due to a very high incidence of renal disease.

In the same study, tumor-promoting activity was observed in mice exposed to undiluted orange oil after pretreating the mice with either dermal or intraperitoneal injections of urethane. The effect was weak compared to the effects observed after DMBA induction.

A similar experiment performed in the same study tested the carcinogenic effects of orange oil without pretreatment with DMBA or urethane. The mice were treated once weekly with 0.25 mL of 40% or 80% orange oil in acetone or undiluted orange oil for 38 (diluted) or 46 (undiluted) weeks. This study found no evidence of direct tumorigenic effects on the treated mouse skin. Urethral orifice tumors were observed in one female mouse of the 40% orange oil group and in one female mouse of the 80% orange oil group. A papilloma was observed on the head of a mouse (outside the treatment site) that was treated with 80% orange oil.[35]

Irritation and Sensitization

Dermal Irritation

Dermal irritation studies are summarized in Table 11. Lemon oil, orange oil, and mandarin peel oil all produced some reaction in irritation studies in animals, but in human patients, no irritation was observed after topical exposure to lemon oil (up to 20%) or mandarin peel oil (8%).[3,4,10,36]

Ocular Irritation

Lemon oil. Lemon oil tested at 5% was not irritating to the eyes of 3 albino rabbits.[3] Each rabbit had 0.1 mL of the test material

Table 12. Sensitization Studies for Citrus-Derived Ingredients.

Test article	Concentration/ dose	Test population	Procedure	Results	Reference
Human: Patch					
Bitter orange oil	2% in paraffin	200 patients with dermatitis tested with 35 essential oils plus an additional 50 patients with balsam sensitivity	Sensitization patch study, details not provided	6 positive reactions	37
Lemon oil	2% in paraffin	200 patients with dermatitis tested with 35 essential oils plus an additional 50 patients with balsam sensitivity	Sensitization patch study, details not provided	4 positive reactions, details not provided	37
Lemon oil	Not reported	100 patients	Marzulli-Maibach sensitization technique; open patches	2% of the patients had a positive skin reaction at the first reading after challenge, no reactions were noted at the 48 and 72 hours readings, study concluded the test material was not sensitizing	3
Mandarin oil, expressed (described as *Citrus reticulate*)	8% in petrolatum	25 patients	Maximization study, details not provided	Not sensitizing	10
Sweet orange oil	2% in paraffin	200 patients with dermatitis tested with 35 essential oils plus an additional 50 patients with balsam sensitivity	Sensitization patch study, details not provided	3 positive reactions, details not provided	37
Sweet orange oil	Not reported	100 patients	Marzulli-Maibach sensitization technique; open patches	4% of the patients had positive skin reaction at the first reading after challenge, no reactions were noted at the 48 and 72 hours readings, study concluded the test material was not sensitizing	4

instilled into the right eye with no further treatment. The left eye served as the control. Eyes were examined every 24 hours for 4 days and then again on the seventh day. No corneal opacity or iris congestion was observed. An intense conjunctival irritation involving chemosis and discharge occurred. Treated eyes were normal on the seventh day.

Orange oil. Orange oil tested undiluted did not induce significant or irreversible damage to the eyes of 3 male New Zealand White rabbits.[4] The test was performed in accordance with Organisation for Economic Co-operation and Development Test Guideline 405. The test material (0.1 mL) was instilled into one eye of each rabbit. The mean scores calculated for the 3 animals across 3 scoring times were 0.0 for corneal opacity and iris lesions and 1.0 for reddening of the conjunctivae.

Sensitization

Sensitization studies are presented in Table 12. Mandarin peel oil (8% in petrolatum) was not sensitizing in human maximization tests. In studies of 250 patients with dermatitis, less than 2.5% had positive reactions to bitter orange oil, lemon oil, or sweet orange oil tested at 2% in paraffin.[3,4,10,37]

Phototoxicity and Photosensitization

Phototoxicity and photosensitization studies are presented in Table 13. Phototoxic potential was observed for sweet orange oil and lemon oil in in vitro studies. No phototoxic responses were noted in some animal studies of lime oil, lemon oil, grapefruit oil, mandarin oil, or tangerine oil; however, signs of phototoxicity were observed in response to undiluted lime oil and lime oil diluted to concentrations of 15%, undiluted bitter orange oil, and undiluted lemon oil and lemon oil diluted to concentrations of 50%. In human studies, phototoxic reactions were observed in response to undiluted bitter orange oil, lemon oil (1%), sweet orange oil (1%), and undiluted and diluted expressed lime oil (30%). Many of the citrus-derived peel oils contain constituents that are photoactive agents, although those noted to be furocoumarin free tended not to induce photosensitization.[19,30,38-41]

Occupational Exposure

In a retrospective study (2001-2010) of professional food handlers in Denmark, 8.5% (16 of 188) of the patients had positive skin prick test reactions to orange peel and 7.9% (15 of 191) of

Table 13. Photosensitization and Phototoxicity Studies.

Test article	Concentration/dose[a]	Test population	Procedure	Results	Reference
Alternative studies					
Sweet orange oil, including deterpenated kind	Concentrations not reported; tested in PBS, ethanol, or DMSO with samples from 3 suppliers	3T3 Balb/c fibroblasts	- 3T3 neutral red uptake phototoxicity test - Light source was a doped mercury-metal halide lamp, filtered with 50% transmission at 335 nm to diminish UVB	Borderline phototoxic, positive phototoxic results observed more frequently with the vehicles PBS and ethanol with certain supplied samples	38
Lemon oil, including deterpenated kind	Same as above	Same as above	Same as above	Borderline phototoxic, positive phototoxic results observed in all 3 vehicles, but were more prominent in the deterpenated sample	38
Sweet orange oil, including deterpenated kind	Up to 3.16% in water with samples from 3 suppliers	Same as above	Same as above	Potential for phototoxicity observed	38
Lemon oil, including deterpenated kind	Same as above	Same as above	Same as above	Cytotoxicity observed with deterpenated lemon oil; potential for phototoxicity observed	38
Nonhuman					
Lime oil, distilled (psoralen-free)	Undiluted; 20 μL	Hairless mice, 6/group	- A single dose was applied to a 2 cm^2 area on the back; animals were exposed to irradiation 30 minutes after dosing - One group was exposed to a compact-arc xenon lamp for 2 minutes (wavelengths $<$295 nm or 320-280 nm excluded) - One group was exposed to a long-arc xenon lamp for 40 minutes at a distance of 1 m; the weighted energy was 0.1667 W/m^2 - One group was exposed to 4 fluorescent black light lamps (UVB eliminated) for 1 hour at an integrated UVA intensity of 3 W/m^2 - Positive controls were treated with 0.01% 8-methoxypsoralen in methanol; negative controls with an appropriate vehicle - Test sites were examined 4, 24, 48, 72, and 96 hours after exposure	- A phototoxic response was not observed with any of the light sources	40
Lime oil, distilled (psoralen-free)	Undiluted; 20 μL	Miniature swine, 2/group	As above	- A phototoxic response was not observed with any of the light sources	40
Lime oil, expressed	Undiluted and diluted; 20 μL	Hairless mice, 6/group	As above	- A phototoxic response was observed with all 3 light sources - The lowest phototoxic concentration was 15%	40
Lime oil, expressed	Undiluted and diluted; 20 μL	Miniature swine, 2/group	As above	- A phototoxic response was observed with all 3 light sources - The lowest phototoxic concentration was 30%	40
Grapefruit oil	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	Not photosensitizing	40

(continued)

Table 13. (continued)

Test article	Concentration/dose[a]	Test population	Procedure	Results	Reference
Lime oil distilled (psoralen-free)	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	Not photosensitizing	[40]
Lime oil; expressed and rectified	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	Not photosensitizing	[40]
Lime oil Persian Florida; expressed and rectified	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	Not photosensitizing	[40]
Mandarin oil	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	Not photosensitizing	[40]
Mandarin oil, Italian	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	Not photosensitizing	[40]
Oil of lemon, California	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	Not photosensitizing	[40]
Oil of lemon, distilled	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	Not photosensitizing	[40]
Oil mandarin, Italian	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	Not photosensitizing	[40]
Oil of tangerine	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	Not photosensitizing	[40]
Orange oil; cold-pressed	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	Not photosensitizing	[40]
California lemon oil	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	- A phototoxic response was observed	[40]
Italian lemon oil	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	- A phototoxic response was observed	[40]
Oil lemon, Greek; cold-pressed	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	- A phototoxic response was observed	[40]
Oil lemon, Italian	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	- A phototoxic response was observed	[40]
Oil lemon, IC (not defined)	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	- A phototoxic response was observed	[40]
Lime oil, expressed	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	- A phototoxic response was observed	[40]
Oil limes, Persian	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	- A phototoxic response was observed	[40]
Oil limes, expressed and rectified	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	- A phototoxic response was observed	[40]
Lime oil, expressed and rectified	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	- A phototoxic response was observed	[40]
Bitter orange oil	Undiluted; 20 μL	6 hairless mice and 2 miniature swine	- As above	- A phototoxic response was observed	[40]
Lemon oil from multiple regional sources	20%, 50%, or 100% in ethanol; 0.02 mL	Albino guinea pigs	- The oil was applied to the shaved back of the animals - The animals were then exposure to UVA radiation (320-400 nm, 13 J/cm^2)	- Concentrations of 50% and 100% elicited phototoxicity in most of the samples tested - Lemon oils from different regions had different phototoxicity potencies	[41]

(continued)

Table 13. (continued)

Test article	Concentration/dose[a]	Test population	Procedure	Results	Reference
			- Erythema was evaluated 24, 48, and 72 hours after irradiation - The samples were then fractionated subsequent phototoxicity testing of the isolated components was performed	- Oxypeucedanin and 5-methoxypsoralen (furocoumarins) were identified as phototoxic	
Human					
Bitter orange peel oil	Undiluted; 5 $\mu L/cm^2$	8 patients	- An occlusive patch was applied to a 2 cm $\times$ 2 cm area - 1 cm site on each patient was exposed to visible light of 20 J/cm^2 UVA - The test sites were scored after 24 and 48 hours	All patients reacted (details not provided)	30
Lemon oil, including deterpenated kind	Up to 1% in water from samples from 3 supplier; dose not provided	5 female patients	-2 occlusive 10 mm diameter Finn Chambers on both sides of the lower back - Exposure time to test material was 1 hour - Irradiation immediately to 1 site after patch removal at a dose of 5 J/cm^2as measured in the UVA range - Test sites scored after 24, 48, and 72 hours - Light source was a doped mercury-metal halide lamp, filtered with 50% transmission at 335 nm to diminish UVB	- Phototoxic reactions concurrent with an irritation reactions were observed in lemon oil at 1% in 4 of 5 patients up to 72 hours after irradiation - Phototoxic reactions were observed in deterpenated lemon oil at 0.1% in 2 of 5 patients at 48 and 72 hours after irradiation - No reactions were observed at concentrations of 0.1% or lower in lemon oil and 0.01% in deterpenated lemon oil	38
Sweet orange oil, including deterpenated kind	Same as above	Same as above	- Same as above	- Phototoxic reaction were observed in orange oil at 1% in 3 of 5 patients at 24 hours and 2 of 5 patients at 48 and 72 hours after irradiation - No reactions were observed at concentrations of 0.1% or lower in orange oil or 0.1% and 0.01% in deterpenated orange oil	38
Lime oil, distilled	Undiluted	10 Caucasian patients	- A single dose was applied to a 2-cm^2 area on the back - 30 minutes after dosing, patients were exposed to sunlight for 30 minutes or a compact-arc xenon lamp for 2 minutes (wavelengths $<$295 nm or 320-280 nm excluded) - Positive controls were treated with 0.01% 8-methoxypsoralen in methanol; negative controls with an appropriate vehicle - Test sites were examined 4, 24, 48, 72, and 96 hours after exposure	No phototoxic response was observed	40

(continued)

Table 13. (continued)

Test article	Concentration/dose[a]	Test population	Procedure	Results	Reference
Lime oil, expressed	Undiluted and diluted; 20 μL	10 Caucasian patients	- A single dose was applied to a 2 cm^2 area on the back - 30 minutes after dosing, 1 treated site and the control untreated site were exposed to sunlight for 30 minutes, a compact-arc xenon lamp for 2 minutes (wavelengths <295 nm or 320-280 nm excluded), or 4 fluorescent black light lamps (UVB eliminated) for 1 hour at an integrated UVA intensity of 3 W/m^2 - Positive controls were treated with 0.01% 8-methoxypsoralen in methanol; negative controls with an appropriate vehicle - Test sites were examined 4, 24, 48, 72, and 96 hours after exposure	- A phototoxic response was observed with all 3 light sources - The lowest phototoxic concentration with the simulated light sources was 30%	[40]

Abbreviations: DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline; UVA, ultraviolet A; UVB, ultraviolet B.
[a]The solvent is specified when known.

the patients had positive skin prick test reactions to lemon peel.[42]

Summary

The 14 citrus-derived peel oils described in this report function primarily as skin conditioning agents-miscellaneous and fragrance. Botanicals such as citrus are composed of hundreds of constituents, some of which have the potential to cause toxic effects; for example, bergapten (as known as 5-MOP) is a naturally occurring, phototoxic furanocoumarin (psoralen) in citrus peel oils. Presently, CIR reviewed the information available on the potential toxicity of each of the citrus peel oil–derived ingredients as a whole, complex substance; CIR did not review the potential toxicity information on the individual constituents of which the citrus-derived ingredients are comprised. Cosmetic Ingredient Review requested information on the concentrations (including ranges, means, upper 95% confidence limits, detection limits, etc) of individual constituents in the citrus peel oil–derived ingredients used in cosmetics, to facilitate the safety assessment of these ingredients. Such information on constituents that have been identified as of concern by the Panel in previous safety assessments, or by other recognized scientific expert review bodies, is especially important.

Citrus oils contain large amounts of monoterpene hydrocarbons; limonene is present in the greatest amount, composing 38.1% to 95.8% of the oils. Citrus oils also contain sesquiterpene hydrocarbons, which are responsible for the characteristic flavor of these oils.

Citrus limon (lemon) peel oil has the most reported uses in cosmetics and personal care products, with a total of 490; more than half of the uses are in leave-on skin care preparations. The range of highest maximum use concentrations for *C limon* (lemon) peel oil is 0.0001% to 0.5%, with 0.5% reported in "other" skin care preparations. *Citrus aurantium dulcis* (orange) peel oil (reported as *C sinensis* [sweet orange] peel oil to the VCRP) has the second greatest number of overall uses reported, with a total of 289; about half of those uses are in leave-on skin care preparations. *Citrus aurantium dulcis* (orange) peel oil had a highest maximum use concentration range of 0.00002% to 29%, with 29% reported in noncoloring hair conditioners.

Under the rules governing cosmetic products in the European Union, citrus-derived ingredients must have furocoumarin content below 1 mg/kg in sun-protection and bronzing products. International Fragrance Association also has issued standards for citrus oils and other furocoumarin-containing essential oils. Finished products that are applied to the skin, excluding rinse-off products like bath preparations and soaps, must not contain more than 0.0015% or 15 ppm 5-MOP. If the level of 5-MOP has not been determined, limits specified for individual oils should be observed, and when such oils are used in combination with other phototoxic ingredients, the potential additive effect should be taken into consideration and use levels should be reduced accordingly. Restrictions for furocoumarin-containing essential oils and limits on the amounts of citrus-derived oils in finished products have been recommended for bitter orange oil expressed, grapefruit oil expressed, lemon oil cold-pressed, and lime oil expressed.

The European Union also set standards for limonene, stating that limonene must be included in the list of ingredients when its concentration exceeds 0.001% in leave-on products and 0.01% in rinse-off products.

The citrus peel oils in this assessment are found in foods, and the daily exposure from food use would result in a much larger systemic dose than that resulting from use in cosmetic products. Essential oils, oleoresins (solvent-free), and natural extractives (including distillates) derived from some citrus fruits are GRAS for their intended use in foods for human and animal consumption.

The dermal LD_{50} of undiluted mandarin peel oil (*C reticulata*) and undiluted sweet orange oil was greater than 5 g/kg in rabbits. In undiluted lemon oil, the dermal LD_{50} was greater than 10 g/kg in rabbits.

No genotoxic effects were observed in lemon oil or sweet orange oil in bacterial reverse mutation assays, mouse lymphoma cell mutation assays, and Chinese hamster chromosome aberration assays. Tumor-promoting activity was observed in mouse skin exposed to undiluted essential oils of orange (sweet), lemon, grapefruit, or lime after pretreatment with DMBA. Related studies of 40%, 80%, or 100% orange oil following pretreatment with DMBA or urethane also reported tumor-promoting activity, although the effect was weaker in the mice induced with urethane. No tumorigenic effects were observed in mice tested with orange oil without pretreatment with DMBA or urethane. Survival rates of the mice, including controls, in these experiments were poor because of a very high incidence of renal disease.

Irritation was observed in animals treated with unspecified concentrations of mandarin peel oil. In human patients, no irritation was observed after topical exposure to lemon oil (up to 20%) or mandarin peel oil (8%).

In rabbits, lemon oil tested at 5% was not irritating and orange oil tested undiluted did not induce significant or irreversible damage to the eyes. Mandarin peel oil (8% in petrolatum) was not sensitizing in human maximization tests. In studies of 250 patients with dermatitis, less than 2.5% had positive reactions to bitter orange oil, lemon oil, or sweet orange oil tested at 2% in paraffin.

Phototoxic potential was observed for sweet orange oil and lemon oil in in vitro studies. No phototoxic responses were noted in some animal studies of lime oil, lemon oil, grapefruit oil, mandarin oil, or tangerine oil; however, phototoxic reactions were observed in response to undiluted lime oil and lime oil diluted to concentrations of 15%, undiluted bitter orange oil, and undiluted lemon oil and lemon oil diluted to concentrations of 50%. In human studies, phototoxic reactions were observed in response to undiluted bitter orange oil, lemon oil (1%), sweet orange oil (1%), and undiluted and diluted expressed lime oil (30%). Many of the citrus-derived peel oils contain constituents that are photoactive agents, although those noted to be furocoumarin free tended not to induce photosensitization.

A retrospective occupational study of food handlers noted positive reactions to orange and lemon peels. No published studies on reproductive and development toxicity of citrus-derived peel oils were discovered and no unpublished data were submitted to address these topics.

Discussion

The citrus ingredients in this assessment are found in foods, and daily exposures from the consumption of foods can be expected to yield much larger systemic exposures to these ingredients than those from the use of cosmetic products. Essential oils, oleoresins (solvent-free), and natural extracts (including distillates) derived from some citrus fruits are GRAS in foods and animal feeds. Additionally, volatile oils of limes, lemons, grapefruits, bitter oranges, oranges, and tangerines are used as flavoring agents. Consequently, the primary focus of this safety assessment is on the potential for irritation and sensitization from dermal exposures to the citrus ingredients.

Most of the reports that the Panel reviewed were not sufficiently detailed to enable determining how well the substances tested represent the cosmetic ingredients included in this safety assessment. For example, the genus and species of the plants from which the test substances were derived, the cultivation methods used to grow the plants, and the methods of extraction are not specified in many of these studies.

The Panel expressed concern about the potential for constituents in citrus-derived peel oils, including the furocoumarin 5-MOP, to cause phototoxicity. International Fragrance Association has issued standards for citrus oils and other furocoumarin-containing essential oils, and the Panel agreed that adherence to the IFRA standards for such constituents will prevent phototoxicity. According to these standards, finished products that are applied to the skin, excluding rinse-off products, must not contain more than 0.0015%, or 15 ppm, 5-MOP. An IFRA standard also has been issued for 7-methoxycoumarin; based on established maximum levels of this substance from commercially available natural sources (like essential oils, extracts, and absolutes), exposure to 7-methoxycoumarin from the use of these oils and extracts is regarded to be acceptable if the level of 7-methoxycoumarin in the finished product does not exceed 100 ppm.

Additionally, based on the findings of a rodent carcinogenicity study in which tumor promotion activity may have been caused by repeated skin irritation and resultant proliferation of DMBA-treated basal cells, the Panel concluded that citrus-derived peel oils could potentially act as tumor promoters if formulated to reach irritant levels. Thus, these botanical ingredients must be formulated to be nonirritating.

