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 FRAGRANCES COMPOUNDS USED IN COSMETIC PRODUCTS

adopted by the SCCNFP during the 12th Plenary meeting
of 3 May 2000

1. Terms of Reference

1.1 Background

In recent years there has been a scientific debate on the safety of fragrance (perfumery) materials. A number of prominent dermatologists have highlighten the risk of delayed contact sensitisation (skin allergy) from perfumes and requested action from legislators. Indeed, several dermatologists state that up to 2% of the general population may exhibit some form of perfume allergy.

Industry disputes these figures claiming that marketplace surveillance findings indicate the true figures to be far lower. In addition, a great deal of proprietary testing of fragrance materials carried out in industry suggests that the materials do not pose such a hazard.

1.2 Current legislation on fragrance materials

Under current legislation, fragrance materials do not fall under all the requirements of Directive 76/768/EEC on cosmetic products. Whilst Article 2 of Directive 76/768/EEC applies to all products, few individual fragrance ingredients are included in the provisions of the directive. Currently, the industry is self-regulated through the code of practice of the International Fragrance Association (IFRA).

Following the 6th Amendment (93/35/EEC) a certificate of conformity must be provided to certify that the compound conforms to IFRA guidelines and the Cosmetics Directive. The 6th amendment also provided 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 the 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 permissable 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 Pleanry 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 related to the first section has been adopted by the SCCNFP during the 10th Plenary meeting of 8 Decmber 1999 (doc. n° SCCNFP/0017/98 Final) and consists of :

- a critical review of the problem of fragrance allergy in consumers;
- identification of those fragrance ingredients which are well-recognised as consumer allergens;
- an opinion as to whether such identification can be related to concentrations present in a product when elicitation levels are known.

Allergy to natural ingredients will be analysed separately.

This opinion relates to the second section and consists of the adoption of the list of perfumery materials to be included in Annex II (List of substances which must not form part of the composition of cosmetic products to Directive 76/768/EEC.

The adoption of the 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 will be the subject of a future opinion.

4. Opinion

On the basis of the assessment of the cutaneous toxicities 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 should not be used as fragrance ingredients in cosmetic products.

Other 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

N°	Substance Name	IFRA Determination
1	Alantroot (Inula helenium) essential oils and derivatives e.g. concrete and absolute CAS n° : 97676-35-2	Prohibited. Should not be used as a fragrance ingredient. Based on findings of RIFM on the sensitising potential (Opdyke,76).
2	Allylisothiocyanate CAS n° : 57-06-7	Should not be used as or in fragrance ingredients. The recommendation is based on the absence of reports on the use of this material as fragrance ingredient and/or inadequate evaluation of potential adverse effects resulting from its use in fragrances. (Annex II, n° 18)
3	Benzyl cyanide CAS n° : 140-29-4	Should not be used as or in fragrance ingredients. The recommendation is based on the absence of reports on the use of this material as fragrance ingredient and/or inadequate evaluation of potential adverse effects resulting from its use in fragrances.
4	p-tert-Butylphenol CAS n° : 98-54-4	Prohibited. Should not be used as a fragrance ingredient. Based on published literature on the sensitisation and depigmentation properties, summarized in (Opdyke,75). (Annex II, n° 340)
5	Chenopodium oil CAS n° : 8006-99-3	Should not be used as or in fragrance ingredients. The recommendation is based on the absence of reports on the use of this material as fragrance ingredient and/or inadequate evaluation of potential adverse effects resulting from its use in fragrances. (Annex II, n° 76)
6	Cyclamen alcohol CAS n° : 4756-19-8	Prohibited. Should not be used as a fragrance ingredient as such.
7	Diethyl maleate CAS n° : 141-05-9	Prohibited. Should not be used as a fragrance ingredient. Based on the sensitizing potential (Opdyke, FCT 14,443(1976)).
8	Dihydrocoumarin CAS n° : 119-84-6	Prohibited. Should not be used as a fragrance ingredient. Based on the sensitizing potential (Opdyke FCT 12,521(1974)).
9	2,4-Dihydroxy-3-methyl-benzaldehyde CAS n° : 6248-20-0	Prohibited. Should not be used as a fragrance ingredient. Based on test results of RIFM on the sensitizing potential of this material (Ford et al. 1988, FCT 26, 303).

N°	Substance Name	IFRA Determination
10	3,7-Dimethyl-2-octen-1-ol (6,7-Dihydrogeraniol) CAS n° : 40607-48-5	Should not be used as or in fragrance ingredients. The recommendation is based on the absence of reports on the use of this material as fragrance ingredient and/or inadequate evaluation of potential adverse effects resulting from its use in fragrances.
11	4,6-Dimethyl-8-tert-butyl-coumarin CAS n° : 17874-34-9	Prohibited. Should not be used as a fragrance ingredient. Based on the potential for inducing photoallergic reactions (Opdyke FCT 18,671 (1980)).
12	Dimethyl citraconate CAS n° : 617-54-9	Prohibited. Should not be used as a fragrance ingredient. Based on the sensitizing potential (Opdyke, FCT 14,749(1976)).
13	7,11-Dimethyl-4,6,10-dodecatrien-3-one CAS n° : 26651-96-7	Prohibited. Should not be used as a fragrance ingredient.
14	6,10-Dimethyl-3,5,9-undecatrien-2-one CAS n° : 141-10-6	Prohibited. Should not be used as a fragrance ingredient.
15	Diphenylamine CAS n° : 122-39-4	Should not be used as or in fragrance ingredients. The recommendation is based on the absence of reports on the use of this material as fragrance ingredient and/or inadequate evaluation of potential adverse effects resulting from its use in fragrances.
16	Ethyl acrylate CAS n° : 140-88-5	Prohibited. Should not be used as a fragrance ingredient. Based on the sensitizing potential (Opdyke FCT 13,801(1975)). Classified by IARC as a carcinogen.
17	Fig leaf, fresh and preparations (Ficus carica) CAS n° : 68916-52-9	Prohibited. Should not be used as a fragrance ingredient. Based on the sensitizing and extreme phototoxic potential (Opdyke FCT 20,691(1982)).
18	trans-2-Heptenal CAS n° : 18829-55-5	Prohibited. Should not be used as a fragrance ingredient. Based on test results of RIFM showing the sensitizing potential of this material (Ford et al. 1988, FCT 26, 331).
19	trans-2-Hexenal diethyl acetal CAS n° : 67746-30-9	Prohibited. Should not be used as a fragrance ingredient. Based on test results of RIFM showing the sensitizing potential of this material (Ford et al. 1988, FCT 26, 345).
20	trans-2-Hexenal dimethyl acetal CAS n° : 18318-83-7	Prohibited. Should not be used as a fragrance ingredient. Based on test results of RIFM showing the sensitizing potential of this material (Ford et al. 1988, FCT 26, 347).
21	Hydroabietyl alcohol CAS n° : 13393-93-6	Prohibited. Should not be used as a fragrance ingredient in cosmetic products. Based on the weak sensitizing potential (Opdyke, FCT 12,919(1974)).

N°	Substance Name	IFRA Determination
22	Hydroquinone monoethyl ether CAS n° : 622-62-8	Prohibited Should not be used as a fragrance ingredient. Based on the depigmenting effect of this material. (Annex II, n° 178)
23	6-Isopropyl-2-decahydronaphthalenol CAS n° : 34131-99-2	Prohibited. Should not be used as a fragrance ingredient. Based on the findings of RIFM on the sensitizing potential of this material (Ford et al 1988, FCT 26, 367).
24	7-Methoxycoumarin CAS n° : 531-59-9	Prohibited. Should not be used as a fragrance ingredient. Based on the findings of RIFM on the potential of inducing allergic and photoallergic reactions of this material (Ford et al 1988, FCT 26, 375).
25	4-Methoxyphenol CAS n° : 150-76-5	Prohibited. Should not be used as a fragrance ingredient. Based on the depigmenting effect (pc to IFRA). (Annex II, n° 178)
26	4-(p-Methoxyphenyl)-3-butene-2-one CAS n° : 943-88-4	Prohibited. Should not be used as a fragrance ingredient. Based on the findings of RIFM on the sensitizing potential (Opdyke,75).
27	1-(p-Methoxyphenyl)-1-penten-3-one CAS n° : 104-27-8	Prohibited. Should not be used as a fragrance ingredient. Based on the sensitizing potential (Opdyke FCT 17,863(1979)). Replaces 11/77.
28	Methyl trans-2-butenoate CAS n° : 623-43-8	Prohibited. Should not be used as a fragrance ingredient. Based on the sensitizing potential (Opdyke FCT 17,865(1979)). Replaces 3/78.
29	6-Methylcoumarin CAS n° : 92-48-8	Prohibited. Should not be used as a fragrance ingredient. Based on the potential for producing photoallergic reactions (Kaidbay,78 & Opdyke FCT 17,275(1979)).
30	7-Methylcoumarin CAS n° : 2445-83-2	Prohibited. Should not be used as a fragrance ingredient. Based on the potential for inducing photoallergic reactions (Opdyke FCT 20,747(1982)).
31	5-Methyl-2,3-hexanedione CAS n° : 13706-86-0	Prohibited. Should not be used as a fragrance ingredient. Based on test results of RIFM on the sensitizing potential (Opdyke,82). Replaces 2/80.
32	Musk ambrette CAS n° : 83-66-9	Prohibited. Musk Ambrette should not be used as a fragrance ingredient. Based on photosensitivity (Cronin,84 Contact Dermatitis 11,88), neurotoxic effects (Spencer, Bischoff-Fenton, Moreno, Opdyke & Ford, 84 Toxicol. Appl. Pharmacol 75,571), and on accumulated evidence that musk ambrette can penetrate human skin and is only slowly excreted (pc to IFRA) (Annex II, n° 414)

N°	Substance Name	IFRA Determination
33	2-Pentylidene cyclohexanone CAS n° : 25677-40-1	Prohibited. Should not be used as a fragrance ingredient. Based on the sensitizing potential (Opdyke FCT 20,797(1982)).
34	4-Phenyl-3-buten-2-one CAS n° : 122-57-6	Prohibited. Should not be used as a fragrance ingredient. Based on RIFM findings on the sensitizing potential of this material (Opdyke,73) and on sensitizing effects on guinea pigs and humans (pc to IFRA). (Annex II, n° 356)
35	3,6,10-Trimethyl-3,5,9-undecatrien-2-one CAS n° : 1117-41-5	Prohibited. Should not be used as a fragrance ingredient, based on the sensitizing potential
36	Verbena (*Lippia citriodora* Kunth.) essential oils and derivatives e.g. concrete and absolute CAS n° : 8024-12-2	Prohibited. Should not be used as a fragrance ingredient, based on the sensitizing potential.

