

**Lemon Oil**  
**[CASRN 8008-56-8]**

**Lime Oil**  
**[CASRN 8008-26-2]**

Prepared October 2000

## SUMMARY

Lemon oil and lime oil are added as fragrances to a number of perfumed cosmetic products. Many of these products are intended for use on sun-exposed skin. Clinical and experimental studies have found that both lemon oil and lime oil are phototoxic. Because of their phototoxicity, the International Fragrance Association has recommended safe use levels for “leave on products” applied to sun-exposed skin. These safe use levels are intended to avoid short-term phototoxicity. However, long-term effects have not been addressed.

The phototoxicity elicited by lemon oil and lime oil has been associated with naturally occurring furocoumarins. Two of these furocoumarins, 5-methoxypsoralen and oxypeucedanin, have been identified as the probable phototoxins in both lemon oil and lime oil. The levels of these furocoumarins have been found to vary significantly with growing conditions.

While the photogenotoxicity of 5-methoxypsoralen is well established, the photogenotoxicity of oxypeucedanin is unknown. Bacterial mutagenesis studies indicated that oxypeucedanin is not mutagenic, however photogenotoxicity studies of oxypeucedanin are not available. Additionally, the mutagenicity and photomutagenicity of mixtures of coumarins and furocoumarins in lemon oil and lime oil have not been assessed.

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## 1.0 BASIS FOR NOMINATION

Lemon oil and lime oil were selected by the Center for Food Safety and Applied Nutrition for photogenotoxicity and photocarcinogenesis studies since these phototoxic oils contain furocoumarins. Furocoumarins such as 5-methoxypsoralen have been shown to be photocarcinogenic and are present in lemon and lime oils.

## 2.0 INTRODUCTION

### 2.1 Chemical Identification

	<b>Lemon Oil</b>	<b>Lime Oil</b>
CASRN:	8008-56-8	8008-26-2
RTECS:	OG8300000	RI7270000

The following synonyms are used for lemon oil:

- *Citrus Medica Limonum* Oil [International Nomenclature of Cosmetic Ingredients (INCI) name, CTFA, 1999]
- Oil of Lemon (Budavari, 1996)

The following synonyms are used for lime oil:

- *Citrus Aurantifolia* Oil (INCI name, CTFA, 1999)
- Oil of Lime (RTECS)

### 2.2 Physical-Chemical Properties of Lemon Oil and Lime Oil (Table 1)

<b>Property</b>	<b>Lemon Oil</b>	<b>Lime Oil</b>
Color <sup>1</sup>	Pale to deep yellow or greenish yellow	Colorless to greenish yellow
Odor <sup>1</sup>	Fresh citrus, intense	Fresh citrus, intense
Specific Gravity <sup>1</sup>	0.849-0.855	0.855-0.863
Refractive Index <sup>1</sup>	1.474-1.467	1.4477-1.4745
Optical Rotation <sup>1</sup>	+57 to +65.6	+34 to +47
Stability <sup>2</sup>	Air/light sensitive	Air/light sensitive
Solubility		
Water <sup>1</sup>	insol.	insol.
Ethanol <sup>1</sup>	sol.	sol.
Propylene glycol <sup>1</sup>	sol.	sol.

<sup>1</sup>Secondini, 1990.

<sup>2</sup>Poucher, 1991.

### 3.0 PRODUCTION PROCESSES

Several processes are used to produce lemon oil and lime oil. Expressed lemon oil and lime oil are produced by pressing the outer rind of the ripe fruits by sponge-press (*i.e.*, by hand) or machines (Poucher, 1991). These oils may be produced more economically using an integrated juice-oil procedure such as the Brown Oil Extractor (Swains and Swains, 1988), where citrus fruit is partially submerged in water and abraded by metal discs. The oil is separated from the juice as a water-emulsion, and further separated using centrifugation to obtain the oil. The yield of expressed oil is approximately 4% for lemons (somewhat less for limes) based on the weight of whole fruit (Burdock, 1995). Lemon and lime oils are also produced by distillation of expressed oils or direct distillation of fruit (Swaine and Swaine, 1988; Burdock, 1995). Lemon oil and lime oil are distilled (rectified) for removal of terpenes in order to improve solubility and permit use for flavoring carbonated beverages (Burdock, 1995). Lemon oil and lime oil can also be steam distilled to remove nonvolatile furocoumarins and are subsequently marketed as “psoralen free” (LOLI Global Industries). The types of lemon oil and lime oil added to cosmetics are not regulated by law, nor limited by recommendations of appropriate trade organizations.