The Panel noted that, because botanical ingredients are complex mixtures, there is concern that multiple botanical ingredients may each contribute to the final concentration of a single constituent. Therefore, when formulating products, manufacturers should avoid reaching levels in final formulation of plant constituents that may cause sensitization or other adverse effects. Specific examples of constituents that could induce adverse effects are limonene, citral, and other monoterpenes, furocoumarins (such as 5-MOP and 7-methoxycoumarin).

Finally, the Panel expressed concern about pesticide residues and heavy metals that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use current good manufacturing practices to limit impurities.

Conclusion

The CIR Expert Panel concluded the citrus-derived peel oils are safe for use in cosmetic products, excluding rinse-off products, do not contain more than 0.0015% (15 ppm) 5-MOP, and when formulated to be nonsensitizing and nonirritating.

C aurantifolia (lime) peel oil*
Citrus aurantium amara (bitter orange) peel oil
Citrus aurantium currassuviensis peel oil*
Citrus aurantium dulcis (orange) peel oil
Citrus clementina peel oil*
Citrus grandis (grapefruit) peel oil
Citrus iyo peel oil*
Citrus junos peel oil
Citrus limon (lemon) peel oil
Citrus medica vulgaris peel oil*
Citrus nobilis (mandarin orange) peel oil
Citrus reticulata (tangerine) peel oil*
Citrus tachibana/reticulata peel oil*
Citrus tangerina (tangerine) peel oil *

*Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.

Author's Note

Unpublished sources cited in this report are available from the Executive Director, Cosmetic Ingredient Review, 1620 L Street, NW, Suite 1200, Washington, DC 20036, USA.

Author Contributions

Burnett, C. contributed to conception and design; acquisition, analysis, and interpretation; drafted manuscript; and critically revised manuscript. Fiume, M., Gill, L., and Heldreth, B. contributed to analysis and interpretation and critically revised manuscript. Bergfeld, W., Belsito, D., Hill, R., Klaassen, C., Liebler, D., Marks, J. Shank, R., Slaga, T., and Snyder, P. contributed to conception and design; analysis and interpretation; and critically revised manuscript; and gave final approval. All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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

CONCERNING

AN INITIAL LIST OF PERFUMERY MATERIALS WHICH MUST NOT FORM PART OF COSMETIC PRODUCTS EXCEPT SUBJECT TO THE RESTRICTIONS AND CONDITIONS LAID DOWN

adopted by the SCCNFP during the 18th Plenary meeting
of 25 September 2001

1. Terms of Reference

In recent years there has been concern on the safety of fragrance (perfumery) materials. Dermatologists have highlighted the frequency of allergic contact dermatitis from perfumes.

Under current legislation, fragrance materials do not fall under all the requirements of Directive 76/768/EEC on cosmetic products. The 6th Amendment (93/35/EEC) provides for the labelling of ingredients on cosmetic products. However, it is not a requirement to label fragrance constituents on the packaging of cosmetic products, current legislation requires only the word *parfum*.

In response to growing concern over this issue, the Commission was asked for positive actions with respect to legislative measures on fragrance materials.

2. Mandate

The SCCNFP has been asked to respond to the following questions :

1. Does the SCCNFP agree to the inclusion of all IFRA restricted materials in the Annex III (List of substances which cosmetic products must not contain except subject to restrictions and conditions laid down)? Are the permitted levels recommended by IFRA suitable for use in the Cosmetics Directive 76/768/EEC ?

2. Does the SCCNFP agree that all materials that IFRA recommend should not be used as fragrance compounds are included in Annex II (List of substances which must not form part of the composition of cosmetic products)?

3. It is proposed that all known fragrance allergens are labelled on cosmetics if used in the products. Does the SCCNFP agree to this proposal? If so :

   - Which chemicals fall under this classification ?
   - Is there a maximum concentration of each chemical permissible without the requirement for labelling ?

4. Restrictions are proposed for the 3 most common fragrance allergens (cinnamic aldehyde, isoeugenol, hydroxycitronellal). Does the SCCNFP agree to restriction on the use of common fragrance allergens (Annex III listing)? If so :

   - Which fragrance materials should be subject to restrictions?
   - What are the conditions for restrictions (maximum concentration, fields of applications, etc) ?

Obviously, in response to each of the questions listed above, a scientific justification will be necessary.

3. Strategy of the SCCNFP

The SCCNFP has considered that this mandate can be usefully divided into two sections (Interim position on Fragrance allergy, document n° SCCNF/0202/99 adopted by the SCCNFP during the 8th Plenary meeting of 23 June 99) :

1. Identification of those fragrance ingredients, which are of concern as allergens for the consumer. Recommendations on informing the consumer of the presence of important allergens to permit the consumer with a known fragrance allergy a means to avoid contact with an allergen. An opinion as to whether such identification can be related to concentrations present in a product when elicitation levels are known.

2. An opinion on the adoption of industry prohibited substances into Annex 2 and adoption of industry restricted substances into Annex 3. Considerations as to whether the concentration limits or other restrictions suggested by industry can be supported or need to be changed if there is such inclusion in Annex 3. Whether there are additional substances which should be subject to inclusion in an Annex.

An opinion concerning fragrance allergy was adopted by the SCCNFP during the 10th Plenary meeting of 8 December 1999 (doc. n° SCCNFP/0017/98 final). During its 12th plenary meeting of 3 May 2000, the SCCNFP adopted an opinion on an initial list of perfumery materials which must not form part of fragrance compounds used in cosmetic products (doc. n° SCCNFP/0320/00 final).

The current opinion consists of the adoption of an initial list of perfumery materials to be included in Annex III - List of substances which cosmetic products must not contain except subject to restrictions and conditions laid down - to Directive 76/768/EEC.

The opinion is based on information submitted as ‘monographs’ (synopses) on behalf of industry.

4. Opinion

On the basis of the available information and assessment of the cutaneous toxicity of the substances tabulated, it is the recommendation of the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) that these substances may be used as ingredients in cosmetic products only under the conditions and restrictions specified in the attached table.

Additional substances will be discussed for possible inclusion at a later date.

Table 1 : List of perfumery materials which must not form part of cosmetic products except subject to the restrictions and conditions laid down

N°	Substance name	Restriction and condition
1	*Abies alba oil from cones (Abies Alba Mill.)* CAS n° : 8021-27-0 *Abies alba oil from needles (Abies Alba Mill.)* CAS n° : 8021-27-0 *Abies sachalinensis oil* CAS n° : Unkown *Fir balsam (Abies balsamea (L.) Mill)* CAS n° : 8021-28-1 *Fir needle oil (Abies sibirica)* CAS n° : 8021-29-2 *Fir needle oil, Canadian (Abies balsamea)* CAS n° : 8021-28-1 *Pine needle, dwarf, oil (Pinus mugo turra var. pumilio (Haenke) Zenari)* CAS n° : 8000-26-8 *Pine needle oil (Abies spp.)* CAS n° : 8021-29-2 *Pine scotch oil (Pinus sylvestris L.)* CAS n° : 8023-99-2 *Pinus nigra oil* CAS n° : 90082-74-9 *Turpentine gum (Pinus spp.)* CAS n° : 9005-90-7 *Turpentine oil* CAS n° : 8006-64-2 *Turpentine oil rectified* CAS n° : 8006-64-2 *Turpentine, steam distilled (Pinus spp.)* CAS n° : 8006-64-2	Essential oils and isolates derived from the Pinacea family, including Pinus and Abies genera, should only be used when the level of peroxides is kept to the lowest practicable level, for instance by adding antioxidants at the time of production. Such products should have a peroxide value of less than 10 millimoles peroxide per liter. Based on the published literature mentioning sensitising properties when containing peroxides (Food and Chemical Toxicology 11,1053(1973); 16,843(1978); 16,853(1978).
2	Acetyl hexamethyl indan CAS n° : 15323-35-0	For applications on areas of skin exposed to sun, excluding bath preparations, soaps and other rinse-off products, limit use to 2%in the finished cosmetic product. Based on the phototoxic potential showing no effects at 10% in human photo-toxicity tests (IFRA guidelines).

N°	Substance name	Restriction and condition
3	*Allyl butyrate* CAS n° : 2051-78-7 *Allyl cinnamate* CAS n° : 1866-31-5 *Allyl cyclohexaneacetate* CAS n° : 4728-82-9 *Allyl cyclohexanepropionate* CAS n° : 2705-87-5 *Allyl heptanoate* CAS n° : 142-19-8 *Allyl hexanoate* CAS n° : 123-68-2 *Allyl isovalerate* CAS n° : 2835-39-4 *Allyl octanoate* CAS n° : 4230-97-1 *Allyl phenoxyacetate* CAS n° : 7493-74-5 *Allyl phenylacetate* CAS n° : 1797-74-6 *Allyl 3,5,5-trimethylhexanoate* CAS n° : 71500-37-3	Use only when the level of free allyl alcohol in the ester is less than 0.1%. Based on the delayed irritant potential of allyl alcohol (Food and Chemical Toxicology 15,611(1977)).
4	*Allyl heptine carbonate* CAS n° : 73157-43-4	Should not be used such that the level in the finished cosmetic products exceeds 0.002 %. Based on test results showing the absence of sensitising reactions for allyl heptine carbonate at low concentrations in a test on humans. This material should not be used in combination with any other 2-alkynoic acid ester (e.g. methyl heptine carbonate). (IFRA guidelines).
5	*Amylcyclopentenone* CAS n° : 25564-22-1	Should not be used such that the level in the finished cosmetic product exceeds 0.1 %. Based on test results showing sensitisation at 10% and no sensitisation at 1 %. (IFRA guidelines).

N°	Substance name	Restriction and condition
6	*Angelica root oil (Angelica archangelica L.)* CAS n° : 8015-64-3 *Bergamot oil* CAS n° : 8007-75-8 *Grapefruit oil, expressed (Citrus paradisi Macf.)* CAS n° : 8016-20-4 *Lemon oil* CAS n° : 8008-56-8 *Lemon oil, cold pressed, California type* CAS n° : 8008-56-8 *Lemon oil, cold pressed, desert type* CAS n° : 8008-56-8 *Lime oil, cold pressed, Mexican* CAS n° : 8008-26-2 *Lime oil, expressed* CAS n° : 8008-26-2 *Lime oil expressed rectified* CAS n° : 8008-26-2 *Orange peel oil, bitter (Citrus aurantium L.)* CAS n° : 68916-04-1 *Rue oil (Ruta graveolens L.)* CAS n° : 8014-29-7	May be used in cosmetic products, provided that the total concentration of furocoumarin-like substances in the finished cosmetic product do not exceed 1ppm.
7	*Balsam oil, Peru (Myroxylon pereirae Klotzsch)* CAS n° : 8007-00-9 *Balsam absolute, Peru* CAS n° : 8007-00-9 *Balsam anhydrol, Peru* CAS n° : 8007-00-9	Extracts and distillates of Peru balsam (the exudation from Myroxylon pereirae (Royle) Klotzsch) should not be used such that the total level exceeds 0.4% in cosmetic products. Based on a wide variety of test results on the sensitising potential of Peru balsam and its derivatives.
8	*p-tert-Butyldihydro-cinnamaldehyde* CAS n° : 18127-01-0	Should not be used such that the level in the finished cosmetic product exceeds 0.6%. Based on test results showing sensitising potential and no sensitisation reactions when tested at 6%. (IFRA guidelines).

N°	Substance name	Restriction and condition
9	Cinnamal CAS n° : 104-55-2 *Cinnamic aldehyde-methyl anthranilate (Schiff base)* CAS n° : 94386-48-8	The concentration of Cinnamic aldehyde in the finished cosmetic product should not exceed 0.1%.
10	*Cassia oil* CAS n° : 8007-80-5 *Cinnamon bark oil* CAS n° : 8015-91 –6	The prime allergen is Cinnamic aldehyde. The concentration of Cinnamic aldehyde in the finished cosmetic product should not exceed 0.1%.
11	Cinnamyl alcohol CAS n° : 104-54-1	Should not be used such that the level in finished cosmetic products exceeds 0.8%. Based on test results showing sensitising potential. (IFRA guidelines).
12	*Cumin oil* CAS n° : 8014-13-9	For applications on areas of skin exposed to sun, excluding bath preparations, soaps and other wash-off products, limit cumin oil to 0.4% in the finished cosmetic product. Based on the photo-toxicity of cumin oil, the observed no-effect level of 25% on the skin of the hairless mouse and on the no-effect level of 5% in tests with humans.
13	*cis-.alpha.-Damascone* CAS n° : 23726-94-5 *trans-. beta.-Damascone* CAS n° : 23726-91-2 *Isodamascone* CAS n° : 39872-57-6 *1-(2,6,6-Trimethylcyclohexa-1,3-dienyl)-2-buten-1-one* CAS n° : 23698-85-7 *1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-l-one* CAS n° : 57378-68-4 *1-(2,6,6-Trimethyl-l-cyclohexen-1-yl)-2-buten-l-one* CAS n° : 23726-92-3 *1-(2,6,6-Trimethyl-2-cydohexen-1-yl)-2-buten-l-one* CAS n° : 43052-87-5	Should not be used as fragrance ingredients such that the total level in finished cosmetic products exceeds 0.02%, individually or in combination. Based on test data showing sensitising potential for these materials and on evidence of cross-reactivity. (IFRA guidelines)
14	*trans-Hexen-2-al* CAS n° : 6728-26-3	Should not be used such that the level in finished cosmetic products exceeds 0.002%. Based on test results of RIFM showing sensitisation reactions at 0.2% and no sensitisation reactions when tested at 0.02% (IFRA guidelines).

N°	Substance name	Restriction and condition
15	Hydroxycitronellal CAS n° : 107-75-5	Should not be used such that the level in finished cosmetic products exceeds 1.0%. Based on a review of a large number of animal and human test data (IFRA guidelines).
16	Isoeugenol CAS n° : 97-54-1	Should not be used such that the level in finished cosmetic products exceeds 0.02%. Based on test results showing sensitising potential (IFRA guidelines).
17	*d-Limonene* CAS n° : 5989-27-5 *l-Limonene* CAS n° : 5989-54-8 dl-Limonene (racemic) CAS n° : 138-86-3	Limonene and natural products containing substantial amounts of it, should only be used when the level of peroxides is kept to the lowest practical level, for instance by adding antioxidants at the time of production. Such products should have a peroxide value of less than 20 millimoles peroxide per liter. (IFRA guidelines).
18	*p-Mentha-1,8-dien-7-al* CAS n° : 2111-75-3	Should not be used such that the level in finished cosmetic products exceeds 0.1%. Based on test results showing sensitisation (IFRA guidelines).
19	*Menthadiene-7-methyl formate* CAS n° : 68683-20-5	Should not be used such that the level in finished cosmetic products exceeds 0.1 %. Based on tests showing sensitisation (IFRA guidelines).
20	*Methoxy dicyclopentadiene carboxaldehyde* CAS n° : 86803-90-9	Should not be used such that the level in finished cosmetic products exceeds 0.5%. Based on test results showing sensitisation potential (IFRA guidelines).
21	*Methyl N-methylanthranilate* CAS n° : 85-91-6	For applications on areas of the skin exposed to sunlight, excluding bath preparations, soaps and other wash-off products, limit to 10**%** in the finished cosmetic product. Based on the phototoxic potential and on the observed no-effect level of approximately 2 mg/cm^2 of the hairless mouse (Food and Chemical Toxicology 17, 273 (1979)).
22	*3-Methyl-2(3)-nonenenitrile* CAS n° : 53153-66-5	Should not be used such that the level in finished cosmetic products exceeds 0.2%. Based on test results showing sensitisation (Food Chemical Toxicology 20, 757 (1982)).
23	*Methyl octine carbonate* CAS n° : 111-80-8 *Methyl heptine carbonate* CAS n° : 111-12-6	Should not be used such that the level in finished cosmetic products exceeds 0.002%. When used alone level in the finished cosmetic products should not exceed 0.01%. When present in combination, the combined level in the finished product should not exceed 0.01% of which methyl heptine carbonate should not be more than 0.002%

N°	Substance name	Restriction and condition
24	*Oakmoss absolute (Evernia spp.)* CAS n° : 9000-50-4 *Oakmoss resinoid (Evernia spp.)* CAS n° : 9000-50-4 *Treemoss absolute (Usnea spp.)* CAS n° : 68648-41-9 *Treemoss concrete (Usnea spp.)* CAS n° : 68648-41-9 *Treemoss resinold (Usnea spp.)* CAS n° : 868648-41-9	Should not be used such that the level in finished cosmetic products exceeds 0.1%. The maximum concentration of Oakmoss and Treemoss in combination in the finished cosmetic product should not exceed 0.1%. The allergenic fractions in these species have not been fully elucidated nor has the relative contribution of the allergenic fractions between the species. Information needs to be obtained on the nature and the importance of the respective allergenic fractions at which time a review of the acceptance of the restriction in levels and relative proportions of the species will be undertaken.
25	*1-Octen-3-yl acetate* CAS n° : 2442-10-6	Should not be used such that the level in finished cosmetic products exceeds 0.3%. Based on test results showing sensitising potential (IFRA guidelines).
26	*3-Propylidenephthalide* CAS n° : 17369-59-4	Should not be used such that the level in finished cosmetic products exceeds 0.01 %. Based on test results showing sensitisation potential (IFRA guidelines).
27	*2,4,6-Trimethyl-3-cyclohexene-1-methanol* CAS n° : 68527-77-5	Should not be used such that the level in finished cosmetic products exceeds 0.5%. Based on test results showing sensitisation potential (IFRA guidelines).
28	*Verbena absolute* CAS n° : 8024-12-2	Verbena absolute obtained from Lippia citriodora Kunth should not be used such that the level in finished cosmetic products exceeds 0.2%. Based on test results showing sensitisation (IFRA guidelines).

EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

Furocoumarins in cosmetic products

Adopted by the SCCP
during the 6th plenary of 13 December 2005

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1. BACKGROUND

Commission Directive 95/34/EC July 10, 1995 adopted an amendment to Annex II, Annex to Cosmetic Directive under reference number 358 as follows: "Furocoumarins (e.g. trioxysalan, 8-methoxypsoralen, 5-methoxypsoralen) except for normal content in natural essences used. In sun protection and in bronzing products, furocoumarins shall be below 1 mg/kg."

The technical adaptation was based on an opinion adopted by the Scientific Committee on Cosmetology (SCC) in 1990. Furocoumarins are recognized to be photomutagenic and photo-carcinogenic. International Agency for Research on cancer (IARC) has classified 5-MOP and 8-MOP plus ultraviolet radiation in group 2A (probably carcinogenic to humans) and in group 1 (carcinogenic to human), respectively.

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted an "Initial List of Perfumery Materials which must not form part of Cosmetic Products except subject to the restrictions and conditions laid down" (SCCNFP/0392/00, final, adopted by the SCCNFP during the 18th Plenary meeting of 25 September 2001). The opinion is based on information submitted as 'monographs' (synopses) on behalf of industry. On the basis of the available information and assessment of the cutaneous toxicity of the substances tabulated in its opinion, it is the recommendation of the SCCNFP that these substances may be used as ingredients in cosmetic products only under the conditions and restrictions specified in the table attached in its opinion.

This opinion under entry nr 6 mentions that the following 11 essential oils: *Angelica root oil (Angelica archangelica L.)* CAS n° 8015-64-3; *Bergamot oil* CAS n° 8007-75-8; *Grapefruit oil, expressed (Citrus paradisi Macf.)* CAS n° 8016-20-4; *Lemon oil* CAS n° 8008-56-8; *Lemon oil, cold pressed, California type* CAS n° 8008-56-8; *Lemon oil, cold pressed, desert type* CAS n° 8008-56-8; *Lime oil, cold pressed, Mexican* CAS n° 8008-26-2; *Lime oil, expressed* CAS n° 8008-26-2; *Lime oil expressed rectified* CAS n° 8008-26-2; *Orange peel oil, bitter (Citrus aurantium L.)* CAS n° 68916-04-1; *Rue oil (Ruta graveolens L.)* CAS n° 8014-29-7 "may be used in cosmetic products, provided that the total concentration of furocoumarin-like substances in the finished cosmetic product does not exceed 1 ppm."
These plant extracts are often used as ingredients in fragrances and cosmetic products.

Submission I from European Flavour & Fragrance Association (EFFA) was submitted to the SCCNFP in November 2002.

Submission II was received in August 2003

The SCCNFP adopted during the 26th plenary meeting December 2003 an opinion concerning Furocoumarins in sun protection and bronzing products (SCCNFP/0765/03). This opinion states that the provided data "*do not justify a higher limit than 1 ppm (not to be intentionally added) for furocoumarins in cosmetics*". The SCCNFP also stated *that the opinion should be interpreted in conjunction with the opinion on CMR substances* (SCCNFP/0474/01, final, adopted 25 September 2001).