Review on the Safety of Perfumery Materials

Appendix 1

Safety data on Perfumery Materials
which must not form part of Fragrance Compounds
used in Cosmetic Products

Entry n° 1 : ALANTROOT OIL

Synonyms : Elecampane oil; oil of Inula.
CAS n° : 97676-35-2

Irritation : Alantroot oil tested at 4% in petrolatum produced no irritation after a 48hr closed-patch test on human subjects (Kligman, 1975).
Sensitisation : A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers. The material was tested at a concentration of 4% in petrolatum and produced extremely severe allergic reactions in 23 out of 25 subjects after the second induction application (Kligman, 1975). Subjects previously sensitized to costus root oil gave severe cross-sensitization responses to alantroot oil (Epstein, 1975).

Alantolactone (one of the main constituents of alantroot) elicited positive patch-test responses in sensitized guinea-pigs (Hausen & Schulz, 1975; Schulz, Hausen, Wallhofer & Schmidt-Loffler, 1975). Two individuals initially sensitized to purified alantolactone (derived from Inula, Compositae family) showed positive patch-test reactions to costus root oil (Mitchell, 1974). Alantolactone produced positive patch-test reactions in five patients who were allergic to Frullania (Mitchell, Fritig, Singh & Towers, 1970). Hjorth (1970) sensitized four patients out of 25 with a single patch test with a 1% petrolatum dispersion of alantolactone. That a patch test with alantolactone can cause sensitization was reported also by Foussereau, Muller & Benezra (1975).

References : Monographs on Fragrance Raw Materials, Food and Cosmetics Toxicology, Volume 14, 1979, p. 307

Entry n° 3 : PHENYL ACETYL NITRILE

Synonyms : Phenylacetonitrile; benzyl cyanide; benzyl nitrile
CAS n° : 140-29-4
Structure : C_6H_5-CH_2-CN

Acute toxicity : The acute oral LD50 in rats has been reported as 0.31-0.38 g/kg (Moreno, 1976 & 1977). Ten male rats dosed orally by intubation with the LDO (0.18g/kg) for 5 days to determine possible cumulative effects all survived and appeared normal throughout the observation period (Moreno, 1977). The acute dermal LD50 has been reported as approximately 0.27 g/kg (0.18-0.41 g/kg) in rabbits (Moreno, 1976 & 1977) and as in the region of 2 ml/kg in rats (Cooke, 1979).

The ip LD50 in mice was reported as 10-25 mg/kg (Doull, Plzak & Brois, 1962) and as 24 mg/kg (Ohkawa, Ohkawa, Yamamoto & Casida, 1972). The single-dose intragastric LD84 for phenyl acetyl nitrile in albino rats was found to be 285 mg/kg, the LD50 was 270 mg/kg and the LD16 was 230 mg/kg (Galibin, Fedorova & Karamzina, 1967). In white mice, the LD84 was 106 mg/kg, the LD50 was 78 mg/kg and the LD16 was 38 mg/kg (Galibin et al. 1967). The sc lethal dose of phenyl acetyl nitrile was reported to be 50 mg/kg in rabbits, 32 mg/kg in mice and 1500 mg/kg in frogs; in pigeons the minimum lethal im dose was reported as 120 mg/kg (Spector, 1956).

Inhalation : In albino rats, the LC84 for a single 2hr exposure of phenyl acetyl nitrile vapour was found to be 0.81 mg/litre, the LC50 was 0.43 mg/litre and the LC16 was 0.21 mg/litre; under similar test conditions, a concentration of 0.1 mg/litre caused the deaths of 50% of the white mice tested (Galibin et al. 1967).

When rats were exposed for 2 hr daily over a 1-month period to a concentration of 0.04-0.08 mg/ litre, they became sluggish, their threshold of excitability rose, arterial pressure dropped by 10-12 mm, the synthetic functions of their livers decreased, their body weights lagged 12% behind those of the controls and they developed catarrhal-desquamative bronchitis and emphysema (Galibin et al. 1967). For rats, a 6-month period of daily 4hr exposure to 0.003 mg/litre caused initial weight loss, a rise in the nervous-excitability threshold, liver-function disturbances and moderate bronchitis and emphysema (Galibin et al. 1967).

Irritation : Phenyl acetyl nitrile applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was slightly irritating (Moreno, 1976). No effect was observed from the application of 1-2 ml phenyl acetyl nitrile to a 10 cm^2 area of rabbit skin, but fur failed to grow back for 2 months (Galibin et al. 1967). Placed in the conjunctival sac of the rabbit eye, this nitrile produced transient hyperaemia and lachrymation (Galibin et al. 1967). Tested at 2% in petrolatum, it produced no irritation after a 48hr closed-patch test on human subjects (Epstein, 1976).

Sensitization : A maximization, test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 27 volunteers. The material was tested at a concentration of 2% in petrolatum and produced no sensitization reactions (Epstein, 1976).

Percutaneous absorption : Phenyl acetyl nitrile was shown to penetrate the intact skin of mouse tails, producing death but no local lesions (Galibin et al. 1967).

Teratogenic effects : Phenyl acetyl nitrile produced some lathyrogenic effects in chick embryos (Levene, 1961).

Detoxication : Phenyl acetyl nitrile is first oxidized to mandelonitrile which then yields benzaldehyde and cyanide (Williams, 1959). The hydrolysis of the cyano group to COOH may also be a minor path.

Treatment : Treatment following inhalation or ingestion of phenyl acetyl nitrile should take account of the lower toxicity of the material compared with ionisable cyanides. This is probably due to the low detoxication rate and consequent low cyanide-ion production. It should be noted, however, that the content of free cyanide ion may vary in different samples. Treatment should be supportive and should not include specific anticyanide therapy unless this is advised by a physician (Cooke, 1979).

Mutagenesis studies : Phenyl acetyl nitrile was not mutagenic to Salmonella typhimurium with or without microsomal activation (Florin, Rutberg, Curvall & Enzell, 1980).

References : Monographs on Fragrance Raw Materials, Food and Chemical Toxicology, Volume 20 Supplement, November 1982, p. 803

Entry n° 4 : p-tert-BUTYLPHENOL

Synonym : 1-Hydroxy-4-tert-butylbenzene.
CAS n° : 98-54-4
Structure : $HO\text{-}C_6H_4\text{-}C(CH_3)_3$

Acute toxicity : The acute oral LD50 in rats was reported as 1-4g/kg (0.56-3.50g/kg) (Denine, 1973). The acute dermal LD50 in rabbits was reported as > 5 g/kg (Denine, 1973).
Irritation : p-tert-Butylphenol applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was irritating (Denine, 1973). Tested at 1% in petrolatum, it produced no irritation after a 48hr closed-patch test on human subjects (Kligman, 1973).
Sensitization and depigmentation of human skin : A maximization test (Kligman, 1966) was carried out on 25 volunteers. The material was tested at a concentration of 1% in petrolatum and produced no sensitization reactions (Kligman, 1973). There is an abundant literature dealing with the well-established sensitization and depigmentation properties of p-tert-butylphenol and/or closely related material (Beetz, 1971; Bleehen, Pathak, Hori & Fitzpatrick, 1968; Calnan, 1973; Calrian & Harman, 1959; Gaul, 1960; Gellin, Possick & Perone, 1970; Hasegawa, Levit & Bluefarb, 1958; Kahn, 1970; McGuire & Hendee, 1971; Malten, 1958, 1964, 1967 & 1973; Malten, Scutter, Hara & Nakajima, 1971; Matz & Blank, 1959; Odom & Stein, 1973).
Metabolism : p-tert-Butylphenol is believed to be excreted by dogs in the form of a conjugate with sulphuric acid; presumably it behaves as a typical phenol (Williams, 1959).
Enzyme induction : The relative abilities of substituted phenols to induce drug-metabolizing enzymes were measured either 24 hr after a single dose or after 6-10 daily doses. The phenols carried various combinations of substituents, one of which was the tert-butyl group. When the effect was measured 24 hr after a single dose, a close relationship was found between the induction of drug-metabolizing enzymes and the lipid-water partition coefficient of the test compound. When the effect was measured after six daily doses, this relationship was less distinct, further dosing could lead either to an accentuation or a diminution of the effect. Many of the substituted phenols also induce uridine diphosphate glucose dehydrogenase, an effect apparently unrelated either to the lipid-water partition coefficient or to the induction of drug-metabolizing enzymes (Gilbert, Martin, Gangolli, Abraham & Golberg, 1969).

References : Monographs on Fragrance Raw Materials, Food and Cosmetics Toxicology, Volume 12, September 1974, p. 835

Entry n° 6 : CYCLAMEN ALCOHOL

Synonyms : α-Methyl-p-isopropylhydrocinnamic alcohol;
2-Methyl-3-cumenylpropanol.
CAS n° : 4756-19-8
Structure : CH_3-(CH_3)CH.C_6H_4-CH_2-CH(CH_3)-CH_2OH

Acute toxicity : Both the acute oral LD50 in rats and the acute dermal LD50 in rabbits exceeded 5g/kg (Levenstein, 1974).
Irritation : Cyclamen alcohol applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was not irritating (Levenstein, 1974). Tested at 20% in petrolatum, it produced no irritation after a 48hr closed-patch test on two different panels of human subjects (Kligman. 1974).
Sensitisation : A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers. The material (RIFM no. 74-66) was tested at a concentration of 20% in petrolatum and produced six sensitization reactions in the 25 subjects tested (Kligman, 1974). When subjected to the same maximization test in 25 new volunteers, again at a concentration of 20% in petrolatum. This material (RIFM no. 74-66) produced sensitization reactions in three subjects (Kligman. 1974). A preparation called 'Cyclamen Aldehyde Special', which contains 50% cyclamen alcohol, has also been tested, at 12% and was found to sensitize three out of 25 subjects (Kligman, 1976). A sample of cyclamen aldehyde containing 1.5% cyclamen alcohol was tested at 12% and produced no senisitization reactions in 25 subjects (Kligman, 1971).
IFRA data : Cyclamen alcohol at concentrations ranging from 3 to 100%, did not sensitize guinea pigs in the Open Epicutaneous Test (OET) but did produce very strong skin irritation at the 30 and 100% levels (H. Geleick and G. Klecak, unpublished communication 1978). At the lowest concentration (3%), very slight skin irritation was produced after a single as well as after repeated applications.

Five intradermal injections each of 0.1 ml of a 5% emulsion of cyclamen alcohol in Freund's Complete Adjuvant did not sensitize the guinea pig. Challenges were made topically on day 21 and 35 (H. Geleick and G. Klecak. unpublished communication 1978).