### 4.0 REGULATORY STATUS

Lemon oil and lime oil are approved as GRAS for food use (CFR, 2000). The Food, Drug and Cosmetic Act does not authorize the Food and Drug Administration to require pre-market approval for ingredients added to cosmetics.

### 5.0 OCCURRENCE IN COSMETICS

Data available from FDA’s Voluntary Cosmetics Registration Program (VCRP), compiled in accordance with Title 21 part 270.4 (d)(1) of the Code of Federal Regulations (Code of Federal Regulations, 2000b), indicate that lemon oil is found in 98 cosmetic formulations and lime oil is in 14 formulations.

Both lemon and lime oil are primarily used as fragrances in perfumed cosmetics (Cosmetic, Toiletry, and Fragrance Association, 1999). The International Fragrance Association (IFRA) has made specific recommendations for use of lemon and lime oil in cosmetic formulations (IFRA, 1992). IFRA recommends that “*for applications on areas of skin exposed to sunshine, excluding bath preparations, soaps and other products which are washed off the skin, lemon oil cold pressed should not be used such that the level in the consumer products exceeds 2%.*” Similar recommendations are made for lime oil except that the level in consumer products is recommended not to exceed 0.7% (IFRA, 1992). IFRA makes no recommendation for removal of nonvolatile photosensitizing components by distillation for either lemon or lime oil.

Typical concentrations in final products are given in Table 2.

**Table 2. Typical Concentrations of Citrus Oils in Finished Products**  
(Opdyke, 1974)

Ingredient	Soap	Detergent	Cream/Lotion	Perfume
	Usual/Maximum	Usual/Maximum	Usual/Maximum	Usual/Maximum
Lemon Oil (Expressed)	0.05/0.4 <sup>1</sup>	0.005/0.04	0.03/0.15	0.5/1.0
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

<sup>1</sup> w/w %

## 6.0 TOXICOLOGICAL ISSUES

### 6.1 Phototoxicity of Lemon Oil and Lime Oil.

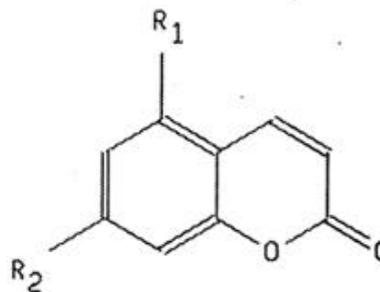
The phototoxicity of lemon oil and lime oil has been demonstrated in both experimental models and humans. Naganuma *et al.*, 1985, studied the phototoxicity of lemon oil obtained from different geographic locations. Lemon oil was diluted in ethanol (100% lemon oil, 50% lemon oil, and 20% lemon oil) and applied to backs of shaved, albino guinea pigs. Animals were then exposed to UVA radiation (320-400 nm, 13 J/cm<sup>2</sup>). Erythema was evaluated at 24, 48 and 72 hr after irradiation. Undiluted lemon oil and 50% lemon oil elicited phototoxicity for most of the samples tested. Lemon oil from Australia and Brazil elicited a weaker phototoxic than lemon oil from the Ivory Coast, Sicily, California, or Argentina. In an effort to identify the phototoxic components in lemon oil, these investigators fractionated samples by solvent extraction and subsequent phototoxicity testing of isolated components. Two phototoxic components were identified: oxypeucedanin and 5-methoxypsoralen (see Figure 1).

Forbes *et al.*, 1977, have investigated the phototoxicity of a large number of fragrance raw materials including lemon oil and lime oil. Fragrance raw materials were tested using humans, pigs and albino, hairless mice. Several irradiation sources were used including sunlight, a solar simulator and a UVA radiation source (black light). Expressed lime oil was phototoxic in all three species under all three radiation sources. Distilled lime oil usually elicited no phototoxic response in any species. Two samples of distilled lime oil (derived from expressed lime oil), however, were found to be phototoxic. Lemon oil from Greece, Italy and from Florida (Persian limes) was reported to be phototoxic.