At the same meeting the SCCNFP also adopted an opinion on furocoumarin Isopimpinellin (SCCNFP/0761/03) stating "*that there is incomplete information on photo-mutagenicity and on*

photo-clastogenicity of isopimpinellin to enable a safety evaluation in order to provide an update of the "Initial List of Fragrance"" for entry n° 6.

At the same meeting the SCCNFP also adopted an opinion on the furocoumarin Bergamottin (SCCNFP/0740/03) stating "*Photo-mutagenicity and photo-carcinogenicity are the main effects of concern in relation to the use of furocoumarins in cosmetics. The data submitted by EFFA on bergamottin is not adequate for evaluation of the safety of the substance in relation to photo-mutagenicity and photo-carcinogenicity."*

Submission III was sent to the Commission in April 2005 by EFFA.

2. TERMS OF REFERENCE

1. *Is the SCCP of the opinion that the limitation to below 1 ppm of furocoumarins should be extended to all finished cosmetic products and not only cover sun protection and bronzing products?*

2. *Does the SCCP recommend any restrictions with regard to the use of the 11 plants extracts, which in the SCCNFP opinion (SCCNFP/0392/00) are known to contain furocoumarins when they are used as ingredients as such in cosmetics and not as ingredients in fragrances?*

3. OPINION

The following documents were submitted to support the terms of references:

* SaitaT, Fujito H and Mori M (2004) Screening of furocoumarin derivatives in citrus fruits by enzyme-linked immunosorbent assay. Biol Pharmacol Bull 27: 974-977.
* Frérot E and Decorzant E (2004) Quantification of total furocoumarins in citrus oils by HPLC coupled with UV, fluorescence and mass detection. J Agr. Food Chem. 52: 6879-6886.
* IFRA/RIFM Furocoumarins Fact Sheet: Furocoumarins in essential oils. (Angelicin, bergamottin, 5-methoxypsoralen, psoralen)

3.1. Screening of furocoumarin derivatives in citrus fruits by enzyme-linked immunosorbent assay. Saita et al (2004) Biol Pharmacol Bull 27: 974-977

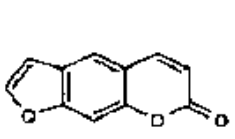
This paper describes an enzyme-linked immunosorbent assay for screening of furocoumarin derivatives as cytochrome P450 3A4 inhibitor in citrus fruits. The antibody used in this assay was produced by immunizing rabbits with 6′,7′-dihydroxybergamottin conjugated with serum albumin. The assay revealed that this antibody could detect both 6′,7′-dihydroxybergamottin and bergamottin, detection limits 800 pg/ml and 4 ng/ml. The specificity testing of the antibody, determined by the cross reactivity with other types of furocoumarin derivatives, revealed 17.2%, 0.28 % and <0.06% (not detectable) cross reaction respectively with bergamottin, 5-methoxy-psoralen and 8-methoxypsoralen for 50% replacement of β-Gal activity. Thus the antibody was

considered to be specific for furocoumarins containing geranyloxy side chain as in bergamottin and 6′,7′-dihydroxybergamottin, the inhibitors of P450 3A4 inhibitor in citrus fruits.

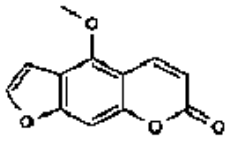
3.2. Quantification of total furocoumarins in citrus oils by HPLC coupled with UV, fluorescence, and mass detection. Frérot and Decorzanr (2004) J Agr. Food Chem. 52: 6879-6886

In this study, an analytical method employing a combination of two high performance liquid chromatography (HPLC) methods and detections by UV diode array, fluorescence and atmospheric pressure chemical ionisation (APCI)-mass spectrometry (MS) was developed for the detection and quantification of 15 furocoumarins (see below). Quantification by UV, fluorescence, or MS was compared in terms of linearity and limit of detection. The method would theoretically allow for the quantification of these 15 furocoumarins at 0.1 ppm level in the sample injected in HPLC. However, some interference was observed when 6 different citrus oils (see below) were analysed employing diode array detection. This problem could be solved by the use of other detection systems, in addition to diode array detection. The authors concluded that the method could be implemented in quality control laboratories. The authors also noted that cold-pressed citrus oils generally contain a high amount of total furocoumarins and would have to be distilled prior to their use in cosmetic products to fall below the permitted level of 1 ppm.

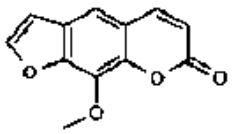
Furocoumarins analysed in the above study are:



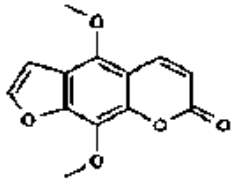
Psoralen



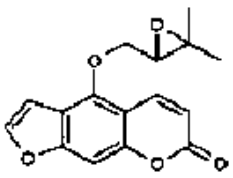
Bergapten



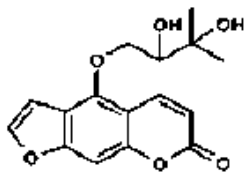
Xanthotoxin



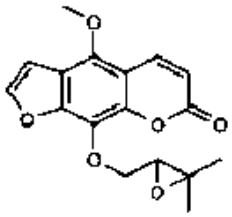
Isopimpinellin



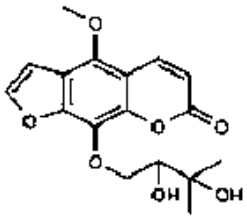
Oxypeucedanin



Oxypeucedanin hydrate



Byakangelicol



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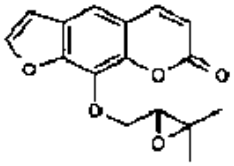
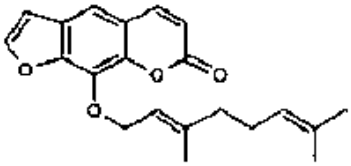
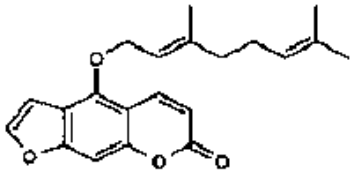


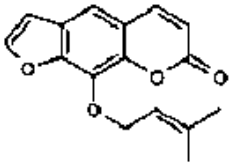
Heraclenin



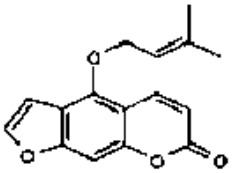
8-Geranyloxypsoralen



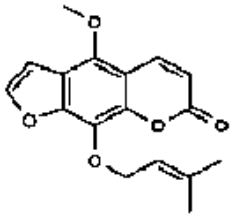
Bergamottin



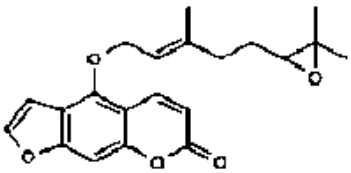
Imperatorin



Isoimperatorin



Phellopterin



Epoxybergamottin

Citrus oils analysed in the above study: Bergamot oil, lemon oil, grapefruit oil, mandarin oil, tangerine oil and bitter orange oil

3.3. IFRA/RIFM Fact Sheet: Furocoumarins in essential oils, Submission Number 3

The Fact Sheet describes the chemical names, CAS No. EINECS No., molecular formula and molecular weight of some furocoumarins. The EU regulation concerning furocoumarins in cosmetic products is also described in the Fact Sheet. Further, it is stated that the amounts of furocoumarins in plant extracts may vary in a wide range. So far only alkoxyfurocoumarins, having alkyl groups of 11 carbons or less, have been identified, but it is completely plausible that other furocoumarins exist in natural essences, which have so far escaped detection. However, reliable methods are available for only the major furocoumarins (Ferrot and Decorzant, 2004). The results of some analyses of these are described in Table 1. The variations shown in Table 1 clearly indicate that there is no single “marker” furocoumarin that can be used to allow

estimation of total furocoumarin levels. It is also mentioned in the Fact Sheet that "it seems that not all furocoumarins have equivalent biological properties"

4. DISCUSSION

The data provided in Submission III has shown that there are many furocoumarin-like substances present in citrus oils. Some of these can be identified and quantified by the newly developed methods. Several furocoumarins have been recognised as phototoxic and SCCNFP has evaluated consumer safety by the use of cosmetic products containing furocoumarins, as already described in the 'Background' to this opinion. The data provided, so far, has not ruled out the photo-toxicity of any of the known furocoumarins. The consumer is exposed to sunlight after application of various types of cosmetic products, not only after the application of bronzing and sun protection products. According to earlier SCCNFP Opinions (SCCNFP/0392/00, SCCNFP/0765/03), the total concentration of furocoumarin-like substances should not exceed 1 ppm in the finished cosmetic product.

In the absence of any additional new data demonstrating the safety of any of the furocoumarin-like substances, the SCCP reemphasise that total concentrations of furocoumarins exceeding 1 ppm in any finished cosmetic product will be of concern with regard to consumer safety.

5. CONCLUSION

The data provided so far has not ruled out the photo-toxicity of any furocoumarin. No new data is provided in Submission III to substantiate consumer safety when using cosmetic products containing furocoumarins.

As the consumer is exposed to sunlight after using various types of cosmetic products, not only bronzing or sun protection products, the SCCP reemphasise that total concentrations of furocoumarins exceeding 1 ppm in any finished cosmetic product will be of concern with regard to consumer safety.

The Opinion concerns the content of furocoumarins in cosmetic formulations, irrespective of the source of these substances.

This Opinion should be interpreted together with the opinion on CMR substances (SCCNFP/0474/01, Final).

IFRA **RIFM**

Furocoumarins – Fact Sheet

Table 1: Summary of furocoumarin analysis in citrus oils

Essential Oil	FUROCOUMARIN							
	PSORALEN	BERGAPTEN (5-MOP)	XANTHOTOXIN (8-MOP)	ISOPIMPINELLIN	BERGAMOTTIN	OXYPEUCEDANIN	ANGELICIN	EPOXY-BERGAMOTTIN
Angelica root oil	up to 112 ppm	up to 78 ppm	not detected	not detected	not detected	?	230 ppm	?
Bergamot oil	0-26 ppm *	up to 2300 ppm (0.23%)	not detected	not detected	up to 2.22%	not detected	not detected	?
Grapefruit oil	not detected	up to 1900 ppm (0.19%)	not detected	< 5 ppm	up to 1100 ppm (0.11%)	not detected	not detected	1126 ppm
Lemon oil, cold pressed	0-3 ppm *	up to 275 ppm	not detected	0-18 ppm *	up to 5412 ppm (0.54%)	up to 8224 ppm (0.82%)	not detected	?
Lime oil, cold pressed	< 5 ppm	up to 2200 ppm (0.22%)	< 5 ppm	2000 (0.2%)	2.5%	1200 ppm (0.12%)	not detected	?
Mandarin oil	not detected	0-3 ppm*	not detected	not detected	0-10 ppm*	not detected	not detected	?
Orange oil bitter	70 ppm	350 ppm (0.035%)	not detected	not detected	5 ppm	not detected	not detected	820 ppm[1]
Rue oil	150 ppm	180 ppm	320 ppm	200 ppm	not detected	?	430 ppm	?
Tagetes oil	110 ppm	not detected	not detected	not detected	not detected	?	not detected	?

All values reported were measured by the fragrance industry unless noted. Furocoumarin peaks were identified by comparing the retention time and the UV-spectrum in the sample solution with that of the standard solution.

* Qualitative determination was performed using the retention time of the reference (the amount was too low for a UV-spectrum)

[1] McHale and Sheridan, 1989. The oxygen heterocyclic compounds of citrus peel oils. Proceedings of the 11th International Congress of Essential Oils. Delhi, India, 12-16 November.

6. MINORITY OPINION

Not applicable

7. REFERENCES

* Furocoumarins in essential oils. Fact sheets (Angelicin, Bergamottin, 5-Methoxypsoralen, Psoralen).
* Frérot E and Decorzant E (2004) Quantification of Total Furocoumarins in Citrus Oils by HPLC Coupled with UV, Fluorescence, and Mass Detection. J. Agric. Food Chem. 2004, 52, 6879-6886.
* SaitaT, Fujito H and Mori M (2004) Screening of Furanocoumarin Derivatives in Citrus Fruits by Enzyme-Linked Immunosorbent Assay. Biol. Pharm. Bull. 27(7) 974-977 (2004).
* Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers concerning an initial list of perfumery materials which must not form part of cosmetic products except subject to the restrictions and conditions laid down. Adopted by the SCCNFP during the 18th Plenary meeting of 25 September 2001. Doc. n° SCCNFP 0392/00
* Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers concerning chemical ingredients in cosmetic products classified as carcinogenic, mutagenic or toxic to reproduction according to the chemicals Directive 67/548/EEC. Adopted by the SCCNFP during the 18th Plenary meeting of 25 September 2001. Doc. n° SCCNFP/0474/01
* Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers concerning furocoumarins in sun protection and bronzing products. Adopted by the SCCNFP during the 26th plenary meeting of 9 December 2003. Doc. n° SCCNFP/0765/03

8. ACKNOWLEDGEMENTS

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

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Effects of Flavoring and Casing Ingredients on the Toxicity of Mainstream Cigarette Smoke in Rats

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A series of in vitro and in vivo studies evaluated the potential effects of tobacco flavoring and casing ingredients. Study 1 utilized as a reference control cigarette a typical commercial tobacco blend without flavoring ingredients, and a test cigarette containing a mixture of 165 low-use flavoring ingredients. Study 2 utilized the same reference control cigarette as used in study 1 and a test cigarette containing eight high-use ingredients. The in vitro Ames *Salmonella typhimurium* assay did not show any increase in mutagenicity of smoke condensate from test cigarettes designed for studies 1 and 2 as compared to the reference. Sprague-Dawley rats were exposed by nose-only inhalation for 1 h/day, 5 days/wk for 13 wk to smoke from the test or reference cigarettes already described, or to air only, and necropsied after 13 wk of exposure or following 13 wk of recovery from smoke exposure. Exposure to smoke from reference or test cigarettes in both studies induced increases in blood carboxyhemoglobin (COHb) and plasma nicotine, decreases in minute volume, differences in body or organ weights compared to air controls, and a concentration-related hyperplasia, squamous metaplasia, and inflammation in the respiratory tract. All these effects were greatly decreased or absent following the recovery period. Comparison of rats exposed to similar concentrations of test and reference cigarette smoke indicated no difference at any concentration. In summary, the results did not indicate any consistent differences in toxicologic effects between smoke from cigarettes containing the flavoring or casing ingredients and reference cigarettes.

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Flavoring ingredients are added to tobacco during the manufacture of many types of commercial cigarettes, and humectants such as glycerol are added to increase the moisture-holding capacity of the tobacco. There has been much speculation about the effect of these added ingredients on the toxicity of the resultant smoke. Wynder and Hoffman (1967) hypothesized that adding

nontobacco ingredients might increase or decrease the toxic effects of inhaled tobacco smoke, and later publications (LaVoie et al., 1980; Hoffman and Hoffman, 1997, 2001; World Health Organization, 2001) supported that hypothesis. Recently published research results (Gaworski et al., 1998; Paschke et al., 2002; Rodgman, 2002a, 2002b; Rodgman and Green, 2002; Carmines, 2002; Rustemeier et al., 2002; Roemer et al., 2002; Vanscheeuwijck et al., 2002; Baker et al., 2004) have presented data from in vitro, and in vivo toxicity studies that indicate the addition of ingredients to tobacco does not increase the toxicity of the smoke. Baker et al. (2004), using a pyrolysis technique that mimics closely the combustion conditions inside burning cigarettes (Baker and Bishop, 2004), studied the effects of pyrolysis on the chemistry, in vitro genotoxicity and cytotoxicity, and inhalation toxicity in rodents of 291 single ingredients added to cigarettes.

The studies described herein were designed to evaluate the potential influence of low-use flavoring ingredients and high-use mixed casing or flavoring ingredients on the biological activity of mainstream cigarette smoke. Test cigarettes containing flavorings or casings were analyzed and compared against an identical reference cigarette respectively produced without flavors or casings.

MATERIALS AND METHODS

Cigarette Design

In study 1, 165 low-use flavoring ingredients were added to a single test cigarette and compared to a reference cigarette without these ingredients. In study 2, eight high-use flavoring or casing ingredients were added to a single test cigarette and compared to the same reference cigarette that was used in study 1. Thus, the design covered these ingredients as well as possible interactions between them and/or their combustion or pyrolysis products. The prototype cigarettes were designed to be representative of commercial, full flavor filter cigarettes. Test and reference cigarettes were constructed with conventional commercial equipment.

The ingredients selected for evaluation in these studies comprise low-use and high-use ingredients normally utilized in the manufacture of commercial cigarettes. The point of addition was chosen to mimic actual process conditions. Study 1 and study 2 ingredients were incorporated into a flavoring or casing system at levels exceeding their normal use. Table 1 outlines the tobacco components of the blend used to construct the cigarettes in both study 1 and study 2. The blends were cased with a mixture of glycerin and water (at a ratio of 2:1) to provide the necessary moisture for standard processing. In preparation of study 1 cigarettes, the ingredients were applied at a rate of 10 kg/1000 kg leaf blend, that is, at 1% on the test cigarettes, and the casing was applied at a rate of 30 kg/1000 kg leaf blend. The study 2 ingredient system was applied at a rate of 31 kg/1000 kg leaf blend (3.1%). The 165 ingredients included in the study 1 mixture appear listed in order of descending application rate in Table 2,

TABLE 1
Blend composition of prototype cigarettes

	Percent of blend component in cigarettes	
Blend components	Tobacco wet weight	Tobacco dry weight
Burley	24	22.9
Virginia	28	25.7
Oriental	14.8	13.6
Reconstituted sheet	23.4	20.1
Expanded tobacco	9.7	8.8

along with the corresponding CAS-Number, regulatory identifiers (where applicable) and application rate. The seven casings and one flavoring included in the study 2 mixture appear listed in order of descending application rate in Table 3. Cellulose acetate filters with 32% average air dilution were used in all cigarettes. Monogram inks were not subject to these studies.

Cigarette Performance

A preliminary cigarette performance evaluation was carried out prior to the toxicology studies. Prior to characterization, the cigarettes were conditioned for a minimum of 48 h at a temperature of $22 \pm 1°C$ and a relative humidity (RH) of $60 \pm 2\%$, in accordance with ISO Standard 3402. Subsequently, the cigarettes were smoked on a 20-port Borgwaldt smoking machine under the conditions stipulated in ISO Standard 3308. Therefore, the puffing regime for mainstream smoke used a 35 ± 0.3 ml puff volume, with 2.0 ± 0.05 s puff duration once every 60 ± 0.5 s. Smoke samples were respectively collected in accordance with the analytical method.

In Vitro Study Design

The mutagenicity of total particulate matter (TPM) in study 1 and 2 cigarettes was investigated using an Ames assay protocol that conformed to OECD Guideline 471. For this purpose, prototype cigarettes containing a mixture of ingredients, reference cigarettes without these ingredients, and 2R4F cigarettes (a standard reference cigarette developed and validated by the University of Kentucky) were smoked on a Borgwaldt RM200 rotary smoking machine under the ISO standard 3308 condition. TPM was collected in a standard fiberglass (Cambridge) trap with dimethyl sulfoxide (DMSO), and the DMSO solution was stored in the dark at $-80°C$ prior to performance of the Ames assay. Each sample was tested with and without S9 metabolic activation in five strains of *Salmonella typhimurium*: TA98, TA100, TA102, TA1535, and TA1537. Evaluation of the Ames assay data was carried out in terms of the mutagenic response, taking into consideration the reproducibly dose-related increase in number of revertants, even if the increase was less than twofold. The mutagenic response to TPM from the reference and test cigarettes was compared using the linear portion of the slope (revertants/mg TPM).