References : Monographs on Fragrance Raw Materials, Food and Cosmetics Toxicology, Volume 17, n° 3, 1979, p. 267

Entry n° 7 : DIETHYL MALEATE

Synonym : Ethyl maleate
CAS n° : 141-05-9
Structure : C_2H_5-OCO-CH=CH-$OCOC_2H_5$

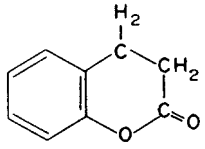
Acute toxicity : The acute oral LD50 value in rats was reported as 3.2g/kg (Fassett, 1963). The actue dermal LD50, value in rats was reported as > 2.5 g/kg (Moreno, 1975) and as 5 ml/kg (Fassett, 1963).
Inhalation toxicity : No death occurred in rats exposed to saturated vapours of diethyl maleate for 8 hr (Fassett, 1963).
Irritation : Diethyl maleate applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was moderately irritating (Moreno, 1975). The ester was reported to be slightly irritating to rabbit skin and eye (Fassett, 1963). Tested at 4% in petrolatum, it produced no irritation after a 48hr closed-patch test on human subjects (Kligman, 1975).
Sensitization : A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers. The material was tested at a concentration of 4% in petrolatum and produced sensitization reactions in all 25 (Kligman, 1975). Diethyl maleate was reported to be a sensitizer in patch tests performed on four men working with unsaturated polyester resins (Malten & Zielhuis, 1964).
Metabolism : α, β-Unsaturated compounds, such as diethyl maleate, react enzymically with glutathione. The reaction has been demonstrated in fractions of rat liver (Boyland & Chasseaud, 1967) and avian liver (Wit & Snel, 1968). The enzyme differs from other known S-alkyl, S-aryl and S-epoxide transferase enzymes responsible for glutathione-conjugate formation (Boyland & Chasseaud, 1967). Diethyl maleate, administered parenterally to rats, reduced the hepatic glutathione content (Boyland & Chasseaud, 1970; Varga, Fischer & Szily, 1974). The latter workers also showed that diethyl maleate pretreatment of rats inhibited the glutathione conjugation of subsequently-administered bromsulphthalein.

Studying this ester's effect on the metabolism of parathion and methyl parathion, Mirer, Levine & Murphy (1975) showed that pretreatment of mice with diethyl maleate (1 mg/kg), 1 hr before challenge, depleted liver glutathione and potentiated parathion and methyl parathion toxicity. In vivo, diethyl maleate potentiated the inhibition of brain cholinesterase by parathion and methyl parathion. Diethyl maleate pretreatment caused a twofold increase in the brain concentrations of parathion and methyl parathion and a large increase in the activation of methyl parathion to methyl paraoxon, and also decreased total degradation. Diethyl maleate inhibited the activation and degradation of parathion.

References : Monographs on Fragrance Raw Materials, Food and Cosmetics Toxicology, Volume 14, 1976, p. 443

Entry n° 8 : DIHYDROCOUMARIN

Synonyms : 3,4-Dihydrocoumarin; hydrocoumarin; 1,2-benzodihydropyrone.
CAS n° : 119-84-6
Structure :

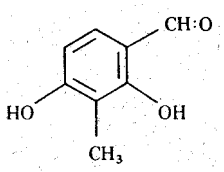


Acute toxicity : The acute oral LD50 value in rats was reported as 1.65 g/kg (1.47-1.83 g/kg) (Moreno, 1972a). The acute dermal LD50 value in rabbits was reported as >5 g/kg (Moreno, 1972b).
Subacute and long-term toxicity : In feeding studies, 1000 and 10 000 ppm fed to rats in the diet for 14 wk produced no effects (Hagan, Hansen, Fitzhugh, Jenner, Jones, Taylor, Long, Nelson & Brouwer, 1967). In a 2-yr study, dogs dosed daily with 50 and 150 mg/kg produced no effects (Hagan et al. 1967).
Irritation : Dihydrocoumarin applied full strength on intact or abraded rabbit skin for 24 hr under occlusion was moderately irritating (Moreno, 1972b). Tested at 20% in petrolatum, it produced no irritation after a 48hr closed-patch test in 25 human subjects (Kligman, 1972).
Sensitization : A maximization test (Kligman, 1966) was carried out on 25 volunteers. The material was tested at a concentration of 20% in petrolatum and produced sensitization reactions in all 25 test subjects (Kligman, 1972).

References : Monographs on Fragrance Raw Materials, Food and Cosmetics Toxicology, Volume 12, 1974, p. 521

Entry n° 9 : 2,4-DIHYDROXY-3-METHYLBENZALDEHYDE

Synonym : 4-Formyl-2-methylresorcinol.
CAS n° : 6248-20-0
Structure :



Acute toxicity : The acute oral LD50 in mice of a 50% w/v mixture in corn oil exceeded 5 g/kg based on 2/10 deaths at that dose, and the acute dermal LD50 in guinea pigs of a 50% w/v mixture in corn oil exceeded 5 g/kg based on 1/10 deaths (Moreno, 1980).
Irritation : A single application of 0.1 ml 2,4-dihydroxy-3-methylbenzaldehyde to the surface of six rabbits' eyes produced no irritation (Fritzsche Dodge & Olcott, 1978). Six rabbits were patch-tested with 0.5 ml on intact and abraded skin for 24 hr under occlusion. Slight irritation was observed with a Primary Irritation Index of 1.63 (Fritzsche Dodge & Olcott, 1978). As part of an acute dermal LD50 study, a 50% w/v mixture in corn oil produced slight irritant effects in guinea pigs patch-tested for 24 hr under occlusion at a dose of 5 g/kg (Moreno, 1980). Guinea pigs were patch-tested on the shaved flank for 6 hr under occlusion. At a dose of 50% in ethyl alcohol, and at 100% no irritation was produced (Fritzsche Dodge & Olcott, 1978).

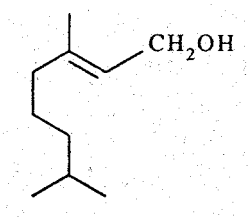
A 48hr closed-patch test at a concentration of 4% in petrolatum on 29 volunteers produced no irritation (Epstein, 1979).
Sensitization : The material was tested on guinea pigs in a delayed hypersensitivity test (Buehler, 1965). Twelve males were given nine topical occluded induction applications over a 21-day period, followed 14 days later by a challenge dose of a 50% solution in ethyl alcohol. No sensitization reactions were produced (Fritzsche Dodge & OLcott, 1978).

A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 29 volunteers. The material was tested at a concentration of 4% in petrolatum; nine sensitization reactions and one clinically irritant reaction were produced (Epstein, 1979). This test concentration was based on a reported maximum concentration of 0.4% in consumer products.

References : Monographs on Fragrance Raw Materials, Food and Chemical Toxicology, Volume 26, n° 4, 1988, p. 303

Entry n° 10 : 6,7-DIHYDROGERANIOL

Synonym : 3,7-Dimethyl-2-octen-l-ol
CAS n° : 40607-48-5
Structure :



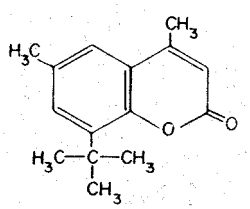
Acute toxicity : The oral LD50 in rats of a 50% solution of 6,7-dihydrogeraniol (in 0.5% aqueous carboxymethyl cellulose) exceeded 5 g/kg based on 2/10 deaths at that dose (BASF, 1985). The dermal LD50 in rats exceeded 2 g/kg based on 0/10 deaths at that dose (BASF, 1985).
Irritation : A 0.1 ml aliquot of undiluted material produced irritation in 6/6 rabbits after it was applied to the surface of the rabbit eye without rinsing (BASF, 1985). The Primary Irritation Index was 18.1. The undiluted material produced irritation on rabbits after a 24hr occluded patch test on intact or abraded skin (BASF, 1985). The Primary Irritation Index was 5.6.
Sensitization : A guinea pig maximization test (OECD Guidelines) with induction by intradermal injection of 5% in olive oil DAB 8 and occluded patch of undiluted material produced no sensitization reactions at a challenge concentration of 80% (in olive oil DAB 8) (BASF, 1985).

In a human repeated-insult patch test (Draize, 1959), 10% in a 3:1 mixture of ethanol and diethyl phthalate produced 5/92 reactions at challenge indicative of hypersensitivity. In addition, 57/106 reactions were observed during the induction phase, 39 of which persisted for more than 48 hr (Billhimer et al., 1988). This study was repeated because methyl 2-nonynoate was tested on this group of subjects and caused strong sensitization reactions during the induction phase. Of the 57 subjects who reacted during the induction phase, 39/57 also reacted to methyl 2-nonynoate. In another human repeated-insult patch test (Draize, 1959) using nine closed 24hr induction applications followed 14 days later by a closed challenge, 10% in 75% ethanol-25% diethyl phthalate produced 3/109 sensitization reactions (Harrison et al., 1988). These three subjects were rechallenged 2 wk after the challenge phase and all three reacted to 10%, but 1% produced only 1/3 sensitization reactions (Harrison et al., 1988). A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 10% in diethyl phthalate on 24 volunteers. One sensitization reaction and one irritation reaction were produced (Fritzsche Dodge & Olcott, Inc., 1985).

References : Monographs on Fragrance Raw Materials, Food and Chemical Toxicology, Volume 30 Supplement, 1992, p. 19S

Entry n° 11 : 4,6-DIMETHYL,8-tert-BUTYLCOUMARIN

CAS n° : 17874-34-9
Structure :



Acute toxicity : Both the acute oral LD50 in mice and the acute dermal LD50 in guinea pigs exceeded 5 g/kg (Moreno, 1978).
Irritation : 4,6-Dimethyl-8-tert-butylcoumarin applied full strength to intact or abraded guinea pig skin for 24 hr under occlusion was slightly irritating (Moreno, 1978). Tested at 8% in petrolatum, it produced no irritation after a 48hr closed-patch test on human subjects (Kligman, 1978).
Sensitization : A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers. The material (RIFM no. 78-83) was tested at a concentration of 8% in petrolatum and produced one sensitization reaction (Kligman, 1978).
Phototoxicity : 4,6-Dimethyl-8-tert-butylcoumarin at a concentration of 5% in hydrophilic ointment did not produce any phototoxic effects on human subjects (Kaidbey, 1979).
Photoallergenicity : 4,6-Dimethyl-8-tert-butylcoumarin produced photoallergenic effects on seven of 25 human subjects when tested at a concentration of 1% in hydrophilic ointment by the photomaximization test (Kligman & Kaidbey, 1978). Human subjects who were photoallergic to 6-methylcoumarin or to 7-methoxycoumarin did not cross-react to 4,6-dimethyl-8-tert-butylcoumarin (Kaidbey, 1979).