**Figure 1. Structures of Major Coumarins and Furocoumarins in Lemon Oil and Lime Oil.**

$R_1 = \text{OCH}_3$ ;  $R_2 = \text{OCH}_3$   
5,7-Dimethoxycoumarin (Citroptene, Limettin)

$R_1 = \text{OCH}_2\text{CH}=\text{C}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}=\text{C}(\text{CH}_3)_2$ ;  
 $R_2 = \text{OCH}_3$   
Geranoxy-7-methoxycoumarin



$R_1 = \text{H}$ ;  $R_2 = \text{H}$   
Psoralen

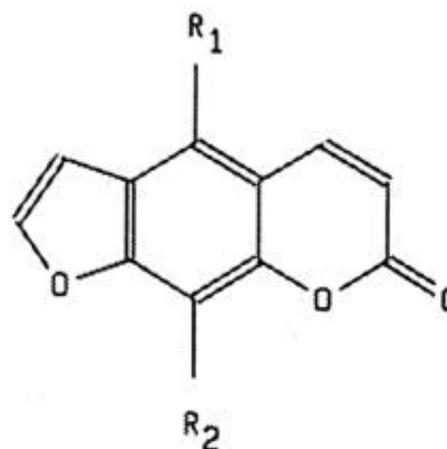
$R_1 = \text{H}$ ;  $R_2 = \text{OCH}_3$   
8-Methoxypsoralen (Xanthotoxin)

$R_1 = \text{OCH}_3$ ;  $R_2 = \text{H}$   
5-Methoxypsoralen (Bergapten)

$R_1 = \text{OCH}_2\text{CH}=\text{C}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}=\text{C}(\text{CH}_3)_2$ ;  
 $R_2 = \text{H}$   
5-Geranoxypsoralen (Bergamottin)

$R_1 = \text{OCH}_3$ ;  $R_2 = \text{OCH}_3$   
5,7-Dimethoxypsoralen (Isopimpinellin)

$R_1 = \text{OCH}_2\text{CH}(\text{O})\text{C}(\text{CH}_3)_2$  (epoxide);  $R_2 = \text{H}$   
5-(Isoamyloxy-2,3-epoxide)psoralen (Oxypeucedanin)



## 6.2 Coumarins and Furocoumarins Occurring in Lemon Oil and Lime Oil

Investigators have examined the level of coumarins and furocoumarins in lemon oil and lime oil (Table 3). A large body of evidence indicates that these secondary metabolites are photo-activated phytoalexins, produced to protect plants from bacterial, fungal, parasitic, and insectile infestations (Downum, 1992; Heitz, 1987). Consistent with their protective function, levels of coumarins and furocoumarins vary widely. Naganuma *et al.*, 1985, found that levels of oxypeucedanin doubled in lemon oil derived from the same growing area in Australia between 1979 and 1980. The levels of oxypeucedanin in lemon oil from the Ivory Coast, Sicily and California diminished nearly three-fold in the same time period.

**Table 3. Levels of Major Coumarins and Furocoumarins in Lemon Oil and Lime Oil.**

Compound	Photosensitizing Activity	% in Lemon Oil <sup>1</sup>	% in Lime Oil
5-Geranoxypsoralen	0 <sup>1</sup>	0.0387	2.2-2.5 <sup>3</sup>
5-Geranoxy-7-Methoxycoumrin	0 <sup>1</sup>	0.0603	2.2-5.2 <sup>3</sup>
5-Geranoxy-8-methoxypsoralen	0 <sup>1</sup>	not analyzed	0.945 <sup>4</sup>
5,7-Dimethoxycoumarin	0 <sup>2</sup>	0.0295	0.464 <sup>4</sup>
5,8-Dimethoxypsoralen	0 <sup>2</sup>	not analyzed	0.508 <sup>4</sup>
Oxypeucedanin	+ <sup>1a</sup>	0.005-0.073	0.0025 <sup>4</sup>
5-Methoxypsoralen	++++ <sup>1</sup>	0.0001-0.0087	0.17-0.33 <sup>3</sup>

<sup>1</sup> Naganuma *et al.* (1985), phototoxicity tested on both guinea pig and rabbit skin.

<sup>1a</sup> Naganuma *et al.* (1985) found that oxypeucedanin was approximately 4 times weaker than 5-methoxypsoralen in eliciting phototoxicity in the guinea pig.

<sup>2</sup> Musajo and Rodighiero (1962), phototoxicity tested on human skin.

<sup>3</sup> Cieri (1969).

<sup>4</sup> Stanley and Vannier (1967).

The photogenotoxicity and photocarcinogenicity of several linear furocoumarins (psoralens) have been well documented (reviewed by Saffran, 1988). 5-Methoxypsoralen, a component of lemon and lime oil, is photogenotoxic in both prokaryotic and eukaryotic test systems. In addition, 5-methoxypsoralen has been found to be photocarcinogenic in an experimental model (Zajdela and Bisagni, 1981). Little is known about the photogenotoxicity and photocarcinogenicity of other furocoumarins found in lemon oil and lime oil. The mutagenicity of oxypeucedanin has been assessed in 6 tester strains of *Salmonella typhimurium* by Uwaifo, 1984. Oxypeucedanin was not mutagenic, however, photomutagenicity was not assessed.