TABLE 2
Ingredients added to test cigarettes in study 1

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
1	Benzyl alcohol	100-51-6	2137	172.515	58c	260
2	Immortelle extract	8023-95-8	2592	182.20	225n	156
3	Coriander oil	8008-52-4	2334	182.20	154n	65
4	Balsam peru resinoid	8007-00-9	2117	182.20	298n	65
5	Anise star oil	8007-70-3	2096	N.A.	238n	65
6	Celery seed oil	89997-35-3	2271	182.20	52n	65
7	Vanillin	121-33-5	3107	182.60	107c	65
8	Potassium sorbate	24634-61-5	2921	182.3640	N.A.	39
9	Propyl *para*-hydroxybenzoate	94-13-3	2951	172.515	N.A.	39
10	Benzoin resinoid	9000-05-9	2133	172.510	439n	26
11	Cedarwood oil	8000-27-9	N.A.	N.A.	252n	26
12	Clary extract	8016-63-5	2321	182.20	415n	26
13	Methylcyclopentenolone	80-71-7	2700	172.515	758c	26
14	Phenethyl alcohol	60-12-8	2858	172.515	68c	26
15	Piperonal	120-57-0	2911	182.60	104c	26
16	Tea extract	84650-60-2	N.A.	182.20	451n	26
17	Vanilla oleoresin	8024-06-4	3106	182.20	474n	26
18	Brandy	N.A.	N.A.	N.A.	N.A.	26
19	*trans*-Anethole	4180-23-8	2086	182.60	183c	19.5
20	Coffee extract	84650-00-0	N.A.	182.20	452n	19.5
21	5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone	698-10-2	3153	N.A.	2300c	19.5
22	Propionic acid	79-09-4	2924	184.1081	3c	13
23	Acetic acid	64-19-7	2006	184.1005	2c	13
24	Amyl formate	638-49-3	2068	172.515	497c	13
25	Angelica root oil	8015-64-3	2088	182.20	56n	13
26	Beeswax absolute	8012-89-3	2126	184.1973	N.A.	13
27	Benzyl benzoate	120-51-4	2138	172.515	262c	13
28	Benzyl propionate	122-63-4	2150	172.515	413c	13
29	Cardamom oil	8000-66-6	2241	182.20	180n	13
30	beta-Carotene	7235-40-7	N.A.	184.1245	N.A.	13
31	Ethyl acetate	141-78-6	2414	182.60	191c	13
32	Ethyl butyrate	105-54-4	2427	182.60	264c	13
33	Ethyl levulinate	539-88-8	2442	172.515	373c	13
34	Eucalyptol	470-82-6	2465	172.515	182c	13
35	Geranium oil	8000-46-2	2508	182.20	324n	13
36	Labdanum resinoid	8016-26-0	2610	172.510	134n	13
37	Lavandin oil	8022-15-9	2618	182.20	257n	13
38	Maltol	118-71-8	2656	172.515	148c	13
39	Spearmint oil	8008-79-5	3032	182.20	285n	13
40	Ethyl hexanoate	123-66-0	2439	172.515	310c	10.4
41	Acetylpyrazine	22047-25-2	3126	N.A.	2286c	9.1
42	Ethylmaltol	4940-11-8	3487	172.515	692c	9.1
43	Chamomile oil, Roman	8015-92-7	2275	182.20	48n	6.5
44	Citronella oil	8000-29-1	2308	182.20	39n	6.5
45	delta-Decalactone	705-86-2	2361	172.515	621c	6.5
46	gamma-Decalactone	706-14-9	2360	172.515	2230c	6.5
47	Ethyl phenylacetate	101-97-3	2452	172.515	2156c	6.5

(Continued on next page)

TABLE 2
Ingredients added to test cigarettes in study 1 *(Continued)*

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
48	Ethyl valerate	539-82-2	2462	172.515	465c	6.5
49	Ethyl vanillin	121-32-4	2464	182.60	108c	6.5
50	Fennel sweet oil	8006-84-6	2485	182.20	200n	6.5
51	Glycyrrhizin ammoniated	53956-04-0	N.A.	184.1408	N.A.	6.5
52	gamma-Heptalactone	105-21-5	2539	172.515	2253c	6.5
53	3-Hexen-1-ol	928-96-1	2563	172.515	750c	6.5
54	3-Hexenoic acid	1577-18-0	3170	N.A.	2256c	6.5
55	Hexyl alcohol	111-27-3	2567	172.515	53c	6.5
56	Isoamyl phenylacetate	102-19-2	2081	172.515	2161c	6.5
57	Methyl phenylacetate	101-41-7	2733	172.515	2155c	6.5
58	Nerol	106-25-2	2770	172.515	2018c	6.5
59	Nerolidol	142-50-7	2272	172.515	67c	6.5
60	Peruvian (bois de rose) oil	8015-77-8	2156	182.20	44n	6.5
61	Phenylacetic acid	103-82-2	2878	172.515	672c	6.5
62	Pyruvic acid	127-17-3	2970	172.515	19c	6.5
63	Rose absolute	8007-01-0	2988	182.20	405n	6.5
64	Sandalwood oil	8006-87-9	3005	172.510	420n	6.5
65	Sclareolide	564-20-5	3794	N.A.	N.A.	6.5
66	Triethyl citrate	77-93-0	3083	184.1911	N.A.	6.5
67	2,3 5-Trimethylpyrazine	14667-55-1	3244	N.A.	735c	6.5
68	Olibanum absolute	8016-36-2	2816	172.510	93n	6.5
69	delta-Octalactone	698-76-0	3214	N.A.	2195c	6.5
70	2-Hexenal	6728-26-3	2560	172.515	748c	5.2
71	Ethyl octadecanoate	111-61-5	3490	N.A.	N.A.	5.2
72	4-Hydroxy-3-pentenoic acid lactone	591-12-8	3293	N.A.	731c	3.9
73	Methyl 2-pyrrolyl ketone	1072-83-9	3202	N.A.	N.A.	3.9
74	Methyl linoleate (48%) methyl linolenate (52%) mixture	112-63-0 301-00-8	3411	N.A.	713c	3.9
75	Petitgrain mandarin oil	8014-17-3	2854	182.20	142n	3.9
76	Propenylguaethol	94-86-0	2922	172.515	170c	3.9
77	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl) but-2-en-4-one	23696-85-7	3420	N.A.	N.A.	3.9
78	2-Propionyl pyrrole	1073-26-3	3614	N.A.	N.A.	3.9
79	Orange essence oil	8008-57-9	2825	182.20	143n	2.6
80	Benzyl phenylacetate	102-16-9	2419	172.515	232c	2.6
81	2,3-Butanedione	431-03-8	2370	184.1278	752c	1.95
82	2,3,5,6-Tetramethylpyrazine	1124-11-4	3237	N.A.	734c	1.95
83	Hexanoic acid	142-62-1	2559	172.515	9c	1.56
84	Cinnamaldehyde	104-55-2	2286	182.60	102c	1.3
85	Acetophenone	98-86-2	2009	172.515	138c	1.3
86	2-Acetylthiazole	24295-03-2	3328	N.A.	N.A.	1.3
87	Amyl alcohol	71-41-0	2056	172.515	514c	1.3
88	Amyl butyrate	540-18-1	2059	172.515	270c	1.3
89	Benzaldehyde	100-52-7	2127	182.60	101c	1.3
90	Butyl butyrate	109-21-7	2186	172.515	268c	1.3
91	Butyric acid	107-92-6	2221	182.60	5c	1.3
92	Cinnamyl alcohol	104-54-1	2294	172.515	65c	1.3

(Continued on next page)

TABLE 2
Ingredients added to test cigarettes in study 1 *(Continued)*

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
93	DL-Citronellol	106-22-9	2309	172.515	59c	1.3
94	Decanoic acid	334-48-5	2364	172.860	11c	1.3
95	para-Dimethoxybenzene	150-78-7	2386	172.515	2059c	1.3
96	3,4-Dimethyl-1,2-cyclopentanedione	13494-06-9	3268	N.A.	2234c	1.3
97	Ethylbenzoate	93-89-0	2422	172.515	261c	1.3
98	Ethyl heptanoate	106-30-9	2437	172.515	365c	1.3
99	Ethyl isovalerate	108-64-5	2463	172.515	442c	1.3
100	Ethyl myristate	124-06-1	2445	172.515	385c	1.3
101	Ethyl octanoate	106-32-1	2449	172.515	392c	1.3
102	Ethyl palmitate	628-97-7	2451	N.A.	634c	1.3
103	Ethyl propionate	105-37-3	2456	172.515	402c	1.3
104	2-Ethyl-3-methylpyrazine	15707-23-0	3155	N.A.	548c	1.3
105	Genet absolute	8023-80-1	2504	172.510	436n	1.3
106	Geraniol	106-24-1	2507	182.60	60c	1.3
107	Geranyl acetate	105-87-3	2509	182.60	201c	1.3
108	gamma-Hexalactone	695-06-7	2556	172.515	2254c	1.3
109	Hexyl acetate	142-92-7	2565	172.515	196c	1.3
110	Isoamyl acetate	123-92-2	2055	172.515	214c	1.3
111	Isoamyl butyrate	106-27-4	2060	172.515	282c	1.3
112	3,7-Dimethyl-1,6-octadiene-3-ol	78-70-6	2635	182.60	61c	1.3
113	Menthyl acetate	89-48-5	2668	172.515	206c	1.3
114	Methyl isovalerate	556-24-1	2753	172.515	457c	1.3
115	Methyl salicylate	119-36-8	2745	175.105	433c	1.3
116	3-Methylpentanoic acid	105-43-1	3437	N.A.	N.A.	1.3
117	gamma-Nonalactone	104-61-0	2781	172.515	178c	1.3
118	Oakmoss absolute	9000-50-4	2795	172.510	194n	1.3
119	Orris absolute	8002-73-1	N.A.	172.510	241n	1.3
120	Palmitic acid	57-10-3	2832	172.860	14c	1.3
121	Phenethyl phenylacetate	102-20-5	2866	172.515	234c	1.3
122	3-Propylidenephthalide	17369-59-4	2952	172.515	494c	1.3
123	Sage oil	8022-56-8	3001	182.20	61n	1.3
124	alpha-Terpineol	98-55-5	3045	172.515	62c	1.3
125	Terpinyl acetate	80-26-2	3047	172.515	205c	1.3
126	gamma-Undecalactone	104-67-6	3091	172.515	179c	1.3
127	gamma-Valerolactone	108-29-2	3103	N.A.	757c	1.3
128	3-Butylidenphthalide	551-08-6	3333	N.A.	N.A.	1.04
129	Davana oil	8016-03-3	2359	172.510	69n	0.65
130	3,5-Dimethyl-1, 2-cyclopentanedione	13494-07-0	3269	N.A.	2235c	0.65
131	Ethyl cinnamate	103-36-6	2430	172.515	323c	0.65
132	Farnesol	4602-84-0	2478	172.515	78c	0.65
133	Geranyl phenylacetate	102-22-7	2516	172.515	231c	0.65
134	alpha-Irone	79-69-6	2597	172.515	145c	0.65
135	Jasmine absolute	8022-96-6	2598	182.20	245n	0.65
136	Kola nut tincture	68916-19-8	2607	182.20	149n	0.65
137	Linalool oxide	1365-19-1	3746	172.515	N.A.	0.65
138	Linalyl acetate	115-95-7	2636	182.60	203c	0.65
139	*para*-Methoxybenzaldehyde	123-11-5	2670	172.515	103c	0.65

(Continued on next page)

TABLE 2
Ingredients added to test cigarettes in study 1 *(Continued)*

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
140	2-Methylbutyric acid	116-53-0	2695	172.515	2002c	0.65
141	Myristic acid	544-63-8	2764	172.860	16c	0.65
142	gamma-Octalactone	104-50-7	2796	172.515	2274c	0.65
143	Opoponax oil	8021-36-1	N.A.	172.510	313n	0.65
144	Tagetes oil	8016-84-0	3040	172.510	443n	0.65
145	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	21835-01-8	3152	N.A.	759c	0.52
146	4-Methylacetophenone	122-00-9	2677	172.515	156c	0.26
147	Isobutyraldehyde	78-84-2	2220	172.515	92c	0.13
148	3-Methylbutyraldehyde	590-86-3	2692	172.515	94c	0.13
149	2,3-Dimethylpyrazine	5910-89-4	3271	N.A.	N.A.	0.13
150	2,5-Dimethylpyrazine	123-32-0	3272	N.A.	2210c	0.13
151	2,6-Dimethylpyrazine	108-50-9	3273	N.A.	2211c	0.13
152	Dimethyltetrahydrobenzofuranone	13341-72-5	3764	N.A.	N.A.	0.13
153	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	3658-77-3	3174	N.A.	536c	0.13
154	4-(*para*-Hydroxyphenyl)-2-butanone	5471-51-2	2588	172.515	755c	0.13
155	alpha-Ionone	127-41-3	2594	172.515	141c	0.13
156	beta-Ionone	8013-90-9	2595	172.515	142c	0.13
157	Isovaleric acid	503-74-2	3102	172.515	8c	0.13
158	Lime oil	8008-26-2	2631	182.20	141n	0.13
159	Mace absolute	8007-12-3	N.A.	182.20	296n	0.13
160	Nutmeg oil	8008-45-5	2793	182.20	296n	0.13
161	Caprylic acid	124-07-2	2799	184.1025	10c	0.13
162	Phenylacetaldehyde	122-78-1	2874	172.515	116c	0.13
163	5,6,7,8-Tetrahydroquinoxaline	34413-35-9	N.A.	N.A.	721c	0.13
164	Thyme oil	8007-46-3	3064	182.20	456n	0.13
165	Valeraldehyde	110-62-3	3098	172.515	93c	0.13

Note. "n" Follows the name of natural source of flavorings and "c" follows the number of chemical substances.
[a]Chemical Abstract Service registry number.
[b]The Flavor and Extract Manufacturers Association reference number.
[c]Code of Federal Regulations reference to Title 21 indicating regulatory status of material.
[d]Council of Europe reference number.

Inhalation Toxicity Study Design

Groups of 30 Sprague-Dawley rats of each sex were exposed by nose-only inhalation for 1 h/day, 5 days/wk for 13 consecutive weeks to concentrations of 0.06, 0.2, or 0.8 mg/L WTPM of smoke from test cigarettes containing flavoring (study 1) or to flavoring or casing ingredients (study 2). Additional groups of 30 rats/sex were exposed to the same concentrations of smoke from reference cigarettes, similar to the test cigarettes but without the flavoring or casing ingredients (as described above), or to filtered air only (sham controls). This exposure regimen (1 h/day, 5 days/wk) reflects current laboratory practices for animal inhalation studies comparing the effects of smoke from test and reference cigarettes, and does not simulate human usage patterns. However, this difference should not influence the validity of the results.

Each group of 30 rats/sex was subdivided into 2 groups: 20 rats/sex scheduled for necropsy immediately after 13 wk of exposure (interim sacrifice) and up to 10 rats/sex scheduled for necropsy following 13 wk of recovery from smoke exposure (final sacrifice). Target smoke concentrations were 0.06, 0.2, or 0.8 mg WTPM/L for the test and reference cigarettes. An additional group of 30 rats/sex served as sham controls.

Biological endpoints for the 13-wk exposure and 13-wk recovery groups included clinical appearance, body weight, organ weights, and gross and microscopic lesions. Plasma nicotine, COHb, and respiratory parameters were measured periodically during the 13-wk exposure period and clinical pathology parameters were measured at the end of the 13-wk exposure period.

Smoke Generation and Exposure System

Animal exposures were conducted in AMESA exposure units (C. H. Technologies, Westwood, NJ). The smoke exposure machines were designed to contain 30 cigarettes on a smoking head that rotated 1 revolution per minute (Baumgartner and Coggins,

TABLE 3
Ingredients added to study 2 test cigarettes

	Ingredient	CAS no.[a]	FEMA no.[b]	CFR[c]	CoE[d]	Application rate (ppm)
1	Invert sugar	8013-17-0	N.A.	184-1859	N.A.	20,000
2	Block chocolate	N.A.	N.A.	N.A.	N.A.	2,500
3	Plum extract	90082-87-4	N.A.	N.A.	371n	2,200
4	Fig extract	90028-74-3	N.A.	N.A.	198n	2,000
5	Molasse extract and tincture	68476-78-8	N.A.	N.A.	371n	2,000
6	Gentian root extract	97676-22-7	2506	172-510	214n	1,000
7	Lovage extract	8016-31-7	2650	172-510	261n	1,000
8	Peppermint oil	8006-90-4	2848	182-20	282n	250

Note. "n" Follows the name of natural source of flavorings and "c" follows the number of chemical substances.
[a]Chemical Abstract Service registry number.
[b]The Flavor and Extract Manufacturer's Association reference number.
[c]Code of Federal Regulations reference to Title 21 indicating regulatory status of material.
[d]Council of Europe reference number.

1980; Ayres et al., 1990). A vacuum port aligned with, and drew a puff from, one test or reference cigarette at a time as the head rotated. Air was drawn through the vacuum port by a peristaltic pump operating at a flow rate of ~1.05 L/min, creating a 2-s, 35-ml puff through each cigarette once each minute. The smoke vacuum flow rate was regulated by a concentration control unit consisting of a real-time aerosol monitor [(RAM)-1; MIE, Inc., Bedford, MA], a computer, and an electronic flow controller (Emerson Electric Co., Brooks Instrument Division, Hatfield, PA). The computer monitored analog voltage output of the RAM and adjusted the amount of smoke that was drawn from the glass mixing bowl by the flow controller until RAM voltage matched the calculated target voltage. The exposure units contained 3 tiers, each with 24 animal exposure ports. The exposure ports were connected to a delivery manifold, which transferred smoke to the animal breathing zone, and to an outer concentric manifold that drew the exhaled and excess smoke to an exhaust duct. Each cigarette was retained for seven puffs.

Exposure Atmosphere Characterization

The protocol-prescribed limits for the smoke concentration (WTPM/L) were target ±10% coefficient of variation (%CV). Smoke exposure concentrations were continuously monitored with a RAM at a representative exposure port. Mean exposure concentration was calculated from the mass collected on the filter and the total volume of air drawn through the filter, which was determined by the sample time and flow rate. RAM voltage readings were recorded during filter sample collection and were used to calculate a RAM response factor for subsequent exposures.

Two filters per exposure group per week were chemically analyzed for total nicotine. Nicotine standard reference material (98%) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). The WTPM:nicotine and CO:nicotine ratios were calculated for the exposure atmospheres. The concentration of CO in the test and reference atmospheres was determined using Horiba PIR-2000 CO analyzers (Horiba Instruments, Inc., Irvine, CA), monitored by DOS-based computers.

Particle size distribution of the smoke was measured using Mercer-style cascade impactors designed specifically for the size range of particles found in cigarette smoke. The mass collected on each impactor stage was analyzed gravimetrically for WTPM and the resulting data were interpreted by probit analysis (NEWCAS; Hill et al., 1977) to obtain the particle size distribution, mass median aerodynamic diameter (MMAD), and geometric standard deviation (GSD). Temperature and RH of the exposure atmospheres were measured from a representative animal exposure port once every 2 wk for each exposure group.

Animals and Animal Care

Sprague-Dawley (Crl:CD) rats 4–5 wk of age were purchased from Charles River Laboratories (Raleigh, NC), held for 13 days in quarantine status prior to initial smoke exposure. Health screens were performed following group assignment and at 24 days after arrival. These health evaluations included necropsy, microscopic examination of selected tissues and examination for parasites. The 24 days after arrival screening included serological testing for antibodies to common viral pathogens. Viral antibody testing was also performed on sera collected from 10 sentinel rats at the end of the 13-wk exposure period and from another 10 at the end of the recovery period. All sera were tested for antibodies to Sendai virus, Kilham's rat virus (KRV)/Toolan's H-1 virus, pneumonia virus of mice (PVM), rat corona virus/sialodacryoadenitis virus, and *Mycoplasma pulmonis*. During the 13-wk exposure period, the animals were housed in individual stainless-steel cages on open racks. During the recovery period, the animals were housed in individual polycarbonate cages (Lab Products, Maywood, NJ) bedded with

ALPHA-dri alpha cellulose bedding (Sheperd Specialty Papers, Kalamazoo, MI). The cage space met the requirements stated in the current *Guide for Care and Use of Laboratory Animals* (National Academy of Sciences, 1996).

Body Weight and Clinical Observations

All rats were observed twice daily for mortality and moribundity. Each rat was examined every 4 wk for clinical signs. Individual body weights were measured during the randomization procedure, on exposure day 1, biweekly thereafter, and at necropsy.

Respiratory Function Measurements

Tidal volume (TV), respiratory rate (RR), and minute volume (MV), derived from flow signals from spontaneously breathing animals, were measured in 4 rats/sex/group during wk 2, 8, and 13 using whole-body phethysmography (Coggins et al., 1981). Each animal was monitored once during a single exposure period. MV and the actual WTPM were used to estimate the average total inhaled mass for the 1-h exposure period for each animal.