References : Monographs on Fragrance Raw Materials, Food and Cosmetics Toxicology, Volume 18, n° 6, 1980, p. 671

Entry n° 12 : DIMETHYL CITRACONATE

Synonym : Dimethyl methyl maleate
CAS n° : 617-54-9
Structure : CH_3-OCO-C(CH_3)=CH-OCO-CH_3

Acute toxicity : Both the acute oral LD50 value in rats and the acute dermal LD50 value in rabbits exceeded 5 g/kg (Moreno, 1974).
Irritation : Dimethyl citraconate applied full strength to intact or abraded rabbit skin for 24hr under occlusion was slightly irritating (Moreno, 1974). Tested at 12% in petrolatum it produced no irritation after a 48hr closed-patch test on two different panels of human subjects (Epstein, 1974; Kligman, 1975).
Sensitization : A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 19 volunteers. The material was tested at a concentration of 12% in petrolatum and produced a questionably positive reaction in one of the 19 subjects (Epstein, 1974). In a second maximization test, the material was retested at a concentration of 12% in petrolatum and produced three sensitization reactions in 25 test volunteers (Kligman, 1975).

References : Monographs on Fragrance Raw Materials, Food and Cosmetics Toxicology, Volume 14 Supplement, December 1976, p. 749

Entry n° 13 : 7,11-DIMETHYL-4,6,10-DODECATRIEN-3-ONE

Synonyms : 2,6-Dimethyldodeca-2,6,8-trien-10-one; pseudo methylionone
CAS n° : 26651-96-7
Structure : CH_3-CH_2-CO-CH=CH-CH=C(CH_3)-CH_2-CH_2-CH=C(CH_3)-CH_3 (a mixture of isomers)

Acute toxicity : The acute oral LD50 in rats exceeded 5 g/kg based on 0/10 deaths at that dose, and the acute dermal LD50 in rabbits exceeded 5 g/kg based on 4/10 deaths (Moreno, 1977).
Irritation : As part of an acute dermal LD50 study, the undiluted material produced severe irritant effects with hardened and thickened skin at necropsy in rabbits patch-tested for 24hr under occlusion at a dose of 5 g/kg (Moreno, 1977). A 48hr closed-patch test at a concentration of 8% in petrolatum on the backs or forearms of 75 volunteers produced seven irritant reactions (Epstein, 1977; Kligman, 1978).
Sensitization : Three maximization tests (Kligman, 1966; Kligman & Epstein, 1975) were carried out on a total of 75 volunteers. The material was tested at a concentration of 8% in petrolatum, and 2/25 (Epstein, 1977), 1/25 (Kligman, 1978) and 8/25 (Kligman, 1978) sensitization reactions were produced. This test concentration was based on a reported maximum concentration of 0.8% in consumer products.
Cytotoxicity : Vapours of 7,11-dimethyl-4,6,10-dodecatrien-3-one incubated in vitro for 5 days with Candida albicans ATCC 10231, Phoma betae ATCC 6504, Geotrichum candidum Coll. no. 4762 and Oospora lactis ATCC 4798 did not inhibit their growth (Maruzzella et al 1961).

References : Monographs on Fragrance Raw Materials, Food and Chemical Toxicology, Volume 26, n° 4, 1998, p. 305

Entry n° 14 : 6,10-DIMETHYL-3,5,9-UNDECATRIENE-2-ONE

Synonyms : Citrylideneacetone; 2,6-dimethylundeca-2,6,8-triene-10-one; pseudoionone
CAS n° : 141-10-6
Structure : CH_3-CO-CH=CH-CH=C(CH_3)-CH_2-CH_2-CH=C(CH_3)-CH_3 (a mixture of isomers)

Acute toxicity : The acute oral LD50 in rats exceeded 5 g/kg based on 0/10 deaths at that dose, and the acute dermal LD50 in rabbits exceeded 5 g/kg based on 1/10 deaths (Moreno, 1976).
Irritation : As part of an acute dermal LD50 study, the undiluted material produced moderate irritant effects in rabbits patch-tested for 24hr under occlusion at a dose of 5 g/kg (Moreno, 1976). A 48hr closed-patch test at a concentration of 8% in petrolatum on the forearms or backs of 108 volunteers produced no irritation (Epstein, 1978; Kligman, 1976).
Sensitization : Four maximization tests (Kligman, 1966; Kligman & Epstein, 1975) were carried out on a total of 108 volunteers. The material was tested at a concentration of 8% in petrolatum, and 2/25 (Kligman, 1976), 4/25 (Epstein, 1978), 2/25 (Kligman, 1976) and 1/33 (Epstein, 1978) sensitization reactions were produced. This test concentration was based on a reported maximum concentration of 0.8% in consumer products.

Mutagenesis studies : In an Ames test (Ames et al. 1975) using Salmonella typhimurium strains TA98, TA 100, TA1535 and TA1537 with and without S-9 activation, 6,10-dimethyl-3,5,9-undecatriene-2-one was not mutagenic at 3 µmol/plate (Florin et al. 1980).
Teratogenesis and reproduction studies : 6,10-Dimethyl-3,5,9-undecatriene-2-one did not inhibit embryonic development in eggs of the insect Earias vittella exposed to various concentrations (concentrations not stated) in isopropyl alcohol (Mehta, 1979). A single dose of 960 mg/kg given orally to female hamsters on day 8 of gestation failed to significantly alter the incidence of abnormal litters or the mean litter foetal body weight (Willhite, 1986). Maternal weight gain was significantly depressed but no other signs of maternal intoxication were observed; no terata were observed.
Cytotoxicity : Cell growth in Ascites sarcoma BP8 mouse cells was inhibited 9% by 0.01 mM 6,10-dimethyl-3,5,9-undecatriene-2-one, while 0.1 and 1 mM inhibited growth by 100% (Pilotti et al. 1975). Oxidative metabolism (noradrenaline-induced respiration) in hamster brown-fat cells was inhibited 70% by 1 mM (Pettersson et al. 1980). The membrane permeability of human lung fibroblasts incubated with 25 mM for 30 min was increased by 68% (Thelestam et al. 1980). A concentration of 5 mM for 23 min caused complete cessation of ciliary activity in chicken embryo tracheal organ cultures (Pettersson et al. 1982). Vapours of pseudoionone incubated in vitro for 5 days with Candida albicans ATCC 10231, Phoma betae ATCC 6504, Geotrichum candidum Coll. no. 4762 and Oospora lactis ATCC 4798 inhibited the growth of P. betae only (Maruzzella et al. 1961).

References : Monographs on Fragrance Raw Materials, Food and Chemical Toxicology, Volume 26, n° 4, 1988, p. 311

Entry n° 15 : DIPHENYLAMINE

CAS n° : 122-39-4
Structure : C_6H_5- NH-C_6H_5

Acute toxicity : The acute dermal LD50 in rabbits exceeded 5g/kg (Levenstein, 1976). The acute oral LD50 in rats was reported as 1.165g/kg by Levenstein (1976) and as 3.2g/kg by Epstein, Saporoschetz & Hunter (1967) and by Volodchenko (1975). The latter author found that administration of 0.5 LD50 decreased haemoglobin and oxyhaemoglobin levels, while increasing methaemoglobin and Heinz-body formation (Volodchenko, 1975). Oral administration of 1 mmol DPA/kg in aqueous suspension to a cat caused considerable methaemoglobin formation (Alexander, Ryan & Wright, 1965). An oral dose of 300 mg/rat was lethal to two of 20 rats in 30 days (Griswold, Casey, Weisburger, Weisburger & Schabel, 1966) while 7500mg/kg is the lowest oral dose reported as being toxic to pregnant rats (National Institute for Occupational Safety and Health, 1976).

Subacute toxicity : DPA given by gavage daily to rats for 14 days at doses above 2 mmol/kg (338 mg/kg) caused necrosis affecting 20% of the papillary apex of the kidney, a reduced capacity
to concentrate urine, polyuria, azotaemia, a reduced ability to secrete acid urine following an oral ammonium-ion load, and grossly elevated absolute kidney weights (Hardy, 1974). Pronounced histological changes occurred in the renal tubules of rats given 0.1-0.2 LD50 for 30 days (Volodchenko, 1974). Severe kidney lesions were produced in dehydrated lambs given DPA in an unstated dose for an unstated period (Salisbury, McIntosh & Staples, 1969).

Chronic toxicity : In common with other diphenyl derivatives, DPA produced a marked, diffuse and progressive renal cystic disease in experimental animals, providing a model for human polycystic disease of the kidney (Eknoyan, Weinman, Tsaparas, Tisher, Yarger, Suki & Martinez-Maldonado, 1976). When 2-5% DPA was added to the diet of rats, a significant defect in maximal urine-concentrating ability was noted within 2wk, and this related morphologically after 3-6wk to changes in the terminal portion of the collecting duct. Arterial blood pressure, water reabsorption and clearance, glucose absorption and bicarbonate absorption remained at normal levels in these animals. The similarities between DPA-induced cystic disease and human polycystic disease have been confirmed by scanning electron-microscopic examination of human polycystic kidneys and of kidneys obtained from rats fed 1% DPA in the diet for 1.5 yr (Evan & Gardner, 1976). Histological changes have been reported in kidneys obtained from rats fed 1% DPA in the diet for 5-20 months (Gardner, Solomon, Fitzgerrel, Evan, Searl & Chavez, 1976).

In a study in which rats were placed on diets containing 0.001, 0.01, 0.1, 0.5 or 1% DPA for 734 days, levels higher than 0.1% caused distinct growth inhibition, while at the 1% level, a moderate degree of anaemia (reductions in haemoglobin and numbers of red cells and an increase in the number of circulating normoblasts) was noted (DeEds, 1963; Thomas, Ribelin, Wilson, Keppler & DeEds, 1967). Survival was not affected by DPA treatment, but at autopsy animals fed levels above 0.01% exhibited cystic dilated renal tubules accompanied by chronic interstitial nephritis. In a parallel study (DeEds, 1963; Thomas, Ribelin, Woodward & DeEds, 1967), in which 0.01, 0.1 or 1% DPA was administered to dogs in the diet for 2 yr, 1% DPA caused severe growth inhibition, low haemoglobin levels and red-cell counts, crenated red cells and an increase in red-cell fragility, and possible impairment of liver function. At autopsy, these dogs were characterized by fatty livers, possible increases in the weight of the kidneys and spleen, and haemosiderosis of the spleen, kidney and bone marrow. The DPA used in both studies was 99.9% pure, as determined by cryoscopy (DeEds, 1963). These studies were considered to

confirm the safety to man of residual levels of DPA used as an antioxidant to protect fruit from scald.

Experimental polycystic renal disease produced in rats by DPA in the diet has been reviewed and studied by a number of other investigators (Kime, McNamara, Luse, Farmer, Silbert & Bricker, 1962; Safouh, Crocker & Vemier, 1970).