## 7.0 REQUESTED STUDIES

Lemon oil and lime oil contain a complex mixture of coumarins and furocoumarins. Two of these compounds, 5-methoxypsoralen and oxypeucedanin, have been identified as phototoxic and implicated in the phototoxicity elicited by these essential oils. While the photogenotoxicity of 5-methoxypsoralen is well established, the photogenotoxicity of oxypeucedanin alone or in mixtures of compounds representative of lemon oil and lime oil has not been assessed. These photogenotoxicity studies are requested. Additional studies on photocarcinogenicity should be conducted if coumarins or furocoumarins are significantly photogenotoxic as individual compounds or in complex mixtures (*e.g.* lemon or lime oil).

## 8.0 REFERENCES

Budavari, S., (Ed.) (1996) **The Merck Index, 12<sup>th</sup> Edition**. Merck & Co., Inc., Whitehouse, N.J.

Burdock, G. A. (Ed.) (1995) **Fenaroli's Handbook of Flavor Ingredients, Volume 1, 3<sup>rd</sup> Edition**. CRC Press, Boca Raton, FL

Cieri, U. R. (1969) Characterization of the Steam Nonvolatile Residue of Bergamot Oil and Some Other Essential Oils. **J. Assoc. Off. Anal. Chem.** 52: 719-728.

Code Federal Regulations (2000) Title 21, Part 182.20, U.S. Government Printing Office, Washington, DC.

Cosmetic, Toiletry, and Fragrance Association, Inc. (1999) **International Cosmetic Ingredient Dictionary and Handbook, Eighth Edition 2000, Volume 2**. Ed: J. A. Wenninger, R. C. Canterbury and G. N. McEwen, Jr., The Cosmetic, Toiletry, and Fragrance Association, Washington, DC.

Downum, K. R. (1992) Light-Activated Plant Defense. **New Phytolog.** 122: 401-420.

Forbes, P. D., F. Urbach and R. E. Davies (1977) Phototoxicity Testing of Fragrance Raw Materials. **Fd. Cosmet. Toxicol.** 15: 55-60.

Heitz, J. R. (1987) Development of Photoactivated Compounds as Pesticides. In: **Light-Activated Pesticides**. Ed: J. R. Heitz and K. R. Downum, ACS Symposium Series 339, American Chemical Society, Washington, DC.

IFRA (1992) Code of Practice, International Fragrance Association, Geneva.

Kaidbey, K. H. and A. M. Kligman (1978) Identification of Topical Photosensitizing Agents in Humans. **J. Invest. Dermatol.** 70: 149-151.

Musajo, L. and G. Rodighiero (1962) The Skin-Photosensitizing Furocoumarins. **Experientia** 18: 153-200.

Naganuma, M., S. Hirose, Y. Nakayama, K. Nakajima and T. Someya (1985) A Study of the Phototoxicity of Lemon Oil. **Arch. Dermatol. Res.** 278: 31-36.

Opdyke, D. L. J. (1974) Monographs on Fragrance Raw Materials. **Fd. Cosmet. Toxicol.** 12: 725-731.

Poucher, W. A. (1991) **Poucher's Perfumes, Cosmetics and Soaps-Vol. 1. The Raw Materials of Perfumery, 9<sup>th</sup> Edition**. Ed.: A. J. Jouhar. Chapman & Hall, NY. pp. 199-201.

Saffran, W. A. (1988) Genotoxic Effects of Psoralen. In: **Psoralen DNA Photobiology. Volume II.** Ed.: F. P. Gasparro, CRC Press, Boca Raton, FL. pp 73-86.

Secondi, O. (1990) **Handbook of Perfumes and Flavors**, Chemical Publishing Co., Inc., NY. pp. 49-50.

Stanley, W. L. and S. H. Vannier (1967) Psoralens and Substituted Coumarins from Expressed Oil of Lime. **Phytochemistry** 6: 585-596.

Swaine, R. L. and R. L. Swaine Jr. (1988) Citrus Oils: Processing, Technology, and Applications. **Perfume & Flavorist** 13: 2-20.

Uwaifo, A. O. (1984) The Mutagenicities of Seven Coumarin Derivatives and a Furan Derivative (Nimbolide) Isolated from Three Medicinal Plants. **J. Toxicol. Environ. Health** 13: 521-530.

Zajdela, F. and E. Bisagni (1981) 5-Methoxypsoralen, the Melanogenic Additive in Suntan Preparations, Is Tumorigenic in Mice Exposed to 365 nm UV Radiation. **Carcinogenesis** 2: 121-127.