Carboxyhemoglobin and Plasma Nicotine Determinations

During wk 2 and 10, blood was collected from designated animals at the end of the 1-h smoke exposure. Animals were removed from the exposure unit and bleeding was initiated within ~5 min. The blood samples were obtained from the retro-orbital plexus of carbon dioxide (CO_2)-anesthetized animals into tubes containing potassium ethylenediaminete traacetic acid (K^+-EDTA). The sample tubes were immediately placed into an ice bath and maintained under these conditions until analyzed for blood carboxyhemoglobin (COHb). Plasma nicotine was quantitatively determined using gas chromatography/mass spectrometry (GC/MS) with selected ion monitoring.

Clinical Pathology

On the day of the 13-wk interim sacrifice, the rats were anesthetized with ~70% CO_2 in room air and blood samples were obtained from the retro-orbital plexus. One sample was collected in a tube (Monoject, Sherwood Medical, St. Louis, MO) containing K^+-EDTA for hematologic determinations. Another sample was collected in a tube devoid of anticoagulant but containing a separator gel (Vacutainer, Franklin Lakes, NJ) for serum chemistry analysis. The following parameters were determined using an Abbott Cell-Dyn 3700 (Abbott Diagnostics Systems, Abbott Park, IL) multiparameter hematology instrument: white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb) concentration, volume of packed red cells (VPRC), the red cell indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], and mean corpuscular hemoglobin concentration [MCHC]), platelet count, and WBC differential counts. Results of the differential cell counts were reported as both relative and absolute values. Reticulocytes were stained supravitally with new methylene blue and enumerated as reticulocytes per 1000 enthrocytes using the Miller disc method (Brecher and Schneiderman, 1950).

A Roche Hitachi 912 system (Roche Diagnostic Corp., Indianapolis, IN) chemistry analyzer was used to determine the following serum analytes: urea nitrogen (BUN), creatinine, glucose, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), sodium, potassium, chloride, calcium, phosphorus, total bilirubin, cholesterol, and triglycerides.

Necropsy and Tissue Collection

A complete necropsy was done on all 13-wk exposure groups and 13-wk recovery group animals. Rats designated for scheduled sacrifices or sacrificed due to moribund condition were weighed and anesthetized with 70% CO_2 in air, followed by exsanguination before cessation of heartbeat. All abnormalities were recorded on the individual animal necropsy forms. Lungs, liver, kidneys, testes, adrenals, spleen, brain, and heart from all scheduled sacrifice animals were weighed. These organ weights and the body weights at necropsy were used to calculate organ:body weight ratios. In addition, organ:brain weight ratios were calculated. The time from removal of the organ until weighing was minimized to keep tissues moist.

A complete set of over 40 tissues was removed from each animal at necropsy and examined. All tissues were fixed in 10% neutral buffered formalin (NBF) except for the eyes, which were fixed in Karnovsky's fixative. After the lungs were weighed, they were perfused with 10% NBF at 25 cm hydrostatic pressure.

Histopathology

All tissues were fixed in 10% NBF for a minimum of 48 h before being trimmed. Paraffin blocks were microtomed at 5 μm. All sections were stained with hematoxylin and eosin (H&E) stains for standard histopathologic evaluation of morphologic changes. Duplicate slides of nasal tissues, larynx, lung, and trachea were stained with periodic acid-Schiff/Alcian blue (PAS/AB) stains for evaluation of goblet cell populations. The lungs, nasal cavity (four sections), nasopharynx, larynx (three cross sections), trachea (three transverse sections), tracheobronchial lymph nodes, mediastinal (thymic) lymph nodes, heart, and all gross lesions were examined microscopically. The lungs were sectioned to present a maximal section of the mainstem bronchi. The nasal cavity was prepared in four sections using the landmarks described by Young (1981). Three transverse laryngeal sections were prepared from the base of the epiglottis, the ventral pouch, and through the caudal larynx at the level of the vocal folds (Renne et al., 1992). In addition, sections of brain, adrenals, spleen, liver, kidneys, and gonads from animals in the sham control and the groups exposed to 0.8 mg/L of smoke from the test or reference cigarettes were examined microscopically. Exposure-related microscopic lesions were observed in the tissues from the rats exposed to 0.8 mg/L; target organs were examined microscopically in the lower concentration groups to ascertain a no-effect concentration.

Evaluation of Cell Proliferation Rates of Respiratory-Tract Tissues

Cell proliferation rates were measured on respiratory tract tissues collected from 10 rats of each sex from each exposure group and the sham controls necropsied immediately after 13 wk of exposure, using a monoclonal antibody to 5-bromo-2′-deoxyuridine (BrdU). Tissues evaluated using the BrdU assay included the respiratory epithelium lining the median nasal septum and distal portions of maxillary and nasal turbinates, the transitional epithelium at the base of the epiglottis, the luminal epithelium dorsolateral to the ventral pouch, the luminal epithelium lining the cranial trachea, the luminal epithelium of the mainstem bronchi and adjacent bronchioles, and selected areas of alveolar epithelium. Data from both sides of bilaterally symmetrical tissues (nose, ventral pouch, mainstem bronchi) were combined for tabulation of results.

Statistical Methods

Body weight, body weight gain, organ:body weight, and organ:brain weight ratios were statistically analyzed for each sex by exposure concentration group using the Xybion PATH/TOX system. Data homogeneity was determined by Bartlett's test. Dunnett's t-test was performed on homogeneous data to identify differences between each concentration group and the sham control group, and between corresponding concentrations of test and reference cigarette smoke-exposed groups. Nonhomogeneous data were analyzed using a modified t-test. Respiratory physiology, clinical pathology, COHb, and plasma nicotine data parameters were statistically evaluated using SAS software (Statistical Analysis System, SAS, Inc., Cary, NC). One-way analysis of variance (ANOVA) between exposure groups was first conducted, followed by Bartlett's test for homogeneity of variance. A two-sided Dunnett's multiple comparison test was employed to determine which exposure groups were different from the controls. An unpaired two-sided t-test was used to compare equivalent exposure groups between cigarette types. Differences were considered significant at $p \leq .05$. The statistical evaluation of incidence and severity of lesions was made using the Kolmogorov–Smirnov two-sample test (Siegel, 1956). All treatment group means were compared to the sham control mean, and means of groups exposed to the test cigarette smoke were compared to the corresponding reference cigarette smoke-exposed group means. Cell proliferation data were compared statistically using Tukey's studentized range test with SAS software.

RESULTS

Cigarette Performance

The results of characterization of the test and reference cigarettes for study 1 and study 2 are presented in Tables 4 and 5. These results show that the filler weight and the number of puffs per cigarette, nicotine yield, and nicotine-free dry particulate matter (NFDPM) were comparable for test and reference cigarettes in both studies. The yields of nicotine and NFDPM and the puff count were also comparable. These results are consistent with the negligible differences in the configuration of both prototype cigarettes, which basically consist of the total relative amount of flavor ingredient contained in the test cigarettes (1% or 3% of the filler weight). A comparison of the burning rates in study 1 illustrates that the addition of the ingredients had little, if any effect on the burning characteristics of the test cigarettes.

TABLE 4
Key parameters for laboratory control of prototype study 1 cigarettes

Parameter	Target	Run average: Test cigarette	Run average: Reference cigarette
Individual weights (g)			
Cigarette weight	1.012	0.963	0.965
Standard deviation	—	0.019	0.018
Non tobacco weight	0.212	0.212	0.215
Net tobacco	0.800	0.751	0.750
Air dilution (%)	32	35	34.1
Standard deviation	—	3.0	3.1
Porosity of cigarette paper (cc/min/cbar/cm^2)	50	49	49
Expanded tobacco (%)	9.7	10.1	9.1
Nicotine (mg/cig)	0.9	0.92	0.97
Nicotine (mg/puff)	n.a.	0.118	0.123
NFDPM (mg/cig)	12.0	11.3	11.5
NFDPM (mg/puff)	n.a.	1.45	1.46
CO (mg/cig)	n.a.	12.4	13.1
CO (mg/puff)	n.a.	1.59	1.66
Puffs/cigarette	n.a.	7.8	7.9
Burning rate (mg tobacco/min)	n.a.	68.1	64.4

Note. Cig, cigarette.

In Vitro Mutagenicity Assays

Figures 1, 2, 3, and 4 summarize the results of Ames assays on test cigarettes from study 1 and 2 with and without metabolic activation. TA100, TA98, and TA1537 strains showed a positive response only with metabolic activation. No response was observed in TA 102 or TA1535. No sporadic responses in revertants were recorded. The highest sensitivity and specificity of the mutagenic response were observed using TA98 with metabolic activation. From the comparison of the data obtained for the test and reference cigarettes, it was concluded that the addition of ingredients did not result in a positive mutagenic response in any of the strains under the conditions already described. Hence, the use of the tested ingredients had no influence on the mutagenic activity of the cigarettes.

TABLE 5
Key parameters for laboratory control of prototype study 2 cigarettes

Parameter	Target	Run average	
		Test cigarette	Reference cigarette
Individual weights (g)			
Cigarette weight	1.012	1.002	1.025
Standard deviation	—	0.0208	0.0173
Nontobacco weight	0.212	0.212	0.212
Net tobacco	0.800	0.790	0.813
Air dilution (%)	32	33.2	36.6
Standard deviation	—	1.6	1.4
Porosity of cigarette paper (cc/min/cbar/cm^2)	50	50	47
Expanded tobacco (%)	9.5	9.6	9.3
Nicotine (mg/cig)	0.9	0.93	0.93
Nicotine (mg/puff)	n.a.	0.112	0.107
NFDPM (mg/cig)	12.0	11.4	11.0
NFDPM (mg/puff)	n.a.	1.37	1.26
CO (mg/cig)	n.a.	12.9	12.8
CO (mg/puff)	n.a.	1.55	1.47
Puffs/cigarette	n.a.	8.3	8.7

Note. Cig, cigarette.

Exposure Atmosphere Characterization

Tables 6 and 7 summarize the exposure data for the inhalation exposure periods for study 1 and study 2. The mean exposure concentrations (WTPM) were all within 3% of the target concentration, with CVs of 6.6%, or less. Nicotine and CO concentrations correlated well with WTPM in reference and test cigarette smoke atmospheres in both study 1 and study 2. Particle sizes were slightly larger in the study 1 test and reference cigarette smokes. All concentrations of the smoke from each cigarette were highly respirable for the rat model under investigation.

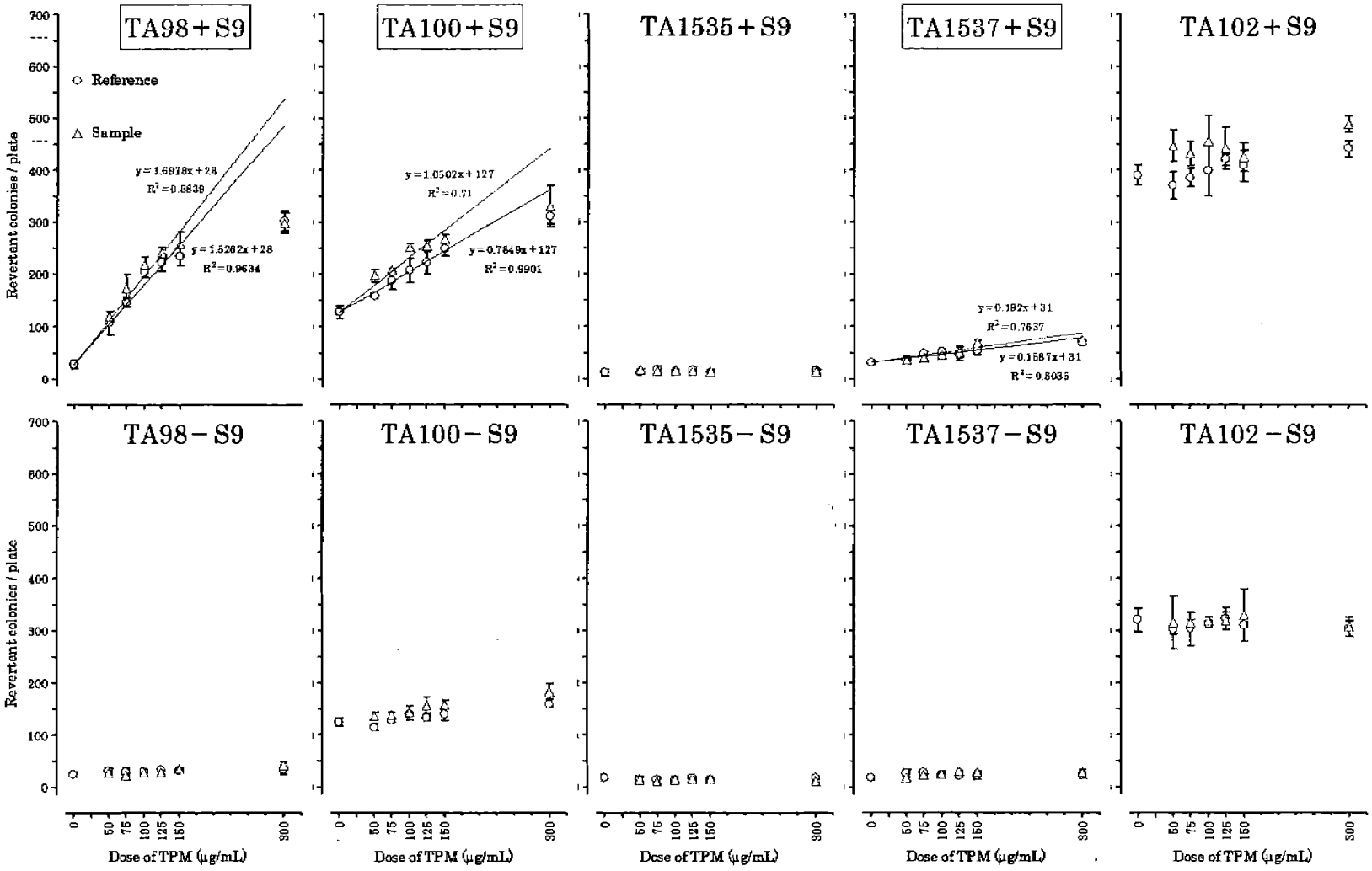
Body Weights and Clinical Observations

No significant mortality occurred in either study. Exposure-related adverse clinical signs were absent. Clinical observations noted were minor in consequence and low in incidence.

Mean body weight data for all groups on study throughout the exposure and recovery periods are illustrated in Figure 5. In study 1, mean body weights were consistently decreased compared to sham controls during the exposure period in male rats exposed to 0.8 mg/L of reference cigarette smoke and in males exposed to all 3 concentrations of test cigarette smoke. With the exception of day 71 (0.8 mg/L test), all female smoke-exposed groups in study 1 were comparable to sham control females throughout the study. In study 2, mean body weights were consistently decreased compared to sham controls in males exposed to 0.8 mg/L of test cigarette smoke and in females exposed to 0.8 mg/L of reference cigarette smoke. Mean body weights of smoke-exposed groups were similar to sham control weights during the recovery period of both study 1 and study 2. The only consistent statistical difference in body weight changes between the test and reference cigarette smoke-exposed groups in either study was the decreased mean body weight in males exposed to 0.8 mg/L of reference cigarette smoke during the exposure period of study 1.

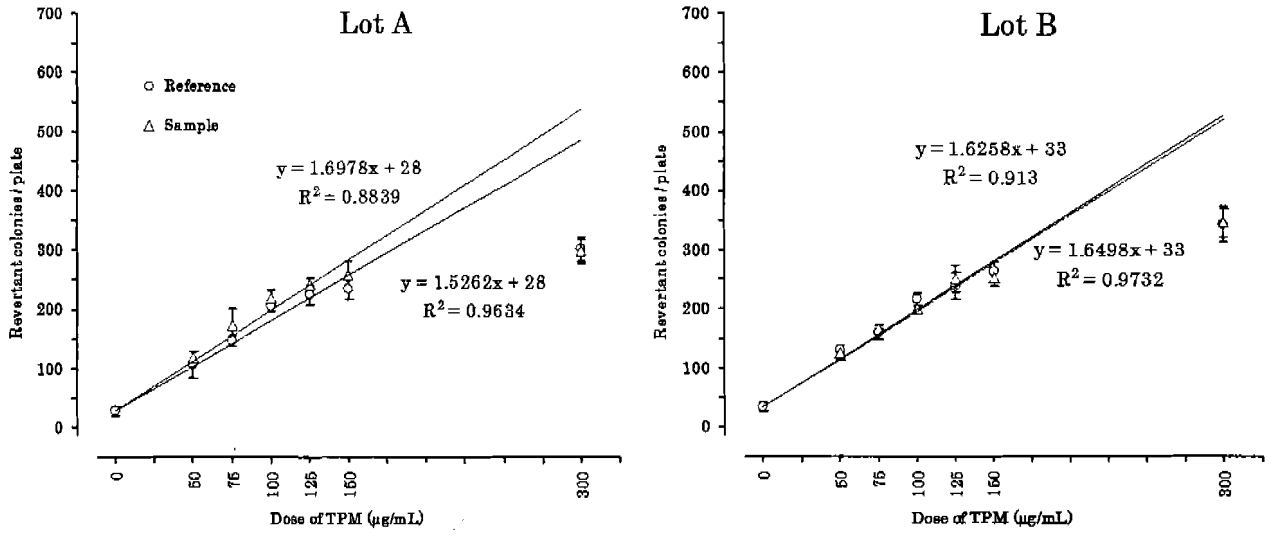
Organ Weights

Comparisons of selected group mean organ weights between smoke-exposed and sham controls in study 1 are presented in Table 8. Statistically significant differences in organ weights in groups of smoke-exposed rats were primarily low mean organ weights compared to their respective sham controls. There was no clear pattern of differences in any absolute or relative organ weight in smoke-exposed groups compared to sham controls, or in groups exposed to test versus reference cigarette smoke at either the interim sacrifice or the recovery sacrifices. Sham controls for the interim sacrifice of study 2 were inadvertently not fasted overnight prior to necropsy, which made comparison of absolute and relative organ weights of smoke-exposed and sham control groups from the interim sacrifice of questionable scientific value; thus these comparisons were not made for study 2. Statistical comparison of absolute and relative organ weights between groups exposed to test and reference cigarette smoke in study 2 showed very few statistically significant differences, none of which were considered toxicologically



N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot.

FIG. 1. Ames assay results, study 1 cigarettes.



MEAN ± SD of Specific Activity (50 to 150 µg/plate)

Reference	1576 ± 141.9	Reference.....	1734 ± 170.9
Sample.....	1783 ± 167.3	Sample.....	1703 ± 151.2

FIG. 2. Ames assay results, study 1 with TA98 metabolic activation.

significant. Comparison of organ weights in rats necropsied following the 13-wk recovery of study 2 indicated no consistent differences between sham control and smoke-exposed groups, or between groups exposed to similar concentrations of test and reference cigarette smoke.

Respiratory Physiology

Reductions in RR and/or TV resulted in consistently lower MV in rats exposed to test or reference cigarette smoke compared to sham controls in both study 1 and study 2. There was no consistent difference in MV between groups of rats exposed to test and reference cigarette smoke in either study. Because the overall MV in study 1 was similar among groups exposed to smoke, total inhaled mass was proportional to increasing smoke concentration in this study. In study 2, decreases in MV in groups exposed to 0.8 or 0.2 mg/L compared to groups exposed to 0.06 mg/L caused total inhaled mass for the high and middle dose groups to be lower in proportion to the exposure concentration of inhaled smoke.