Irritation : DPA applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was not irritating (Levenstein, 1976). Tested at 1% in petrolatum, it produced no irritation after a 48hr closed-patch test on human subjects (Epstein, 1976).

Sensitisation : A maximization test (Kligman, 1966, Kligman & Epstein, 1975) was carried out on 30 volunteers. The material (RIFM no. 76-92) was tested at a concentration of 1% in petrolatum and produced no sensitization reactions (Epstein, 1976).

Metabolism : In man, unchanged DPA and the 4-hydroxy and 4,4'-dihydroxy derivatives were detected in urine: N-hydroxylated derivatives were not recovered even when N-hydroxy DPA was administered (Alexander et al. 1965). Nitrosodiphenylamine, which has been characterized as a neoplastic agent (National Institute for Occupational Safety and Health, 1976), was found in the stomach of patients given DPA in a solution of nitrite (Sander & Schweinsberg, 1972). The urinary and biliary metabolites of DPA in the rat were identified as the 4-hydroxy and 4,4'-dihydroxy derivatives (Alexander et al. 1965), elimination of ^{14}C-DPA was rapid in urine and bile. In the rabbit, these metabolites, plus the 2-hydroxy derivative and unchanged DPA, were recovered from the urine (Alexander et al. 1965). Metabolites of DPA detected in the urine and faeces of dogs receiving DPA were 4-hydroxy DPA and its sulphate and N-glucosiduronide derivatives and 4,4'-dihydroxy DPA (DeEds, 1963). When a Holstein cow was fed DPA at a level of 5 ppm for 4 days, DPA residues were not detected in the milk or urine, but small amounts (1.4% of the total dose) were recovered in the faeces (Gutenmann & Lisk, 1975).

Threshold limit value : The threshold limit value for DPA has been set at 10mg/m^3 (American Conference of Governmental Industrial Hygienists, 1973).

Carcinogenicity : The incidence of both benign and malignant tumours found at autopsy after a 2-yr feeding study of DPA in rats was not considered to be related to treatment (Thomas et al. 1967). No neoplastic changes were reported in 20 rats within 6 months of the oral administration of a single dose of 300mg/rat (Griswold et al. 1966). A bioassay for antioxidants, based on the protection of isolated rat-liver mitochondria from the photodynamic toxicity of benzo[a]pyrene was carried out on 92 antioxidants, including DPA, by Epstein, Saporoschetz, Katsioules & Bishop (1971). At a concentration of 10 μg/ml, DPA gave protection against photodynamic injury of the mitochondria by benzo[a]pyrene, at a concentration of 100 μg DPA/ml slight photodynamic effects were seen. In a host-mediated in vivo-in vitro bioassay for identifying potential carcinogenicity for mammalian cells, DPA at doses of 5-20 mg/kg administered ip to pregnant hamsters was classified as a non-carcinogen (DiPaolo, Nelson, Donovan & Evans, 1973).

Teratogenic effects : The effects of aged DPA in pregnant rats have been studied (Crocker, Brown, Borch & Vernier, 1972, Crocker & Vernier, 1970; Gibson, 1976). The results confirmed the nephrotoxicity of commercial DPA at 2.5% in the diet, and cystic abnormalities of the proximal tubule of the kidney were seen in newborn rats delivered from mothers fed DPA as a part of the diet or given DPA by gavage for 6-7 days. The exact nature of the abnormality appeared to depend on the time of administration during the gestation period. The production of cystic changes was associated with an unidentified contaminant isolated from DPA by chromatographic techniques. This unknown compound, when fed to pregnant rats in 50 μg quantities daily, induced changes similar to those caused by 20mg commercial aged DPA. When rats of both sexes maintained on diets containing 99.9% pure DPA at a level of 0.1, 0.25 or 0.5%

were mated twice and their offspring were mated, the only observation was that the average size of the litters decreased as the concentration of DPA in the diet increased (DeEds, 1963; Thomas et al. 1967).

Chemotherapy : DPA administered orally at 100mg/kg to infected rats or mice exhibited weak anthelmintic effects (Selivanova, Molodykh & Kudryavtsev, 1972). Similarly, oral administration at the 200mg/kg level showed weak anthelmintic activity in infected mice (Izard-Verchere, Cavier, Morin, Manuel-Menillet & Viel, 1971). DPA, in two oral doses of 300 mg/kg, was inactive in rabbits, sheep and cattle infected with liver fluke (Lämmler & Loewe, 1962).

Cytotoxicity : The median growth-inhibiting dose (ID50) of DPA to the ciliate Tetrahymena pyriformis was 25 μg/ml (Epstein et al. 1967). When tested for toxicity to ascites sarcoma BP8 cell cultures, DPA showed 100% inhibition at a dilution of 1 mM and 15% inhibition at 0.1 mM (Pilotti, Ancker, Arrhenius & Enzell, 1975).

Physiology : When the olfactory bulb of the burbot (a fish) was exposed to a 10^{-3} M solution of DPA, an afferent neuron monitored for response produced three excitatory, two inhibitory and three negative responses in eight trials (Döving, 1966).

Pharmacology : DPA administered orally to either rats or mice at 100 mg/kg showed weak anticholinesterase activity (Selivanova et al. 1972). A mixture of DPA, ethyleneglycol tetraacetate and cytochrome C administered iv to dogs for 6-10 days prevented the development of experimentally induced liver necrosis (Zarinskaya & Krcnili, 1975).

References : Monographs on Fragrance Raw Materials, Food and Cosmetics Toxicology, Volume 16 Supplement 1, December 1978, p. 723

Entry n° 16 : ETHYL ACRYLATE

Synonym : Ethyl propenoate.
CAS n° : 140-88-5
Structure : CH_3-CH_2-OCO-CH=CH_2.

Acute toxicity : The oral LD50 value was reported as 1000 mg/kg in rats and 400 mg/kg in rabbits (Fassett, 1963). The inhalation LC50 value in rats was reported as < 1000 ppm (4 mg/litre) after 4 hr (Fassett, 1963).
Chronic toxicity : Rats were fed 6-7, 60-70 or 2000 ppm in the diet for 2 yr (Borzelleca, Larson, Hennigar. Huf. Crawford & Smith, 1964). At 2000 ppm, a decrease in fluid intake was observed in males and females; for the females decreases in food intake and body weight were recorded. Body weights of males were decreased only in yr 1. Body-weight ratios for heart, spleen, liver and testes were normal. Haematological values were within normal ranges and semiquantitative tests for urinary concentrations of protein and reducing substances were negative. Histopathology. which was carried out on all groups except those given the lowest concentration, showed no abnormalities.

Dogs were fed 10, 100 or 300-1000 ppm in the diet for 2 yr (Borzelleca et al. 1964). The highest dose level (1000 ppm) was not tolerated at first (some dogs vomited). It was reduced to 300, therefore, and then raised gradually for 16 wk. No gross effects observed. At the highest dose, less food was consumed and less weight was gained. Organ weight/body weight ratios were normal at all dose levels. Histopathological examination revealed no lesions and haematological findings and urinary concentrations of reducing substances and proteins were within normal ranges.
Irritation : Ethyl acrylate tested at 4% in petrolatum produced no irritation after a 48hr closedpatch test on human subjects (Epstein, 1974).
Sensitiation : A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 24 volunteers. The material was tested at a concentration of 4% in petrolatum and produced sensitization reactions in ten of the 24 (Epstein, 1974).
Threshold limit value :The threshold limit value for ethyl acrylate has been set at 25 ppm (American Conference of Governmental Industrial Hygienists, 1973).

References : Monographs on Fragrance Raw Materials, Food and Cosmetics Toxicology, Volume 13 Supplement, December 1975, p. 801

Entry n° 17 : FIG LEAF ABSOLUTE

CAS n° :

Irritation : Fig leaf absolute applied undiluted to the backs of hairless mice was irritating (Forbes & Davies, 1979). Tested at 5% in petrolatum, it produced one irritant reaction after a 48hr closed-patch test on two different panels of human subjects (Epstein, 1979).
Sensitization : A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 28 volunteers. The material (RIFM no. 78-37) was tested at a concentration of 5% in petrolatum and produced two sensitization reactions (Epstein, 1979). A second maximization test was carried out on another group of 25 volunteers using a new sample of fig leaf absolute. The material (RIFM no. 79-PROD-R) was tested at a concentration of 5% in petrolatum and produced two idiosyncratic reactions (Epstein, 1979).
Phototoxicity : Fig leaf absolute (RIFM 78-37) produced strong phototoxic effects on the hairless mouse even at a 1:8 dilution (Forbes, Urbach & Davies, 1979). The later sample of fig leaf absolute (RIFM 79-PROD-R) at full strength was also strongly phototoxic on the hairless mouse (Forbes & Davies, 1979). A 0.001% concentration still produced phototoxic reactions in three of six mice (Forbes & Davies, 1980), as compared with 0.0025% 8-methoxypsoralen which produced no reactions.
Therapeutic use : The leaves, bark and fruits of the fig plant, Ficus carica, have been used in Oriental folk medicine for the treatment of haemorrhoids, swelling and parasitism (Shibata, Isomura, Inoue & Nagai, 1976).
Pharmacology : Several fractions were prepared by extraction of the leaves of Ficus carica with n-hexane, alcohols and water. Anti-oedematous effects were observed following oral administration (2g/kg) of an n-butanol extract to rats with carrageenan-induced oedema. An ethanol extract and a hexane-soluble, ether-insoluble residue had delayed anti-oedematous effects, while an aqueous extract had no effect. Oral administration (2 g/kg) of the ethanol extract to mice increased the pain threshold in a tail-pressure test but did not show any analgesic effect on acetic acid-induced tail writhing. Lactic acid-induced anal ulceration in rats was decreased following oral administration of the ethanol extract in a dose of 2 g/kg/day for 2 days (Shibata et al. 1976).
Photosensitization : Although no references to leaf extracts have been found, it should be pointed out that fig leaves contain furocournarins (see Additional references : Composition) and that fresh fig fruit has been mentioned as a photosensitizing agent (Brun, 1976).

References : Monographs on Fragrance Raw Materials, Food and Cosmetics Toxicology, Volume 20 Supplement, November 1982, p. 691

Entry n° 18 : trans-2-HEPTENAL

Synonyms : 3-Butylacrolein; β-butylacrolein; (E)-2-hepten-l-al.
CAS n° : 18829-55-5
Structure : CH_3-$(CH_2)_3$-CH=CH-CHO

Acute toxicity : The acute oral LD50 in rats was reported as 1.30 g/kg (1.06-1.60 g/kg) (Moreno, 1980) and as 1.3 g/kg (0.85-2.0 g/kg) (Moreno, 1982). The acute dermal LD50 in guinea pigs has been reported as 1.53 g/kg (1.02-2.28 g/kg) (Moreno, 1980). The acute dermal LD50 in rabbits has been reported as 0.86 g/kg (0.41-1.80 g/kg) (Moreno, 1982).
Irritation : As part of an acute dermal LD50 study, the undiluted material produced irritant effects and skin abnormalities at necropsy in rabbits patch-tested for 24 hr under occlusion at doses of 0.313-5.0 g/kg (Moreno, 1980 & 1982). Similarly, in an acute dermal LD50 study on guinea pigs, doses of 1.25-5.0 g/kg produced irritant effects and skin abnormalities at necropsy (Moreno, 1980).

A 48hr closed-patch test at a concentration of 4% in petrolatum on the backs of 29 (Epstein, 1980) or 27 (Epstein, 1985) volunteers produced 2/29 irritant reactions and 0/27 reactions, respectively.
Sensitization : Maximization tests (Kligman, 1966; Kligman & Epstein, 1975) were carried out on 56 volunteers in two separate panels. The material was tested at a concentration of 4% in petrolatum, and 1/29 (Epstein, 1980) and 8/27 (Epstein, 1985) sensitization reactions were produced. This test concentration was based on a reported maximum concentration of 0.4% in consumer products.
Mutagenesis studies : 2-Heptenal (isomer not specified) was tested in an Ames test (Ames et al. 1975) using Salmonella typhimurium strains TA104 and TA102 and a liquid pre-incubation procedure. No mutagenic effects were produced (Marnett et al. 1985).

References : Monographs on Fragrance Raw Materials, Food and Chemical Toxicology, Volume 26, n° 4, 1988, p. 331

Entry n° 19 : trans-2-HEXENAL DIETHYL ACETAL

Synonym : 1,1-Diethoxy-trans-2-hexene.
CAS n° : 67746-30-9
Structure : CH_3-CH_2-CH_2-CH=CH-CH(OCH_2CH_3)$_2$

Acute toxicity : The acute oral LD50 in rats has been reported as 0.86 g/kg (0.49-1.51 g/kg) (Moreno, 1977) and as 1.71 g/kg (1.12-2.62 g/kg) (Moreno, 1980). The acute dermal LD50 in guinea pigs exceeded 5 g/kg based on 0/4 deaths at that dose (Moreno, 1980). The acute dermal LD50 in rabbits has been reported as approximately 5 g/kg with 2/4 deaths at that dose (Moreno, 1980) and exceeded 5 g/kg based on 0/8 deaths (Moreno, 1977).
Irritation : As part of an acute dermal LD50 study, the undiluted material produced irritant effects (Moreno, 1977 & 1980) with skin and subcutaneous tissue abnormalities at necropsy (Moreno, 1980) in rabbits patch-tested for 24 hr under occlusion at a dose of 5 g/kg. Similarly, in an acute dermal LD50 study on guinea pigs, doses of 2.5 and 5.0 g/kg produced irritant effects (Moreno, 1980). As part of an irritation screen for the Closed Epicutancous Test (CET), application in Finn Chambers to the shaved flank of guinea pigs for 48 hr caused irritation at a concentration of 10% in petrolatum. No irritation was observed at 1 and 3% (Buehler et al. 1985b). Guinea pigs were patch-tested on the shaved flank for 6 hr under occlusion in an irritation screen for the Buehler delayed hypersensitivity test (Buehler, 1965; Ritz & Buehler, 1980). Irritation with erythema was observed at 100%, at 1.0-50% in 80% ethanol-20% distilled water and at 25-50% in diethyl phthalate. No irritation was observed at 0.5% in 80% ethanol-20% distilled water or at 0.25-10% in diethyl phthalate (Buehler et al. 1986). In an irritation screen prior to a maximization test, guinea pigs were given intradermal injections on the shaved flank at doses of 0.625-100% in physiological saline. Irritation was produced at all dose levels (Buehler et al. 1985a).

A 48hr closed-patch test at a concentration of 8% in petrolatum on the backs of 30 (Epstein, 1976), 26 (Epstein, 1980) or 25 (Epstein, 1985) volunteers produced 5/30, 0/26 and 0/25 irritation reactions, respectively. A concentration of 4% in petrolatum produced 1/30 irritation reactions (Epstein, 1977).
Sensitization : When tested on guinea pigs in a delayed hypersensitivity test (Buehler, 1965; Ritz & Buehler, 1980) with induction at 2.5% in ethanol-water and challenge in diethyl phthalate, no reactions were produced at 1%, but 3 and 10% produced 1/0 and 3/19 sensitization reactions, respectively (Buehler et al. 1986). A Closed Epicutaneous Test (CET) in guinea pigs (Ishihara et al. 1985) with induction at 3% in petrolatum and challenge concentrations in petrolatum of 3% (the maximum non-irritating concentration), 1 and 0.3% elicited 8/20 sensitization reactions at 3%, 3/20 at 1%, and 1/20 at 0.3% (Buehler et al. 1985b). A guinea pig maximization test (Magnusson & Kligman, 1969) with induction via intradermal injection and occluded patch at 10%, and a topical occluded challenge in petrolatum, produced 1/20, 2/20, and 1/20 sensitization reactions at 3, 0.9 and 0.3%, respectively (Buehler et al. 1985a).

Three maximization tests (Kligman, 1966; Kligman & Epstein, 1975) were carried out on a total of 81 volunteers. The material was tested at a concentration of 8% in petrolatum, and 1/30 (Epstein, 1976), 7/26 (Epstein, 1980) and 16/25 (Epstein, 1985) sensitization reactions were produced. This test concentration was based on a reported maximum concentration of 0.8% in consumer products. In another maximization test, a concentration of 4% in petrolatum on 30 volunteers produced no sensitization reactions (Epstein, 1977).

References : Monographs on Fragrance Raw Materials, Food and Chemical Toxicology, Volume 26, n° 4, 1988, p. 345

Entry n° 20 : trans-2-HEXENAL DIMETHYL ACETAL

Synonym : 1,1-Dimethoxy-trans-2-hexene
CAS n° : 18318-83-7
Structure : CH_3-CH_2-CH_2-CH=CH-CH$(OCH_3)_2$

Acute toxicity : The acute oral LD50 in rats was reported as 1.70 g/kg (1.36-2.12 g/kg) (Moreno, 1980). The acute dermal LD50 in guinea pigs exceeded 5 g/kg based on 0/4 deaths at that dose (Moreno, 1980). In rabbits, 5 g/kg applied to intact and abraded skin produced 2/2 deaths (Moreno, 1980).

Irritation : As part of an acute dermal LD50 study, the undiluted material produced irritant effects with skin abnormalities at necropsy in rabbits patch-tested for 24 hr under occlusion at a dose of 5 g/kg (Moreno, 1980). Similarly, in an acute dermal LD50 study on guinea pigs, doses of 1.25-5.0 g/kg produced irritant effects (Moreno, 1980).

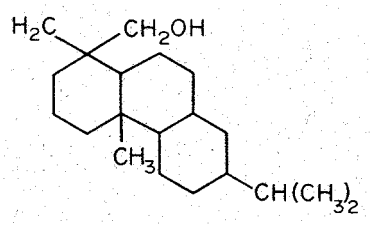
A 48hr closed-patch test at a concentration of 8% in petrolatum on the backs of 26 (Epstein, 1980) or 22 (Epstein, 1985) volunteers produced no irritation.

Sensitization : Two maximization tests (Kligman, 1966; Kligman & Epstein, 1975) were carried out on a total of 48 volunteers. The material was tested at a concentration of 8% in petrolatum, and 1/26 (Epstein, 1980) and 11/22 (Epstein, 1985) sensitization reactions were produced. This test concentration was based on a reported maximum concentration of 0.8% in consumer products.

References : Monographs on Fragrance Raw Materials, Food and Chemical Toxicology, Volume 26, n° 4, 1988, p. 347

Entry n° 21 : HYDROABIETYL ALCOHOL

CAS n° : 13393-93-6
Structure : Main component



Acute toxicity : Both the acute oral LD50 value in rats and the acute dermal LD50 value in rabbits exceeded 5 g/kg (Shelanski, 1972).
Irritation : Hydroabietyl alcohol applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was not irritating (Shelanski, 1972). Tested at 10% in petrolatum, it produced no irritation after a 48hr closed-patch test on human subjects (Kligman, 1972).
Sensitization : A maximization test (Kligman, 1966) was carried out on 25 volunteers. The material was tested at a concentration of 10% in petrolatum and produced sensitization in three of those tested (Kligman, 1972).

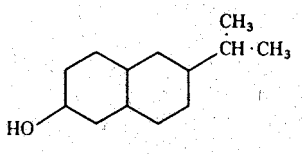
References : Monographs on Fragrance Raw Materials, Food and Cosmetics Toxicology, Volume 12, 1974, p. 919

Entry n° 23 : 6-ISOPROPYL-2-DECAHYDRONAPHTHALENOL

Synonyms : Decahydro-6-isopropyl-2-naphthol; 6-isopropyidecalol
decahydro-6-(1-methylethyl)-2-naphthalenol; decatol

CAS n° : 34131-99-2

Structure :



Acute toxicity : The acute oral LD50 in rats has been reported (Givaudan Corporation, 1973) as 4.2 ml/kg (3.75-4.70 ml/kg), and as approximately 5 g/kg with 5/10 deaths at that dose (Moreno, 1978). The acute dermal LD50 in rabbits has been reported (Moreno, 1978) as 3.5 g/kg (2.0-6.2 g/kg).

Irritation : No irritation was produced in three rabbits that received 0.1 ml of a 0.5% solution of 6-isopropyl-2-decahydronaphthalenol in propylene glycol applied to the surface of the eye (Givaudan Corporation, 1973). As part of an acute dermal LD50 study, the undiluted material produced irritant effects with moderate oedema and redness in rabbits patch-tested for 24 hr under occlusion at doses of 1.25 to 5.0 g/kg (Moreno, 1978).

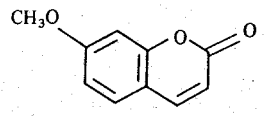
A closed-patch test at a concentration of 2% in dimethyl phthalate on ten volunteers produced no irritation (Givaudan Corporation, 1973). A 48hr closed-patch test at a concentration of 10% in petrolatum on the backs of 33 volunteers (Epstein, 1978), or at a concentration of 4% in petrolatum on the forearms of 25 volunteers (Kligman, 1979) produced no irritation.

Sensitization : When the material was tested in a human repeated insult patch test at a concentration of 2% in dimethyl phthalate (10 or 11 closed-patch induction applications followed 11 days later by a closed challenge patch) no reactions were produced in 54 volunteers (Givaudan Corporation, 1973). A maximization test (Kligman, 1966; Kligman & Epstein, 1975) carried out at a concentration of 4% in petrolatum produced 3/25 sensitization reactions (Kligman, 1979), while at a concentration of 10% it produced 4/33 reactions (Epstein, 1978).