Clinical Pathology

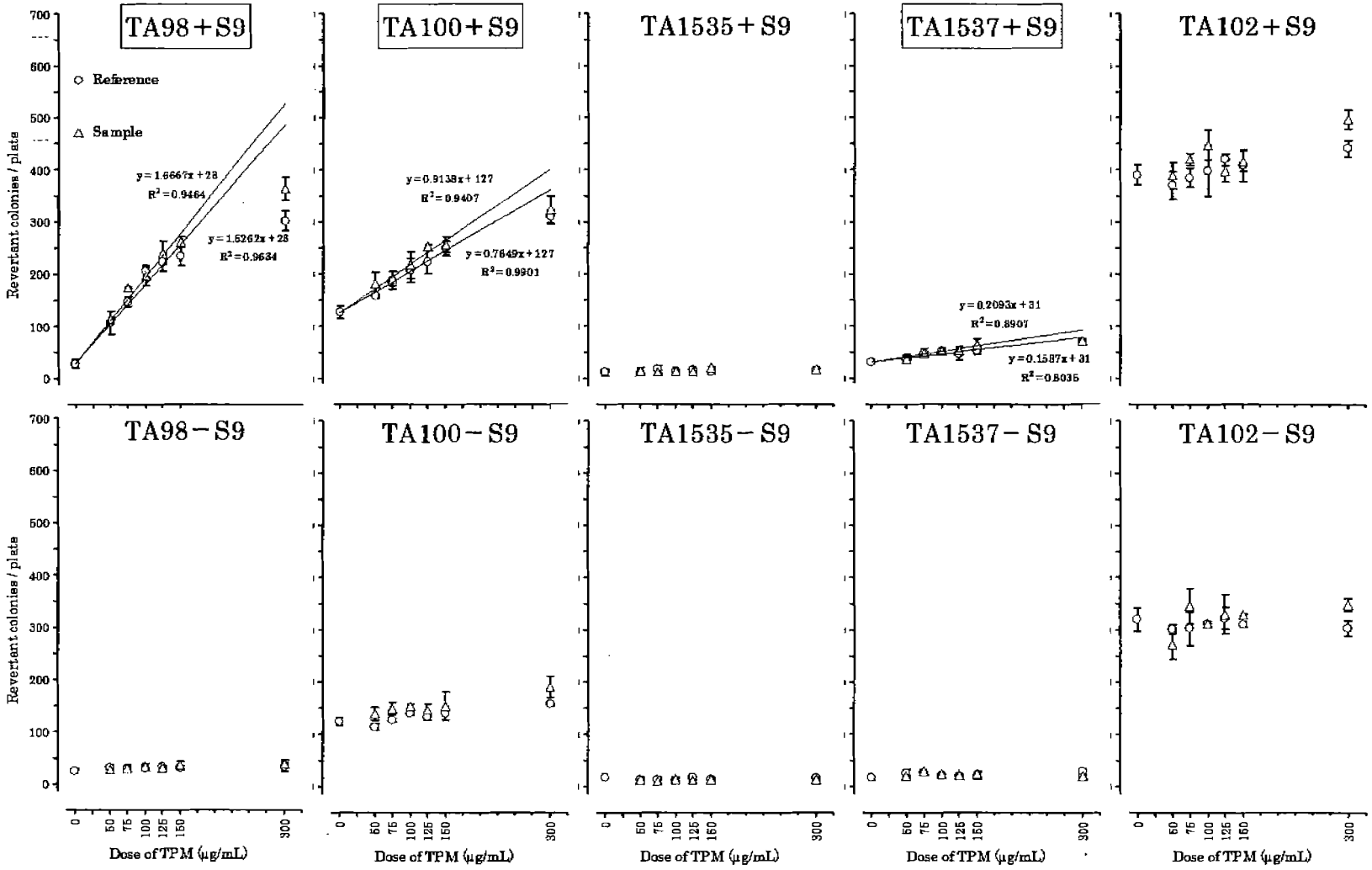
There were occasional statistically significant differences in hematology and clinical chemistry parameters from control values in groups exposed to smoke from test or reference cigarettes in both study 1 and study 2. These differences did not occur in a dose-response pattern and were well within ±2 standard deviations of historic values for control Sprague-Dawley rats of comparable age. There were also statistically significant differences in several hematology and clinical chemistry parameters between groups exposed to similar concentrations of test and reference cigarette smoke. These differences are not considered to be of toxicologic significance, nor were they exposure related.

Whole-blood COHb levels were increased in a graded dose-response fashion as a function of exposure concentration for all test and reference cigarette smoke-exposed groups in both studies. In study 2 rats bled during exposure wk 2, there was a statistically significant decrease in COHb levels in both sexes exposed to 0.8 mg/L of test cigarette smoke and in females exposed to 0.2 mg/L of test cigarette smoke, compared to groups exposed to reference cigarette smoke. There were no other clear differences in whole blood COHb levels between the test and reference cigarette groups at equivalent exposure levels in either study.

Plasma nicotine levels increased in a graded dose-response fashion for test and reference males and female groups in both studies. In study 2, test female groups exposed to 0.8 mg/L had significantly lower plasma nicotine levels than the 0.8 mg/L reference females at both 2- and 10-wk sampling. Comparing males to females at all exposure levels for test and reference cigarettes, the females consistently had higher plasma nicotine levels in both studies.

Pathology

Few gross lesions were observed in either study, with no evidence of changes attributable to exposure to smoke from the test



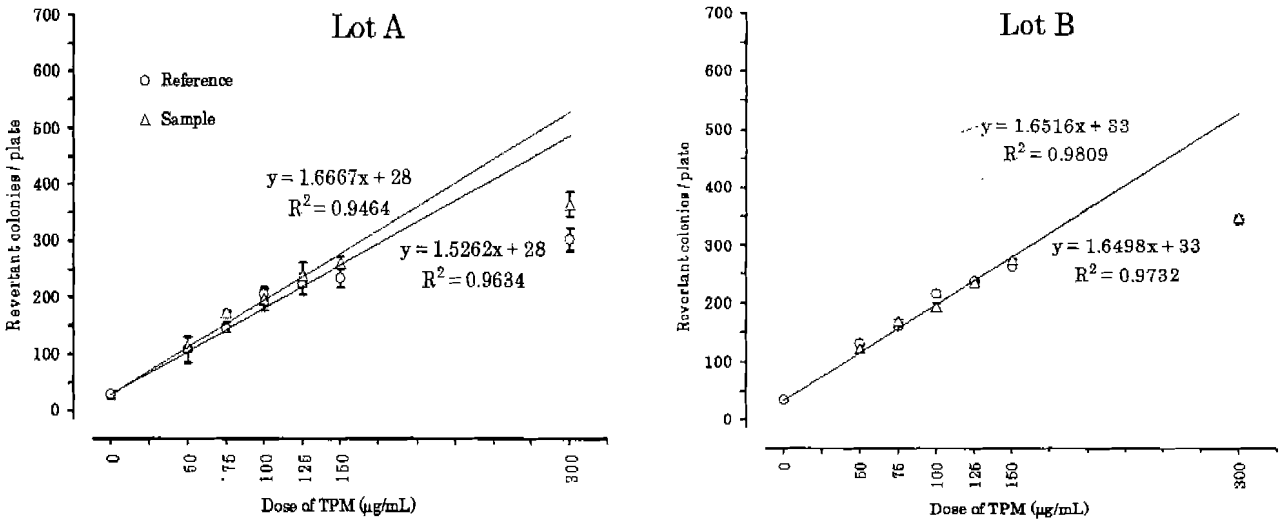
N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot .

FIG. 3. Ames assay results, study 2 cigarettes.

TABLE 6
Study 1, exposure concentration data for rats exposed to mainstream smoke from test or reference cigarettes

	Concentration [mean ± SD (%CV)]				
	Measured exposure concentration (mg WTPM/L; $n = 126$)	Nicotine concentration (μg/L; $n = 28$)	CO concentration (ppm; $n = 63$)	Percent of target WTPM concentration (mean ± SD)	Particle size (MMAD, μm)
Test target exposure concentration (mg WTPM/L)					
0.800	0.787 ± 0.035 (4.4)	68.2 ± 2.5 (3.7)	584 ± 27 (4.6)	98.4 ± 4.3	0.73 ± 0.08
0.200	0.199 ± 0.009 (4.5)	15.5 ± 1.0 (6.5)	144 ± 6 (4.2)	99.3 ± 4.3	0.74 ± 0.12
0.060	0.061 ± 0.004 (6.6)	4.4 ± 0.5 (11.4)	47 ± 3 (6.4)	101 ± 6	0.69 ± 0.09
Reference target exposure concentration (mg WTPM/L)					
0.800	0.795 ± 0.023 (2.9)	70.1 ± 2.1 (2.9)	608 ± 20 (3.3)	99.4 ± 2.7	0.74 ± 0.08
0.200	0.202 ± 0.004 (2.0)	15.8 ± 0.7 (4.5)	147 ± 4 (2.7)	101 ± 2	0.72 ± 0.07
0.060	0.060 ± 0.002 (3.3)	4.4 ± 0.4 (9.8)	50 ± 2 (4.8)	100 ± 4	0.74 ± 0.10

Note. CO, carbon monoxide; WTPM, wet total particulate matter.



MEAN±SD of Specific Activity (50 to 150 µg/plate)

Reference.....	1576±141.9	Reference.....	1734±170.9
Sample.....	1726±138.6	Sample-1.....	1701±107.9

FIG. 4. Ames assay results, study 2 cigarettes with TA98 metabolic activation.

TABLE 7
Study 2, exposure concentration data for rats exposed to smoke from test or reference cigarettes

	Concentration [mean ± SD (%CV)]				
	Measured exposure concentration (mg WTPM/L; $n = 134$)	Nicotine concentration (μg/L; $n = 28$)	CO concentration (ppm; $n = 67$)	Percent of target WTPM concentration (mean ± SD)	Particle size (MMAD, μm)
Test target exposure concentration (mg WTPM/L)					
0.8	0.798 ± 0.040 (5.0)	56.8 ± 2.6 (4.6)	646 ± 34 (5.3)	100 ± 5	0.65 ± 0.01
0.2	0.194 ± 0.007 (3.6)	12.9 ± 0.6 (4.7)	158 ± 9 (5.7)	97 ± 4	0.62 ± 0.04
0.060	0.060 ± 0.002 (3.3)	4.0 ± 0.2 (5.0)	54 ± 3 (5.6)	100 ± 3	0.66 ± 0.03
Reference target exposure concentration (mg WTPM/L)					
0.8	0.784 ± 0.031 (4.0)	55.1 ± 2.3 (4.2)	676 ± 31 (4.6)	98 ± 4	0.57 ± 0.03
0.2	0.201 ± 0.004 (1.8)	13.0 ± 0.4 (3.4)	170 ± 15 (8.7)	100 ± 2	0.64 ± 0.07
0.060	0.060 ± 0.002 (3.3)	4.1 ± 0.2 (4.4)	57 ± 3 (5.8)	99 ± 3	0.66 ± 0.06

Note. CO, carbon monoxide; WTPM, wet total particulate matter.

or the reference cigarettes. Exposure to smoke from reference or test cigarettes in both studies induced concentration-related proliferative, metaplastic, and inflammatory microscopic lesions in the respiratory tract after 13 wk of exposure. The incidence of exposure-related respiratory-tract lesions observed at microscopic examination of tissues from rats necropsied at the interim sacrifice immediately following 13 wk of exposure is summarized in Table 9 for study 1 and Table 10 for study 2.

Hyperplasia of respiratory epithelium lining the anterior nasal cavity was present in all rats exposed to 0.8 mg/L in both studies, a few rats exposed to 0.2 mg/L in both studies, and in 3/40 rats exposed to 0.06 mg/L in study 1. Areas most severely and most frequently affected were the distal portions of the nasal and maxillary turbinates in sections of nose just caudal to the incisor teeth. In affected rats, the epithelium in the distal turbinates was up to six cells thick. There was also a clear dose response in the severity of nasal respiratory epithelial hyperplasia, with severity ranging from minimal to moderate. Comparison of incidence and severity data for nasal respiratory epithelial hyperplasia in rats exposed to similar concentrations of smoke from the test and reference cigarettes did not indicate any statistically significant differences in either study. Minimal goblet-cell hyperplasia was observed in the mucosal epithelium lining the median nasal septum in some smoke-exposed and sham control rats. Although not statistically significant compared to concurrent sham controls, the incidence of nasal goblet cell hyperplasia in male rats exposed to the 0.8-mg/L concentration of smoke from the reference cigarette or test cigarette in study 1 were considered to be toxicologically significant. There was no clear difference in the incidence of goblet cell hyperplasia between groups exposed to similar concentrations of reference and test cigarette smoke in either study.

Exposure to smoke from the reference or test cigarette in both study 1 and study 2 induced squamous metaplasia, hyperplasia, and hyperkeratosis of the transitional epithelium lining the base of the epiglottis and the epithelium lining the dorsal border of the ventral pouch and the adjacent laryngeal lumen. In control rats, the epithelium lining the base of the epiglottis was a mixture of ciliated columnar epithelium and slightly flattened, oval, rounded, or cuboidal cells one or two cells thick over a poorly defined basal cell layer (Renne et al., 1992). In affected smoke-exposed rats, the base of the epiglottis was covered by a stratified squamous epithelium up to eight cells thick with a variably keratinized surface layer and a distinct basal cell layer. There was a concentration-related increase in severity of squamous metaplasia and hyperplasia of epiglottis epithelium in rats exposed to test or reference cigarette smoke. Statistical analysis did not indicate any significant differences in incidence or severity of these lesions between test and reference cigarette smoke-exposed groups in either study. Hyperkeratosis (accumulation of keratinized squamous cells on the surface) was observed in association with squamous metaplasia of the epithelium lining the base of the epiglottis in most rats exposed to smoke from reference or test cigarettes. Comparison of incidence/severity of hyperkeratosis in the epiglottis between test and reference cigarette smoke-exposed groups indicated a statistically

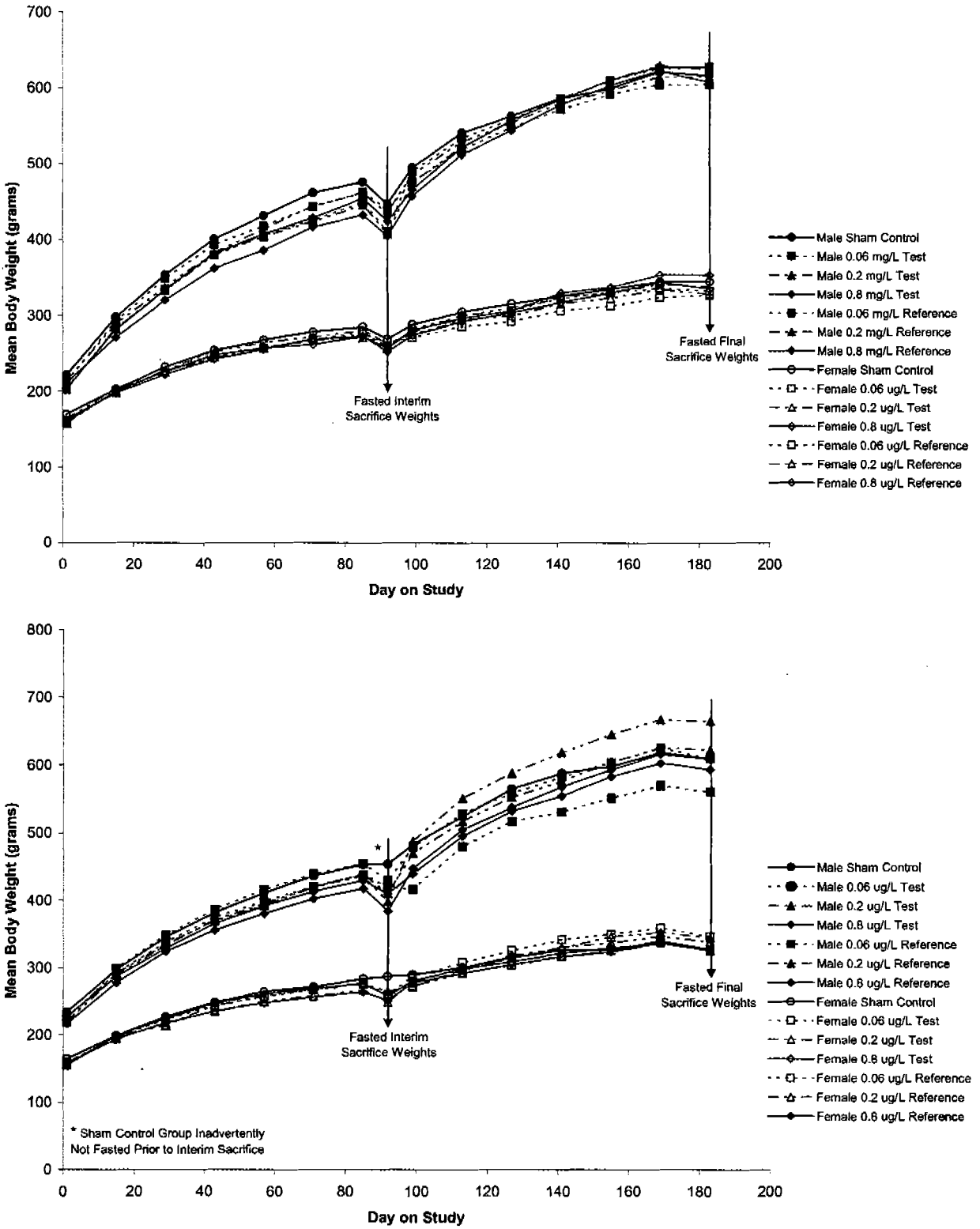


FIG. 5. Body weights, study 1 (top) and study 2 (bottom).

TABLE 8
Organ weights for rats exposed to smoke from study 1 cigarettes ($n = 20$, g ± SD)

		Test			Reference		
	Sham control	0.06 mg WTPM/L	0.2 mg WTPM/L	0.8 mg WTPM/L	0.06 mg WTPM/L	0.2 mg WTPM/L	0.8 mg WTPM/L
Males							
Heart	1.60 ± 0.16	1.48 ± 0.15[a,b]	1.43 ± 0.16[a,c]	1.55 ± 0.15	1.60 ± 0.13	1.57 ± 0.16	1.52 ± 0.15
Kidneys	3.39 ± 0.33	3.17 ± 0.39	2.92 ± 0.30[a,c]	3.05 ± 0.33[a]	3.38 ± 0.33	3.20 ± 0.31	3.02 ± 0.27[a]
Lungs	1.95 ± 0.22	1.89 ± 0.17	1.82 ± 0.23[c]	1.93 ± 0.14	2.02 ± 0.28	1.98 ± 0.26	1.89 ± 0.15
Adrenals	0.066 ± 0.010	0.066 ± 0.012	0.059 ± 0.010	0.064 ± 0.012	0.062 ± 0.007	0.064 ± 0.008	0.063 ± 0.008
Females							
Heart	1.06 ± 0.09	1.02 ± 0.10	1.00 ± 0.10[c]	1.05 ± 0.12	1.03 ± 0.09	1.07 ± 0.09	1.09 ± 0.12
Kidneys	2.18 ± 0.21	2.02 ± 0.24	1.90 ± 0.19[a]	1.93 ± 0.18[a]	2.04 ± 0.21	1.99 ± 0.19[a]	1.95 ± 0.19[a]
Lungs	153 ± 0.13	1.50 ± 0.13	1.52 ± 0.17[c]	1.52 ± 0.15	1.55 ± 0.14	1.50 ± 0.17	1.60 ± 0.19
Adrenals	0.080 ± 0.010	0.081 ± 0.011	0.078 ± 0.008	0.082 ± 0.012	0.078 ± 0.008	0.080 ± 0.010	0.081 ± 0.013

[a] $p < .05$, Dunnett's t-test of significance, compared to sham control.
[b] $p < .05$, Dunnett's t-test of significance, compared to 0.06 reference group.
[c] $p < .05$, Dunnett's t-test of significance, compared to 0.2 reference group.

significant difference only in the 0.06-mg/L groups from study 1, in which females exposed to test cigarette smoke had a higher incidence/severity than females exposed to reference cigarette smoke. Chronic inflammation was present in the submucosa of the epiglottis in some rats exposed to reference or test cigarette smoke in study 1, most frequently in rats exposed to the 0.8 mg/L smoke concentration. Squamous metaplasia, hyperplasia, and hyperkeratosis were also present in the epithelium lining the opening of the ventral pouch and the adjacent laryngeal lumen in most rats exposed to smoke from the test or reference cigarette in both studies. In control rats, the epithelium lining the opening of the ventral pouch and adjacent laryngeal lumen was slightly flattened, oval, rounded, or cuboidal cells one or two cells thick with no discernible basal cell layer (Renne et al., 1992). In affected smoke-exposed rats, this area was covered by a stratified squamous epithelium from three to six cells thick with a variably keratinized surface layer and a distinct basal cell layer. Comparison of incidence/severity of lesions at this site between test and reference cigarette smoke-exposed groups did not indicate any statistically significant differences in either study. Minimal or mild squamous metaplasia of the mucosal epithelium lining the caudal larynx was observed in 2/20 rats exposed to the 0.8 mg/L concentration of smoke from the test cigarette and 1/20 rats exposed to the 0.8 mg/L concentration of smoke from the reference cigarette in study 1.

Exposure to smoke from reference or test cigarettes induced a dose-related increase in minimal hyperplasia of the mucosal epithelium lining the tracheal lumen in both sexes of rats in study 1 and in males in study 2. Comparison of incidence in groups exposed to similar concentrations of smoke from test and reference cigarettes did not indicate any statistical differences in either study.

There were increased numbers of macrophages diffusely scattered through the pulmonary alveoli of rats exposed to smoke from reference or test cigarettes in both studies, compared to concurrent controls. There was some evidence of a dose response in the incidence and severity of macrophage accumulation in alveoli of smoke-exposed rats. This increase was graded as minimal in the vast majority of affected rats. Comparison of incidence and severity data for macrophages in alveoli of rats exposed to smoke from the test and reference cigarettes did not indicate any statistically significant differences. Minimal goblet-cell hyperplasia was observed in AB/PAS-stained sections of the mainstem bronchi of some rats exposed to smoke from reference or test cigarettes in both studies. There was some evidence of a dose response in the incidence of this lesion. Analysis of data indicated a statistically significant increase compared to controls in rats of both sexes exposed to the 0.8 mg/L concentration of smoke from reference cigarettes and in female rats exposed to the 0.8-mg/L concentration of smoke from the test cigarette in study 1, and in both sexes exposed to 0.8 mg/L of reference cigarette smoke in study 2. The incidence (7/20) of goblet-cell hyperplasia in males exposed to the 0.8-mg/L concentration of smoke from the test cigarette in both studies, although not statistically significant, was considered to be toxicologically significant. The incidence of bronchial goblet-cell hyperplasia was slightly higher in male rats exposed to smoke from reference cigarettes compared to similar concentrations of smoke from test cigarettes, but comparison of incidence in groups exposed to similar concentrations of smoke from test and reference cigarettes did not indicate any statistical differences. There was a very low incidence of a variety of microscopic lesions in other tissues examined in both studies, with no evidence of an effect of exposure to smoke from the reference or test cigarette on these tissues.

TABLE 9
Study 1, summary of microscopic observations with average severity in rats

Organ/diagnosis	Incidence of lesions (mean severity, if applicable) by target exposure concentration (mg WTPM/L)						
		Test			Reference		
	Sham controls	0.06	0.2	0.8	0.06	0.2	0.8
				Males			
Nose/turbinates	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Respiratory epithelium, hyperplasia	0[b] (0.0)	2 (0.2)	4 (0.3)	20 (2.2)	1 (0.1)	8 (0.4)	20 (2.1)
Goblet-cell hyperplasia	2 (0.1)	6 (0.3)	3 (0.2)	9 (0.5)	5 (0.3)	5 (0.3)	10 (0.5)
Suppurative inflammation	2 (0.2)	2 (0.3)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)
Larynx	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epiglottis, squamous metaplasia	0 (0.0)	20 (2.2)	20 (2.9)	20 (3.0)	20 (2.1)	20 (2.9)	20 (3.1)
Epiglottis, epithelial hyperplasia	0 (0.0)	20 (2.2)	20 (2.9)	20 (3.0)	20 (2.1)	20 (2.9)	20 (3.0)
Epiglottis, hyperkeratosis	0 (0.0)	9 (0.5)	20 (1.4)	19 (1.9)	16 (0.9)	20 (1.8)	20 (1.9)
Ventral pouch, squamous metaplasia	0 (0.0)	12 (0.7)	20 (2.4)	20 (2.8)	7 (0.5)	19 (2.7)	20 (2.9)
Ventral pouch, epithelial hyperplasia	0 (0.0)	12 (0.7)	20 (2.4)	20 (2.8)	7 (0.5)	19 (2.7)	20 (2.9)
Ventral pouch, hyperkeratosis	0 (0.0)	0 (0.0)	9 (0.6)	19 (1.4)	1 (0.2)	17 (1.4)	18 (1.5)
Chronic inflammation	0 (0.0)	2 (0.1)	8 (0.4)	16 (0.9)	0 (0.0)	4 (0.2)	13 (0.7)
Caudal larynx, squamous metaplasia	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Trachea	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epithelial hyperplasia	1 (0.1)	6 (0.3)	6 (0.3)	18 (0.9)	5 (0.3)	12 (0.6)	16 (0.8)
Lung	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Alveoli, macrophages	3 (0.2)	15 (0.8)	14 (0.7)	20 (1.4)	8 (0.4)	11 (0.6)	20 (1.1)
Bronchi, goblet-cell hyperplasia	0 (0.0)	1 (0.1)	1 (0.1)	7 (0.4)	3 (0.2)	4 (0.2)	11 (0.6)
Alveoli, hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)
				Females			
Nose/turbinates	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Respiratory epithelium, hyperplasia	0[b] (0.0)	0 (0.0)	7 (0.4)	20 (2.0)	0 (0.0)	3 (0.2)	20 (2.1)
Goblet-cell hyperplasia	2 (0.1)	2 (0.1)	2 (0.1)	7 (0.4)	2 (0.1)	2 (0.1)	4 (0.2)
Suppurative inflammation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Larynx	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epiglottis, squamous metaplasia	0 (0.0)	20 (2.2)	20 (3.0)	20 (3.1)	20 (2.2)	20 (2.6)	20 (3.1)
Epiglottis, epithelial hyperplasia	0 (0.0)	20 (2.2)	20 (3.0)	20 (3.1)	20 (2.2)	20 (2.6)	20 (3.0)
Epiglottis, hyperkeratosis	0 (0.0)	19 (1.4)[c]	20 (2.2)	20 (2.2)	13 (0.7)	20 (2.0)	20 (2.1)
Ventral pouch, squamous metaplasia	0 (0.0)	10 (0.6)	20 (2.7)	20 (3.0)	12 (0.8)	20 (2.7)	20 (2.9)
Ventral pouch, epithelial hyperplasia	0 (0.0)	10 (0.6)	20 (2.7)	20 (3.0)	12 (0.8)	20 (2.7)	20 (2.9)
Ventral pouch, hyperkeratosis	0 (0.0)	0 (0.0)	15 (1.3)	20 (1.8)	1 (0.1)	18 (1.5)	18 (1.5)
Chronic inflammation	0 (0.0)	3 (0.2)	2 (0.2)	10 (0.6)	0 (0.0)	4 (0.2)	17 (1.0)
Caudal larynx, squamous metaplasia	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)
Trachea	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epithelial hyperplasia	1 (0.1)	2 (0.1)	8 (0.4)	12 (0.6)	3 (0.2)	7 (0.4)	18 (0.9)
Lung	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Alveoli, macrophages	3 (0.2)	10 (0.5)	13 (0.7)	20 (1.2)	12 (0.6)	17 (0.9)	20 (1.3)
Bronchi, goblet-cell hyperplasia	0 (0.0)	2 (0.1)	3 (0.2)	10 (0.5)	1 (0.1)	4 (0.2)	13 (0.7)
Alveoli, hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Note. Severity: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.
[a]Number of tissues or animals examined.
[b]Number of diagnoses made.
[c]$p < .05$, Kolmogorov–Smirnov test, compared to 0.06-mg/L reference group.

TABLE 10
Study 2, summary of microscopic observations with average severity in rats

Organ/diagnosis	Incidence of lesions (mean severity, if applicable) by target exposure concentration (mg WTPM/L)						
		Test			Reference		
	Sham controls	0.06	0.2	0.8	0.06	0.2	0.8
				Males			
Nose/turbinates	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Respiratory epithelium, hyperplasia	0[b] (0.0)	0 (0.0)	2 (0.1)	20 (2.0)	0 (0.0)	4 (0.2)	20 (1.9)
Goblet-cell hyperplasia	2 (0.1)	3 (0.2)	3 (0.2)	3 (0.2)	3 (0.2)	4 (0.2)	3 (0.2)
Suppurative inflammation	0 (0.0)	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)
Larynx	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epiglottis, squamous metaplasia	0 (0.0)	20 (1.8)	20 (2.4)	20 (3.0)	20 (1.9)	20 (2.5)	20 (3.0)
Epiglottis, epithelial hyperplasia	0 (0.0)	20 (1.8)	20 (2.4)	20 (3.0)	20 (1.9)	20 (2.5)	20 (3.0)
Epiglottis, hyperkeratosis	0 (0.0)	6 (0.4)	15 (1.2)	20 (2.0)	13 (1.0)	20 (1.8)	20 (2.1)
Ventral pouch, squamous metaplasia	0 (0.0)	1 (0.1)	18 (1.4)	20 (1.8)	1 (0.1)	16 (1.2)	20 (1.8)
Ventral pouch, epithelial hyperplasia	0 (0.0)	1 (0.1)	18 (1.4)	20 (1.8)	1 (0.1)	16 (1.2)	20 (1.8)
Ventral pouch, hyperkeratosis	0 (0.0)	0 (0.0)	6 (0.4)	16 (1.2)	0 (0.0)	5 (0.4)	16 (1.0)
Trachea	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epithelial hyperplasia	2 (0.1)	8 (0.4)	9 (0.5)	11 (0.6)	6 (0.3)	8 (0.4)	10 (0.5)
Lung	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Alveoli, macrophages	4 (0.2)	11 (0.6)	16 (0.9)	20 (1.4)	11 (0.6)	14 (0.7)	20 (1.4)
Alveoli, hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Chronic inflammation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Bronchi, goblet-cell hyperplasia	0 (0.0)	1 (0.1)	1 (0.1)	4 (0.2)	0 (0.0)	1 (0.1)	9 (0.5)
				Females			
Nose/turbinates	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Respiratory epithelium, hyperplasia	0[b] (0.0)	0 (0.0)	4 (0.2)	20 (1.5)	0 (0.0)	4 (0.2)	20 (1.6)
Goblet-cell hyperplasia	3 (0.2)	3 (0.2)	5 (0.3)	5 (0.3)	5 (0.3)	2 (0.1)	8 (0.4)
Suppurative inflammation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)
Larynx	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epiglottis, squamous metaplasia	0 (0.0)	20 (1.9)	20 (2.8)	20 (2.8)	20 (1.8)	20 (2.6)	20 (2.6)
Epiglottis, epithelial hyperplasia	0 (0.0)	20 (1.9)	20 (2.8)	20 (2.8)	20 (1.8)	20 (2.6)	20 (2.6)
Epiglottis, hyperkeratosis	0 (0.0)	16 (1.0)	20 (2.0)	20 (2.2)	15 (0.9)	20 (1.6)	20 (2.4)
Ventral pouch, squamous metaplasia	0 (0.0)	1 (0.1)	15 (1.2)	19 (1.9)	2 (0.1)	16 (1.1)	20 (2.0)
Ventral pouch, epithelial hyperplasia	0 (0.0)	1 (0.1)	14 (1.1)	19 (1.9)	2 (0.1)	16 (1.1)	20 (2.0)
Ventral pouch, hyperkeratosis	0 (0.0)	0 (0.0)	6 (0.5)	18 (1.4)	0 (0.0)	9 (0.6)	20 (1.7)
Trachea	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Epithelial hyperplasia	1 (0.1)	0 (0.0)	1 (0.1)	2 (0.1)	2 (0.1)	1 (0.1)	2 (0.1)
Lung	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]	20[a]
Alveoli, macrophages	3 (0.2)	9 (0.5)	10 (0.5)	19 (1.1)	10 (0.5)	10 (0.5)	17 (1.0)
Perivascular lymphoid infiltrate	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Alveoli, hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Chronic inflammation	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Bronchi, goblet-cell hyperplasia	0 (0.0)	1 (0.1)	0 (0.0)	7 (0.4)	3 (0.2)	4 (0.2)	10 (0.5)

Note. Severity: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.
[a]Number of tissues or animals examined.
[b]Number of diagnoses made.

Examination of tissue sections from rats necropsied at the end of the recovery period demonstrated nearly complete regression of nasal and tracheal lesions and a substantial decrease in the incidence and severity of smoke-induced lesions in the larynx and lungs in rats exposed to smoke from test or reference cigarettes in both studies. Macrophages observed in alveoli of smoke-exposed and control recovery group rats were in small focal aggregates, as opposed to the diffuse distribution of macrophages in lungs of rats necropsied at the interim sacrifice. There was no statistically significant difference in the incidence or severity of respiratory-tract lesions between recovery group rats previously exposed to similar concentrations of test and reference cigarette smoke in either study.

Evaluation of Cell Proliferation Rates

There was a dose-related trend toward higher mean nuclear labeling rates in the epithelium lining the median nasal septum in groups exposed to progressively higher concentrations of test or reference cigarette smoke compared to sham controls, but the increases were statistically significant only in females exposed to 0.8 mg/L of test cigarette smoke in study 1 and males exposed to 0.8 mg/L of reference cigarette smoke in study 2. Mean nuclear labeling rates of nasal epithelium lining the distal portions of the nasal and maxillary turbinates were statistically increased compared to control rates in both sexes of rats exposed to 0.8 mg/L of smoke from the test or reference cigarettes in both studies. Mean labeling rates in nasal and maxillary turbinates of study 1 males exposed to 0.8 mg/L of test cigarette smoke were statistically increased compared to labeling rates at these sites in males exposed to the same concentration of reference cigarette smoke.

Mean nuclear labeling rates in laryngeal epithelium were increased compared to sham control groups at all dose levels in both studies. Labeling rates in laryngeal epithelium were statistically different between several test and reference cigarette smoke-exposed groups in both studies, with no clear trend. The histopathology findings of laryngeal epithelial hyperplasia in smoke-exposed rats confirmed the relative sensitivity of these laryngeal sites to smoke-induced hyperplastic changes.

Mean nuclear labeling rates in the tracheal epithelium of rats exposed to smoke from test or reference cigarettes were not clearly different from those of sham controls of the same sex in either study. Labeling rates of bronchial, bronchiolar, and alveolar epithelium in both studies were difficult to evaluate due to wide standard deviations, low labeling rates, and variable sample sizes, and therefore labeling data from these sites were not used in evaluating effects of smoke exposure.

DISCUSSION

The studies described here were designed to evaluate the potential influence of ingredients on the chemical composition and the biological activity of mainstream cigarette smoke. Test cigarettes containing flavorings or casings were analyzed and compared against reference cigarettes identical except produced without flavors or casings. The configuration and ISO-condition tar, nicotine, and CO yields of all cigarettes investigated are representative of American blend cigarettes. Both test and reference cigarettes had the same tobacco blend and humectant composition (glycerine plus water) and were prepared by the same manufacturing process. Similarly, identical nontobacco materials (NTM) were used throughout. The weight of the filler remained constant between test and reference cigarettes. These studies illustrate that the application of 165 low-use flavoring or 8 high-use flavoring or casing ingredients had little, if any, observable effect on the deliveries or physical parameters of the cigarettes.

From comparison of the mutagenicity data obtained in Ames assays of studies 1 and 2 test and reference cigarettes, it was concluded that the addition of these ingredients did not increase the mutagenic response of any of the strains of *Salmonella typhimurium* under the conditions described, and the results did not suggest any mutagenic activity of the added ingredients.

The objectives of the two inhalation toxicity studies were to compare the biologic activity of mainstream smoke from the two test cigarettes with reference cigarettes in a series of two 13-wk inhalation exposures, each followed by a 13-wk recovery period. Data collected during the 13-wk exposures confirmed that both the particulate (WTPM, nicotine) and vapor (CO) phases of the inhalation atmospheres presented to the rats were well controlled and provided appropriate data for comparison of the responses of the study animals to smoke from the two cigarettes under investigation in each of the two studies. WTPM was used as the basis for exposure concentration in these studies, since the predominant known toxicologic effects of cigarette smoke are associated with the mainstream particulate phase (Coggins et al., 1980).

Blood COHb concentrations demonstrated that exposure of rats to smoke from either the test or reference cigarette resulted in reproducible biomarkers of exposure consistent with the concentration of CO in the smoke. Samples taken for plasma nicotine analysis confirmed exposure to nicotine in test or reference smoke, which resulted in exposure-related increases in plasma nicotine concentrations.

The only occurrence during either study that affected the utility of the data was the failure to fast the sham control rats prior to necropsy at the interim sacrifice immediately following the exposure period in study 2. This error did not allow direct comparison of the body and organ weights of controls with smoke-exposed groups sacrificed at that time point.

Other investigations have noted effects similar to those we observed of cigarette smoke exposure on body weight, including the relative resistance of females to this change (Coggins et al., 1989; Baker et al., 2004). We concluded that the decreased body weights in smoke-exposed groups in both studies compared to sham controls were the result of smoke exposure. However, we do not consider these effects on body weight to be toxicologically significant due to their recovery after smoke exposure was terminated, and due to the lack of any concurrent clinical observations that would indicate any significant dysfunction.

In study 1 there were a number of statistically significant differences in absolute or relative organ weights between test or reference cigarette smoke-exposed groups and sham controls necropsied immediately following 13 wk of smoke exposure. However, these statistical differences showed no clear dose-response pattern, and no exposure-related histopathologic effects were observed in any weighed organ except the lungs. It is possible that the increased lung/body weight ratios in study 1 rats exposed to 0.8-mg/L of smoke from test or reference cigarettes were related to the minimal increase in numbers of macrophages in alveoli of these rats. These increases in lung/body weight ratio more likely reflect the decreased body weight in these groups at the interim sacrifice. In any case, these and the other statistical differences in absolute or relative organ weights in smoke-exposed rats compared to sham controls are not considered toxicologically significant. There was no consistent difference in organ weights between groups of rats exposed to similar concentrations of test and reference cigarette smoke in either study. Increases in total inhaled mass were proportional to increasing exposure concentration in study 1, but in study 2 decreases in MV in groups exposed to 0.8- or 0.2-mg/L relative to groups exposed to 0.06 mg/L caused total inhaled mass for the high and middle dose groups to be lower in proportion to exposure concentration of smoke.

Inhalation exposure to smoke from test or reference cigarettes in both studies clearly induced microscopic changes in the nasal cavity, larynx, trachea, and lungs of exposed rats. Results of histopathologic examination of the recovery groups illustrated that these respiratory-tract lesions were either completely resolved or in the process of resolving by 13 wk after cessation of smoke exposure, and thus represent an adaptive response to the inhaled smoke. The nasal cavity and larynx were much more affected by inhaled smoke than the lungs in our studies, and the mucosal epithelium lining the base of the epiglottis and adjacent ventral pouch was the most affected site. The extreme susceptibility of the rodent laryngeal mucosa to inhaled smoke and other xenobiotics has been described in detail (Lewis, 1980, 1991; Gopinath et al., 1987; Burger et al., 1989). Since the most notable cellular changes observed in the respiratory tract of rodents in response to inhaled smoke involve cellular proliferation and metaplasia, a quantitative measure of cell turnover in affected tissue is a useful tool to measure the effect of exposure. Cell proliferation rate measurements in nasal turbinates and laryngeal epithelium using nuclear labeling with BrdU correlated well with histopathology data, reinforcing the conclusion that exposure to smoke from test or reference cigarette smoke for 13 wk clearly induced epithelial hyperplasia at these sites. Results of BrdU labeling in the trachea and lungs were less clear, and probably reflect the more subtle effects of inhaled smoke on the epithelium at these sites.

The effects of inhaled cigarette smoke on the respiratory tract of rats in both the studies described herein are similar to those described in a number of previously reported cigarette smoke inhalation studies in rats (Dalbey et al., 1980; Gaworski et al., 1997; Coggins et al., 1989; Ayres et al., 2001; Vanscheeuwijck et al., 2002) and hamsters (Lewis, 1980; Wehner et al., 1990). Four recently published papers have described studies similar to those presented here, in which smokes from cigarettes with and without flavoring or casing ingredients were compared on the basis of chemical composition and biologic effects on rodents (Gaworski et al., 1998; Paschke et al., 2002; Carmines, 2002; Baker et al., 2004). Results of the studies presented here are consistent with the conclusions of these authors that the presence of flavoring and casing ingredients studied to date did not significantly change the type or extent of toxicologic effects observed in rodents inhaling cigarette smoke.

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SAFETY DATA SHEET
Lime Oil Distilled Organic

1. Identification

Product identifier

Product name Lime Oil Distilled Organic

Product number 12-4211-OB

Synonyms; trade names Citrus aurantifolia

CAS number 8008-26-2

Recommended use of the chemical and restrictions on use

Application Fragrance Household Products Flavors

Details of the supplier of the safety data sheet

Supplier

Global Essence Inc.
Sean Grimes
8 Marlen Drive
Hamilton, New Jersey 08691
USA
1-732-677-1100
1-732-677-1107
regulatory@globalessence.com

Emergency telephone number

Emergency telephone INFOTRAC 1-800-535-5053

2. Hazard(s) identification

Classification of the substance or mixture

OSHA Regulatory Status This Product is Hazardous under the OSHA Hazard Communication Standard.