References : Monographs on Fragrance Raw Materials, Food and Chemical Toxicology, Volume 26, n° 4, 1988, p. 367

Entry n° 24 : 7-METHOXYCOUMARIN

Synonym : Herniarin
CAS n° : 531-59-9
Structure :



Acute toxicity : The acute oral LD50 in rats was reported as approximately 4.3 g/kg (Moreno, 1979). The acute dermal LD50 in guinea pigs exceeded 5.0 g/kg with 0/4 deaths at that dose (Moreno, 1979). Dogs injected iv with 10 mg 7-methoxycoumarin/kg (solvent unknown) had a 13.04 + 7% fall in blood pressure within a 5-min duration (Thakur et al. 1978).
Irritation : As part of an acute dermal LD50 study, the undiluted material produced irritant effects in guinea pigs patch-tested for 24 hr under occlusion at a dose of 5.0 g/kg (Moreno, 1979).

A 48hr closed-patch test at a concentration of 8% in petrolatum on the backs of 27 volunteers produced no irritation (Epstein, 1978).
Sensitization : Guinea pigs were tested in an open epicutaneous test. A total of 21 daily induction applications of 5.7% in acetone/ethanol (4:1) followed 11 days later by an open challenge application produced no effects (Hausen & Schmieder, 1986). Guinea pigs were also tested in a Freund's Complete Adjuvant Test. Induction injections with 7-methoxycoumarin and Freund's Complete Adjuvant were made on 6 days with 2-day intervals; open epicutaneous challenge was at a concentration of 1 %, and no sensitization effects were produced (Hausen & Schmieder, 1986).

A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 27 volunteers. The material was tested at a concentration of 8% in petrolatum and produced two sensitization reactions (Epstein, 1978). This test concentration was based on a reported maximum concentration of 0.8% in consumer products.
Phototoxicity : The photoreactivity of 7-methoxycoumarin with calf-thymus DNA and yeast-ribosomal RNA was studied. Slight but well-defined photoreactivity was observed (Marciani et al. 1971).

7-Methoxycoumarin tested at a concentration of 5% on humans produced 10/10 phototoxic reactions after irradiation with 20 J/cm^2 of UVA (Kaidbey, 1979). Human subjects were patch-tested with 7-methoxycoumarin at concentrations of 5, 1 and 0.1 % for 24 hr under occlusion and were then irradiated with 15 J/cm^2 of UVA. Phototoxic reactions were observed at a concentration of 5% (10/10) only. After a 2-4 hr occlusion and irradiation with 15 J/ cm^2, 10/10 phototoxic reactions were observed at a concentration of 5% and 8/10 were observed at 1%. No reactions were observed at a concentration of 0.1% (Kaidbey, 1979). A 6hr closed-patch test at a concentration of 5% (in hydrophilic ointment), followed by 20 J/cm^2 of UVA exposure, produced 10/10 phototoxic reactions in humans (Kaidbey & Kligman, 1980 & 1981).
Photosensitization : A photomaximization. test (Kaidbey & Kligman, 1980) was carried out on 25 volunteers. The material was tested at a concentration of 5% in hydrophilic ointment and produced eleven photosensitization reactions (Kaidbey & Kligman, 1981; Kligman & Kaidbey, 1979). Ethanolic solutions of 7-Methoxycoumarin were tested in a UVA dose response study on subjects who were previously photosensitized to it. Photosensitization reactions were produced

by 0.001 % 7-Methoxycoumarin at 20 J/cm^2, 0.01 % at 10-20 J/cm^2, 0.1% at 10-15 J/cm^2, and 1.0 % at 0.1-0.4 J/cm^2 (Kaidbey, 1979; Kaidbey & Kligman, 1981). Human subjects who were previously photosensitized to 6-methylcoumarin by the photomaximization procedure (Kaidbey & Kligman, 1980) were patch-tested with 7-methoxycoumarin at a concentration of 1 % in hydrophilic ointment. Cross reactions occurred in 3/4 subjects (Kaidbey, 1978; Kaidbey & Kligman, 1981). Cross reactivity was studied in six human subjects photosensitized to 7-methoxycoumarin by the photomaximization procedure (Kaidbey & Kligman, 1980). At a concentration of 1% in hydrophilic ointment, two subjects cross reacted to coumarin, three cross reacted to 6-Methylcoumarin, and five cross reacted to 7-Methylcoumarin. No cross reactions occurred with 4,6-Dimethyl-8-tert-butylcoumarin, Octahydrocoumarin or dicumarol (Kaidbey, 1979; Kaidbey & Kligman, 1981).

Mutagenesis studies : 7-Methoxycoumarin (5 µmol/plate) was tested in an Ames test (Ames et al. 1975) using Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 with and without S-9 activation.
It was mutagenic in strain TA 100 with metabolic activation (Stoltz & Scott, 1980).
7-Methoxycoumarin in dimethylsulphoxide was tested in a modified (pre-incubation with S-9 and bacteria) Ames test using Salmonella typhimurium strains TA100 and TA98 with and without S-9 activation. No mutagenic effects were produced at concentrations up to 300 µg 7-methoxycoumarin/plate (Nagao et al. 1981).
Review : A review on the genetic effects of coumarins (Grigg, 1978) has been published.

References : Monographs on Fragrance Raw Materials, Food and Chemical Toxicology, Volume 26, n° 4, 1988, p. 375

Entry n° 26 : ANISYLIDENE ACETONE

Synonyms : Methyl-p-methoxycinnamylketone; 4-(p-Methoxyphenyl)-3-buten-2-one.
CAS n° : 943-88-4
Structure : CH_3-O-C_6H_4-CH=CH-CO-CH_3

Acute toxicity : Both the acute oral LD50 value in rats and the acute dermal LD50 value in rabbits exceeded 5g/kg (Wohl, 1974).
Irritation : Anisylidene acetone applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was not irritating (Wohl, 1974). Tested at a concentration of 2% in petrolatum, it produced no irritation after a 48hr closed-patch test on human subjects (Epstein, 1974).
Sensitization : A maximization test (Kligman, 1966, modified) was carried out on 22 volunteers. The material was tested at a concentration of 2% in petrolatum and produced two sensitization reactions out on the 22 tested (Epstein, 1974).
Antitumour activity : In vitro studies indicated weak anti-tumour activity for anisylidene acetone (Doré, 1973).
Metabolism : When the side chain of a mixed ketone contains a double bond, both the keto group and the double bond are potentially reducible in vivo. In a related material, methyl styryl ketone (C_6H_5-CH=CH-CO-CH_3), the keto group appears to be more readily reduced than the double bond, with reduction via C_6H_5-CH=CH-CH(OH)-CH_3 to the completely reduced carbinol compound C_6H_5-$(CH_2)_2$-CH(OH)-CH_3, which is found as the main product (Fischer & Bielig, 1940). The ether link is relatively stable in substituted anisoles, such as anethole (p-CH_3-OC_6H_4-CH=CH-CH_3), containing a potential carboxyl group attached to the aromatic ring (Williams, 1959).

References : Monographs on Fragrance Raw Materials, Food and Cosmetics Toxicology, Volume 13, 1975, p. 456

Entry n° 27 : α-METHYLANISALACETONE

Synonym : 1-(4-Methoxyphenyl)-l-penten-3-one
CAS n° : 104-27-8
Structure : $H_3CO-C_6H_4-CH=CH-CO-CH_2-CH_3$

Acute toxicity : Both the acute oral LD50 in rats and the acute dermal LD50 in rabbits exceeded 5 g/kg (Moreno, 1977).
Subacute toxicity : A short-term feeding study was carried out in male and female rats in which α-methylanisalacetone in cottonseed oil was added to the diet to provide an expected dose of 13.5 mg/kg/day, a level estimated to be more than 100-fold greater than the daily dietary intake by man. Male and female rats actually received 12.7 and 15.2 mg/kg/day, respectively. There were no significant differences between treated and control animals with respect to body-weight gain, efficiency of food utilization, or blood chemistry and no significant gross pathological changes were observed at autopsy (Oser, Carson & Oser, 1965).
Irritation : α-Methylanisalacetone applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was mildly irritating (Moreno, 1977). Tested at 8% in petrolatum, it was moderately irritating to human subjects after a 48hr closed-patch test (Epstein, 1977). Even after the concentration was halved (4%) it still produced irritation in five subjects (Epstein, 1977).
Sensitization : A maximization test (Kligman. 1966; Kligman & Epstein, 1975) was carried out on 24 volunteers. The material (RIFM no. 77-154) was tested at a concentration of 8% in petrolatum and produced 16 sensitization reactions (Epstein, 1977).

References : Monographs on Fragrance Raw Materials, Food and Cosmetics Toxicology, Volume 17 Supplement, December 1979, p. 863

Entry n° 28 : METHYL CROTONATE

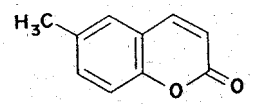
Synonyms : Methyl α-crotonate; methyl trans-2-butenoate
CAS n° : 623-43-8
Structure : CH_3-OOC-CH=CH-CH_3

Acute toxicity : The acute oral LD50 values in rats and mice were reported as >3.2 g/kg and 1.6-3.2 g/kg, respectively (Fassett, 1963). The acute dermal LD50 was reported as 10-20 ml/kg in guinea pigs (Fassett, 1963) and as > 5 g/kg in rabbits (Moreno, 1976).
Inhalation : All rats exposed to 19 000 ppm methyl crotonate for 6 hr survived (Fassett, 1963).
Irritation : Methyl crotonate was reported to be moderately irritating to rabbit skin and slightly irritating to the rabbit eye (Fassett, 1963). Applied full strength to intact or abraded rabbit skin for 24 hr under occlusion, the ester was moderately irritating (Moreno, 1976). Tested at 6% in petrolatum, it produced no irritation after a 48hr closed-patch test on human subjects (Kligman, 1976).
Sensitization : A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers. The material (RIFM no. 76-197) was tested at a concentration of 6% in petrolatum and produced sensitization reactions in two of the test subjects (Kligman, 1976).
Nutrition : Chicks were able to utilize methyl crotonate as an energy source when it was added to their diet at the 5% level (Yoshida, Morimoto & Oda, 1970).