Physical hazards Flam. Liq. 3 - H226

Health hazards Skin Irrit. 2 - H315 Skin Sens. 1 - H317 Asp. Tox. 1 - H304

Environmental hazards Aquatic Chronic 1 - H410

Label elements

Pictogram

Signal word Danger

Hazard statements H226 Flammable liquid and vapor.
H304 May be fatal if swallowed and enters airways.
H315 Causes skin irritation.
H317 May cause an allergic skin reaction.
H410 Very toxic to aquatic life with long lasting effects.

Lime Oil Distilled Organic

Precautionary statements P210 Keep away from heat, sparks, open flames and hot surfaces. No smoking.
P273 Avoid release to the environment.
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
P403+P235 Store in a well-ventilated place. Keep cool.
P501 Dispose of contents/ container in accordance with national regulations.

Contains Lime Oil

3. Composition/information on ingredients

Mixtures

Lime Oil **100.0%**

CAS number: 8008-26-2

M factor (Chronic) = 1

Classification
Flam. Liq. 3 - H226
Skin Irrit. 2 - H315
Skin Sens. 1 - H317
Asp. Tox. 1 - H304
Aquatic Chronic 1 - H410

The full text for all hazard statements is displayed in Section 16.

Ingredient notes 100% Organic USDA-NOP

4. First-aid measures

Description of first aid measures

Inhalation Remove affected person from source of contamination. Move affected person to fresh air at once.

Ingestion If swallowed, do NOT induce vomiting. Give victim a glass or water or milk. Call a physician or poison control center immediately. Never give anything by mouth to an unconscious person.

Skin Contact Remove contaminated clothing immediately and wash skin with soap and water.

Eye contact Rinse immediately with plenty of water. Remove any contact lenses and open eyelids wide apart. Continue to rinse for at least 15 minutes.

Most important symptoms and effects, both acute and delayed

Inhalation May cause an asthma-like shortness of breath.

Ingestion May cause discomfort if swallowed.

Skin contact Prolonged skin contact may cause redness and irritation.

Eye contact May cause temporary eye irritation.

5. Fire-fighting measures

Extinguishing media

Suitable extinguishing media Extinguish with foam, carbon dioxide or dry powder.

Unsuitable extinguishing media Do not use water jet as an extinguisher, as this will spread the fire.

Special hazards arising from the substance or mixture

Lime Oil Distilled Organic

Hazardous combustion products Does not decompose when used and stored as recommended.

Advice for firefighters

Protective actions during firefighting Fight fire from safe distance or protected location.

Special protective equipment for firefighters Use protective equipment appropriate for surrounding materials.

6. Accidental release measures

Personal precautions, protective equipment and emergency procedures

Personal precautions Wear protective clothing as described in Section 8 of this safety data sheet.

Environmental precautions

Environmental precautions Do not discharge into drains or watercourses or onto the ground.

Methods and material for containment and cleaning up

Methods for cleaning up Contain spilled liquid with sand or earth. DO NOT use combustible materials such as sawdust. Retain all contaminated water for removal and treatment.

Reference to other sections Wear protective clothing as described in Section 8 of this safety data sheet.

7. Handling and storage

Precautions for safe handling

Usage precautions Avoid spilling. Avoid contact with skin and eyes. Keep away from heat, sparks and open flame.

Conditions for safe storage, including any incompatibilities

Storage precautions Keep container tightly closed. Keep away from heat, sparks and open flame.

8. Exposure Controls/personal protection

Exposure controls

Protective equipment

Eye/face protection Wear tight-fitting, chemical splash goggles or face shield.

Hand protection Chemical-resistant, impervious gloves complying with an approved standard should be worn if a risk assessment indicates skin contact is possible.

Other skin and body protection Wear rubber apron.

Hygiene measures Wash contaminated clothing before reuse.

Respiratory protection If ventilation is inadequate, suitable respiratory protection must be worn.

9. Physical and Chemical Properties

Information on basic physical and chemical properties

Appearance Liquid.

Lime Oil Distilled Organic

Color	Colorless to Yellow
Odor	Characteristic.
Flash point	46°C CC (Closed cup).
Relative density	0.853 to 0.921 @ 20°C
Refractive index	1.469 to 1.489 @ 20*C

10. Stability and reactivity

Reactivity	There are no known reactivity hazards associated with this product.
Stability	Stable at normal ambient temperatures and when used as recommended.
Possibility of hazardous reactions	None if used according to storage & handling conditions & identified uses None under normal conditions and storage reccomendations
Conditions to avoid	Avoid heat. Avoid contact with the following materials: Strong oxidizing agents.
Materials to avoid	Strong oxidizing agents.
Hazardous decomposition products	Does not decompose when used and stored as recommended.

11. Toxicological information

Information on toxicological effects

Acute toxicity - oral

Acute toxicity oral (LD_{50} mg/kg)	5,000.0
Species	Rat
Notes (oral LD_{50})	>93*C / >200*F

Acute toxicity - dermal

Acute toxicity dermal (LD_{50} mg/kg)	5,000.0
Species	Rabbit
Notes (dermal LD_{50})	>93*C / >200*F

Acute toxicity - inhalation

Notes (inhalation LC_{50})	Not available.

Inhalation	Vapor may irritate respiratory system/lungs.
Ingestion	May cause discomfort if swallowed.
Skin Contact	Prolonged and frequent contact may cause redness and irritation.
Eye contact	May cause temporary eye irritation.

12. Ecological Information

Ecotoxicity	The product contains substances which are toxic to aquatic organisms and which may cause long-term adverse effects in the aquatic environment.

Lime Oil Distilled Organic

13. Disposal considerations

Waste treatment methods

General information Waste is classified as hazardous waste. Dispose of waste to licensed waste disposal site in accordance with the requirements of the local Waste Disposal Authority.

Disposal methods Dispose of waste to licensed waste disposal site in accordance with the requirements of the local Waste Disposal Authority.

14. Transport information

Road transport notes Avoid releasing into the environment.

Rail transport notes Avoid releasing into the environment.

Sea transport notes Do not release into the environment.

Air transport notes Avoid releasing into the environment.

UN Number

UN No. (IMDG) 1169 EXTRACTS, AROMATIC, LIQUID (limonene)

UN No. (ICAO) 1169 Extracts, aromatic, liquid

UN No. (DOT) Not regulated.

UN proper shipping name

Proper shipping name (IMDG) EXTRACTS, AROMATIC, LIQUID (limonene)

Proper shipping name (ICAO) Extracts, aromatic, liquid

Proper shipping name (DOT) Not regulated.

Transport hazard class(es)

IMDG Class 3

ICAO class/division 3

Transport labels

Packing group

IMDG packing group III

ICAO packing group III

Environmental hazards

Environmentally Hazardous Substance

Special precautions for user

IMDG Code segregation group Category A

EmS F-E, S-D

Lime Oil Distilled Organic

15. Regulatory information

Regulatory Status	This material is Hazardous under the OSHA Hazard Communication Standard
Regulatory References	29 CFR 1910.1010 Federal Regulations (OSHA STANDARD)

16. Other information

General information	FEMA# 2631 Tariff Code: 3301192000 FDA# 182.20 COE# 141
Revision comments	Revised by KB
Issued by	DH
Revision date	7/27/2020
Revision	4
Supersedes date	2/23/2018
Hazard statements in full	H226 Flammable liquid and vapor. H304 May be fatal if swallowed and enters airways. H315 Causes skin irritation. H317 May cause an allergic skin reaction. H410 Very toxic to aquatic life with long lasting effects.
NFPA - instability hazard	Normally stable. (0)
NFPA - health hazard	Irritation, minor residual injury. (1)
NFPA - flammability hazard	Burns only if heated moderately. (2)

SAFETY DATA SHEET.

Version 2

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND OF THE COMPANY/UNDERTAKING

Product identifier

Product Code(s) 150465
Product name OIL LIME EXPRESSED TERPENELESS EXTRA

EC-No 290-010-3
CAS-No 68916-84-7
FEMA Numbers 2632

Recommended use of the chemical and restrictions on use

Recommended Use No information available.
Uses advised against No information available

Manufacturer

C & A Service Inc. as agents for
Citrus and Allied Essences, Ltd
4620 Mercedes Drive
Belcamp, MD 21017
(410) 273-9500

Emergency telephone

Chemtrec: 1-800-424-9300 for US/ 703-527-3887 outside US

2. HAZARDS IDENTIFICATION

Classification

Skin corrosion/irritation	Category 2
Serious eye damage/eye irritation	Category 2
Skin Sensitization	Category 1
Aspiration toxicity	Category 1
Flammable liquids	Category 4

GHS Label elements, including precautionary statements

Emergency Overview

Danger

Hazard Statements
Causes skin irritation
Causes serious eye irritation
May cause an allergic skin reaction
May be fatal if swallowed and enters airways
Combustible liquid

Appearance clear **Physical state** liquid **Odor** Strong folded cold pressed lime

Precautionary Statements - Response
Specific treatment (see .? on this label)
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
If eye irritation persists: Get medical advice/attention
IF ON SKIN: Wash with plenty of soap and water
Take off contaminated clothing and wash before reuse
If skin irritation or rash occurs: Get medical advice/attention
IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician
Do NOT induce vomiting
In case of fire: Use CO2, dry chemical, or foam for extinction

Precautionary Statements - Storage
Store locked up
Store in a well-ventilated place. Keep cool

Precautionary Statements - Disposal
Dispose of contents/container to an approved waste disposal plant

Hazards not otherwise classified (HNOC)
Other information
• May be harmful if swallowed
• May be harmful in contact with skin
• Toxic to aquatic life

3. COMPOSITION/INFORMATION ON INGREDIENTS

Chemical Name	CAS-No	Weight %	Trade Secret
GERANIAL	141-27-5	15-20%	*
BETA BISABOLENE	495-61-4	15-20%	*
NERAL	106-26-3	10-15%	*
CARYOPHELLENE BETA FCC (NATURAL)	87-44-5	5-10%	*

4. FIRST AID MEASURES

First aid measures for different exposure routes

General advice If symptoms persist, call a physician.

Eye contact If symptoms persist, call a physician. Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Immediately flush with plenty of water. After initial flushing, remove any contact lenses and continue flushing for at least 15 minutes. Keep eye wide open while rinsing.

Skin contact If skin irritation persists, call a physician. Wash off immediately with plenty of water for at least 15 minutes. Wash off immediately with soap and plenty of water. Immediate medical attention is not required. Wash off immediately with soap and plenty of water while removing all contaminated clothes and shoes.

Inhalation If symptoms persist, call a physician. Move to fresh air. Immediate medical attention is not required. Move to fresh air in case of accidental inhalation of vapors or decomposition products.

Ingestion Do NOT induce vomiting. Drink plenty of water. Immediate medical attention is not required. Rinse mouth. Clean mouth with water and drink afterwards plenty of water. Never give anything by mouth to an unconscious person. Call a physician.

Self-protection of the first aider Use personal protective equipment.

Most important symptoms/effects, acute and delayed

Main Symptoms Hives.

Indication of immediate medical attention and special treatment needed, if necessary

Note to physicians Treat symptomatically. May cause sensitization in susceptible persons.

5. FIRE-FIGHTING MEASURES

Suitable Extinguishing Media
Use. Dry chemical. Carbon dioxide CO_2. Water spray. Alcohol-resistant foam.

Unsuitable extinguishing media
Decomposition by contact with water may generate vapors which can be ignited by heat or open flame.

Specific hazards arising from the chemical
Keep product and empty container away from heat and sources of ignition. Risk of ignition. Thermal decomposition can lead to release of irritating gases and vapors. In the event of fire and/or explosion do not breathe fumes. May cause sensitization in susceptible persons.

Explosion data
Sensitivity to Mechanical Impact none.
Sensitivity to Static Discharge Yes.

Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions, protective equipment and emergency procedures

Personal precautions Use personal protective equipment. Avoid contact with eyes and skin. Evacuate personnel to safe areas. Keep people away from and upwind of spill/leak. Remove all sources of ignition. Pay attention to flashback. Take precautionary measures against static discharges.

Environmental precautions

Environmental precautions Prevent entry into waterways, sewers, basements or confined areas. Do not flush into surface water or sanitary sewer system. Prevent further leakage or spillage if safe to do so. Prevent product from entering drains. Beware of vapors accumulating to form explosive concentrations. Vapors can accumulate in low areas.

Methods and materials for containment and cleaning up

Methods for containment Prevent further leakage or spillage if safe to do so.

Methods for cleaning up Soak up with inert absorbent material. Pick up and transfer to properly labeled containers. After cleaning, flush away traces with water. Prevent product from entering drains. Dam up. Take precautionary measures against static discharges.

7. HANDLING AND STORAGE

Precautions for safe handling

Advice on safe handling Use personal protective equipment as required. Ensure adequate ventilation. Use only in area provided with appropriate exhaust ventilation. To avoid ignition of vapors by static electricity discharge, all metal parts of the equipment must be grounded. Keep away from heat, sparks and open flame. No smoking. Do not breathe dust/fume/gas/mist/vapors/spray. Take necessary action to avoid static electricity discharge (which might cause ignition of organic vapors).

Conditions for safe storage, including any incompatibilities

Storage Conditions Keep out of the reach of children. Keep container tightly closed. Keep containers tightly closed in a cool, well-ventilated place. Keep away from heat. Keep in properly labeled containers.

Incompatible products None known based on information supplied.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Control parameters

Exposure Guidelines This product, as supplied, does not contain any hazardous materials with occupational exposure limits established by the region specific regulatory bodies.

Chemical Name	ACGIH TLV	OSHA PEL	NIOSH IDLH
NERAL 106-26-3	TWA: 5 ppm inhalable fraction and vapor S*	-	

Exposure controls

Engineering Controls Showers
Eyewash stations
Ventilation systems.

Individual protection measures, such as personal protective equipment

Eye/face protection Tightly fitting safety goggles.

Skin and body protection Chemical resistant apron.

Respiratory protection If exposure limits are exceeded or irritation is experienced, NIOSH/MSHA approved respiratory protection should be worn. Positive-pressure supplied air respirators may be required for high airborne contaminant concentrations. Respiratory protection must be provided in accordance with current local regulations.

General Hygiene Considerations When using, do not eat, drink or smoke. Wash contaminated clothing before reuse. Provide regular cleaning of equipment, work area and clothing.

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical and chemical properties

Physical state	liquid
Appearance	clear
Odor	Strong folded cold pressed lime
Color	Pale yellow or almost colorless

Property	**Values**	**Method**
pH		No information available
Melting/freezing point		FCC Method
Boiling point/boiling range		FCC Method
Flash Point	61 °C / 141 °F	Closed cup
Evaporation rate	No information available	No information available
Flammability (solid, gas)	No information available	No information available
Flammability Limits in Air		No information available
Upper flammability limit	No information available	
lower flammability limit	No information available	
Vapor pressure mm Hg 20°C		No information available
Vapor density		No information available
Specific Gravity @ 25C	0.880 - 0.902	FCC Method
Specific Gravity @ 20C	0.883 - 0.905	FCC Method
Refractive Index	1.479 - 1.491	FCC Method
Water solubility	Practically insoluble	
Solubility in other solvents		No information available
Partition coefficient: n-octanol/water		No information available
Autoignition temperature	No information available	No information available
Decomposition temperature		No information available
Viscosity, kinematic	No information available	No information available
Viscosity, dynamic	No information available	
Explosive properties	No information available	
Oxidizing Properties	No information available	

Other information

Softening point	No information available
Molecular Weight	No information available
VOC Content(%)	No information available
Density VALUE	No information available
Bulk Density VALUE	No information available

10. STABILITY AND REACTIVITY

Reactivity
No data available

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions
None under normal processing.

Conditions to Avoid
Heat, flames and sparks.

Incompatible Materials
None known based on information supplied.

Hazardous Decomposition Products
None known based on information supplied.

11. TOXICOLOGICAL INFORMATION

Information on likely routes of exposure

Product Information Product does not present an acute toxicity hazard based on known information

Inhalation There is no data available for this product.

Eye contact There is no data available for this product.

Skin contact There is no data available for this product.

Ingestion There is no data available for this product.

Chemical Name	Oral LD50	Dermal LD50	LC50 Inhalation
GERANIAL 141-27-5	500 mg/kg (Rat)	-	-
NERAL 106-26-3	4950 mg/kg (Rat)	2000 mg/kg (Rat) 2250 mg/kg (Rabbit)	-

Information on toxicological effects

Symptoms No information available.

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Sensitization No information available.
Germ Cell Mutagenicity No information available.

Reproductive toxicity No information available.
Specific target organ systemic toxicity (single exposure) No information available.
Specific target organ systemic toxicity (repeated exposure) No information available.
Chronic toxicity Repeated contact may cause allergic reactions in very susceptible persons. Avoid repeated exposure.
Aspiration hazard No information available.

Numerical measures of toxicity - Product Information

The following values are calculated based on chapter 3.1 of the GHS document
. mg/kg

12. ECOLOGICAL INFORMATION

Ecotoxicity

Toxic to aquatic life

33% of the mixture consists of components(s) of unknown hazards to the aquatic environment

Persistence and degradability
No information available.

Bioaccumulation
No information available.

Chemical Name	log Pow
NERAL 106-26-3	2.76

Other adverse effects

No information available

13. DISPOSAL CONSIDERATIONS

Waste treatment

Waste Disposal Methods It must undergo special treatment, e.g. at suitable disposal site, to comply with local regulations.

Contaminated packaging Do not reuse container.

14. TRANSPORT INFORMATION

DOT/ADR Not regulated

ICAO/IATA Not regulated

IMDG / IMO Not regulated

15. REGULATORY INFORMATION

International Inventories

TSCA	-
DSL/NDSL	-
EINECS/ELINCS	-
ENCS	-
IECSC	-
KECL	-
PICCS	-
AICS	-

Legend:

TSCA - United States Toxic Substances Control Act Section 8(b) Inventory
DSL/NDSL - Canadian Domestic Substances List/Non-Domestic Substances List
EINECS/ELINCS - European Inventory of Existing Chemical Substances/European List of Notified Chemical Substances
ENCS - Japan Existing and New Chemical Substances
IECSC - China Inventory of Existing Chemical Substances
KECL - Korean Existing and Evaluated Chemical Substances
PICCS - Philippines Inventory of Chemicals and Chemical Substances
AICS - Australian Inventory of Chemical Substances

U.S. Federal Regulations

SARA 313

Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 (SARA). This product does not contain any chemicals which are subject to the reporting requirements of the Act and Title 40 of the Code of Federal Regulations, Part 372.

SARA 311/312 Hazard Categories

Acute Health Hazard	Yes
Chronic Health Hazard	no
Fire Hazard	Yes
Sudden Release of Pressure Hazard	no
Reactive Hazard	no

Clean Water Act
This product does not contain any substances regulated as pollutants pursuant to the Clean Water Act (40 CFR 122.21 and 40 CFR 122.42).

CERCLA
This material, as supplied, does not contain any substances regulated as hazardous substances under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302) or the Superfund Amendments and Reauthorization Act (SARA) (40 CFR 355). There may be specific reporting requirements at the local, regional, or state level pertaining to releases of this material.

U.S. State Regulations

California Proposition 65
This product is not, to the best of our knowledge, subject to the labeling requirements under California Proposition 65

U.S. State Right-to-Know Regulations

U.S. EPA Label information

16. OTHER INFORMATION

NFPA	**Health Hazard** 2	**Flammability** 2	**Instability** 0	**Physical and chemical hazards** *
HMIS	**Health Hazard** 2	**Flammability** 2	**Physical Hazard** 0	**Personal protection** b

Revision Date 28-Mar-2017
Revision Note No information available

Revision# 2

WARNING/DISCLAIMER:
This ingredient(s) has not been tested, nor has it been deemed safe, for inhalation or use in electronic smoking devices electronic nicotine delivery systems, electronic cigarettes or other similar devices (collectively "E-Cigarettes"). In supplying this ingredient, Citrus and Allied instructs, and by receiving this ingredient recipient confirms, that this ingredient will not be used in connection with the manufacture and distribution of E-Cigarettes or any component thereof.

Disclaimer
The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text.

end