References : Monographs on Fragrance Raw Materials, Food and Cosmetics Toxicology, Volume 17 Supplement, December 1979, p. 865

Entry n° 29 : 6-METHYLCOUMARIN

Synonyms : 6-Methyl-1,2-benzopyrone
CAS n° : 92-48-8
Structure :



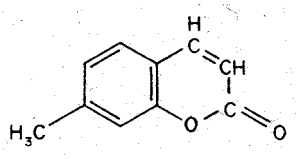
Acute toxicity : The acute oral LD50 value in rats was reported to be 1.68 g/kg (1.43-1.93 g/kg) (Moreno, 1973). The acute dermal LD50 value in rabbits exceeded 5 g/kg (Moreno, 1973).
Subacute and long-term toxicity : In a 2-yr feeding study, five groups of rats, each containing 25 males and 25 females were given 6-methylcoumarin at levels of 500, 1000, 3500, 7500 and 15000 ppm in the diet. No effects were seen at the 500, 1000, and 3500 ppm levels, but at 7500 ppm growth depression was observed in males and at 15000 ppm growth depression in both males and females, microscopic liver changes and moderate atrophy were observed (Hagan, Hansen, Fitzhugh, Jenner, Jones, Taylor, Long, Nelson & Brouwer, 1967). In another feeding study involving groups of ten male and ten female rats, neither 1000 nor 10000 ppm fed in the diet for 14 wk had any effects (Hagan et al. 1967).

A male dog fed 150 mg methylcoumarin/kg had to be killed after 39 days because of weakness, emaciation and dehydration, and showed moderate to severe hepatitis and slight to moderate muscle atrophy (Hagan et al. 1967). In a 2-yr feeding study involving one male and one female dog given 50 mg/kg/day, no effects were produced (Hagan et al. 1967).
Irritation : 6-Methylcoumarin applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was mildly irritating (Moreno, 1973). Tested at 4% in petrolatum, it produced no irritation after a 48hr closed-patch test on human subjects (Kligman, 1973).
Sensitization : A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 human volunteers. The material was tested at a concentration of 4% in petrolatum and produced no sensitization reactions (Kligman, 1973).

References : Monographs on Fragrance Raw Materials, Food and Cosmetics Toxicology, Volume 14, 1976, p. 605

Entry n° 30 : 7-METHYLCOUMARIN

Synonym : 7-Methyl-2H-1-benzopyran-2-one
CAS n° : 2445-83-2
Structure :



Acute toxicity : The acute oral LD50 in rats was reported as 3.8 g/kg (2.9-5.0 g/kg) and the acute dermal LD50 in rabbits exceeded 5 g/kg (Moreno, 1979).
Irritation : 7-Methylcoumarin applied full strength to intact or abraded rabbit skin for 24hr under occlusion was slightly irritating (Moreno, 1979). Tested at 8% in petrolatum, it produced no irritation after a 48hr closed-patch test on human Subjects (Kligman, 1978).
Sensitization : A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers. The material (RIFM no. 78-85) was tested at a concentration of 8% in petrolatum and did not produce any sensitization reactions (Kligman, 1978).
Phototoxicity : 7-Methylcoumarin at a concentration of 5% in hydrophilic ointment did not produce any phototoxic effects on human subjects (Kaidbey, 1979).
Photoallergenicity : 7-Methylcoumarin produced photoallergenic effects on six out of 25 human subjects when tested at a concentration of 1% in hydrophilic ointment by the photomaximization test (Kaidbey, 1978). Five subjects who were photoallergic to 7-Methoxycoumarin (Kaidbey, 1979) and two who were photoallergic to 6-Methylcoumarin (Kaidbey, 1978) cross-reacted to 7-Methylcoumarin.

References : Monographs on Fragrance Raw Materials, Food and Cosmetics Toxicology, Volume 20 Supplement, November 1982, p. 747

Entry n° 31 : ACETYL ISOVALERYL

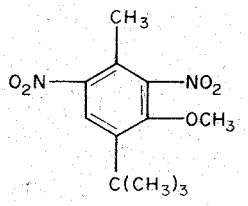
Synomyns : 5-Methyl-2,3-hexanedione, acetylisopentanoyl.
CAS n° : 13706-86-0
Structure : CH_3-CO-CO-CH_2-CH(CH_3)-CH_3

Acute toxicity : Both the acute oral LD50 value in rats and the acute dermal LD50 value in rabbits exceeded 5 g/kg (Moreno, 1979).
Irritation : Acetyl isovaleryl applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was slightly to moderately irritating (Moreno, 1979). Tested at 5% in petrolatum, it produced no irritation after a 48hr closed-patch test on two different panels of human subjects (Epstein, 1979; Kligman, 1978).
Sensitization : A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers. The material (RIFM no. 78-92) was tested at a concentration of 5% in petrolatum and produced two sensitization reactions (Kligman, 1978). The maximization test was repeated in 29 different volunteers using a new sample of acetyl isovaleryl. This material (RIFM no. 79-5943) was also tested at a concentration of 5% in petrolatum and produced one sensitization reaction (Epstein, 1979).

References : Monographs on Fragrance Raw Materials, Food and and Cosmetics Toxicology, Volume 20 Supplement, November 1982, p. 637

Entry n° 32 : MUSK AMBRETTE

Synonym : 2,6-Dinitro -3-methoxy-4-tert-butyltoluene
CAS n° : 83-66-9
Structure :

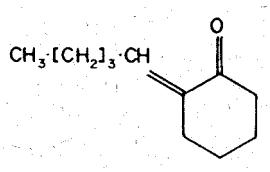


Acute toxicity : The acute oral LD50 in rats has been reported as 339 mg/kg (Jenner, Hagan, Taylor, Cook & Fitzhugh, 1964) and as 4.8 g/kg (4.3-5.3 g/kg) (Moreno, 1972a). The acute dermal LD50 in rabbits exceeded 2 g/kg (Moreno, 1972b).
Sub-acute toxicity : In a 12-wk feeding study in rats the no-effect level was 0.76 mg/kg (Bär & Griepentrog, 1967). In another feeding study, diets containing 500, 1500, 2500 or 4000 ppm were fed to male rats for 50 wk and to female rats for 20 wk (Davis, Taylor, Jones & Brouwer, 1967). All but the lowest dose level caused marked loss in weight, progressive weakness of the hind quarters, leading to complete loss of the use of the legs after 10-40 wk as a result of histologically confirmed muscular atrophy, and blood changes, including a decrease in erythrocyte count and clotting time and an icteric plasma (which was also observed at 500 ppm). The toxic effects observed in this study occurred at dietary levels 2500 times greater than those likely to be encountered in every-day human consumption.
Irritation : Musk ambrette applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was moderately irritating (Moreno, 1972b). Tested at 20% in petrolatum, it produced no irritation after a 48hr closed-patch test on human subjects (Kligman, 1972).
Sensitization : A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers. The material was tested at a concentration of 20% in petrolatum and produced no sensitization reactions (Kligman, 1972).

References : Monographs on Fragrance Raw Materials, Food and and Cosmetics Toxicology, Volume 13 Supplement, December 1975, p. 875

Entry n° 33 : 2-PENTYLIDENECYCLOHEXANONE

CAS n° : 25677-40-1
Structure :



Acute toxicity : The acute oral LD50 in rats has been reported as approximately 5 g/kg and the acute dermal LD50 in rabbits as 3.5 g/kg (2.1- 5.9 g/kg) (Moreno, 1978).
Irritation : Applied full strength to intact or abraded rabbit skin for 24 hr under occlusion, 2-pentylidenecyclohexanone was moderately to severely irritating (Moreno, 1978). Tested at 10% in petrolatum, it produced no irritation after a 48hr closed-patch test on human subjects (Epstein, 1978).
Sensitization : A maximization test (Kligman, 1966; Kligman & Epstein, 1975) was carried out on 25 volunteers. The material (RIFM no. 78-79) was tested at a concentration of 10% in petrolatum and produced 21 sensitization reactions (Epstein, 1978).

References : Monographs on Fragrance Raw Materials, Food and and Cosmetics Toxicology, Volume 20 Supplement, November 1982, p. 797

Entry n° 34 : BENZY1LIDENE ACETONE

Synonyms : 4-Phenyl-3-buten-2-one; benzalacetone
CAS n° : 122-57-6
Structure : C_6H_5–CH=CH-CO-CH_3

Acute toxicity : The acute oral LD50 was reported as > 5 g/kg in the -rat (Levenstein, 1972a). The acute dermal LD50 was reported as > 3 g/kg in the rabbit (Levenstein, 1972b).
Irritation : Benzylidene acetone applied full strength to intact or abraded rabbit skin was mildly irritating (Levenstein, 1972b). Tested at a 2 % concentration in petrolatum it produced no irritation in a 48hr closed-patch test in 25 human subjects (Kligman, 1972).
Sensitization : A maximization test (Kligman, 1966) was carried out on 25 volunteers. The material was tested at a 2% concentration in petrolatum and produced sensitization reactions in 12 out of 25 subjects (Kligman, 1972).
Additional published data : Benzylidene acetone is listed as a strongly irritant perfume material (Thomssen, 1947), and has been cited many times in the literature as a skin irritant.

References : Monographs on Fragrance Raw Materials, Food and and Cosmetics Toxicology, Volume 11, 1973, p. 1021

Entry n° 35 : 3,6,10-TRIMETHYL-3,5,9-UNDECATRIEN-2-ONE

Synonyms : Methylisopseudoionone; 2,6,9-trimethylundeca-2,6,8-trien-10-one; pseudo-α-isomethylionone
CAS n° : 1117-41-5
Structure : CH_3-CO-C(CH_3)=CH-CH=C(CH_3)-CH_2-CH_2-CH=C(CH_3)-CH_3 (a mixture of isomers)

Acute toxicity : The acute oral LD50 in rats exceeded 5 g/kg based on 1/10 deaths at that dose and the acute dermal LD50 in rabbits exceeded 2.5 g/kg based on 0/4 deaths (Moreno, 1977).
Irritation : As part of an acute dermal LD50 study, the undiluted material produced moderate to severe irritant effects with hardened and thickened skin at necropsy in rabbits patch-tested for 24 hr under occlusion at doses of 2.5 and 5.0 g/kg (Moreno, 1977).

A 48hr closed-patch test at a concentration of 8% in petrolatum on the backs of 25 (Kligman, 1977) or 30 (Epstein, 1978) volunteers produced no irritation.
Sensitization : Two maximization tests (Kligman, 1966; Kligman & Epstein, 1975) were carried out on a total of 55 volunteers. The material was tested at a concentration of 8% in petrolatum and sensitization reactions were produced in 6/25 (Kligman, 1977) and 0/30 (Epstein, 1978) volunteers. This test concentration was based on a reported maximum concentration of 0.8% in consumer products.

References : Monographs on Fragrance Raw Materials, Food and Chemical Toxicology, Volume 26, n° 4, 1988, p. 413

Entry n° 36 : VERBENA OIL

Synonyms : Lippia citriodora oils
CAS n° : 8024-12-2

Acute toxicity : The oral LD50 in rats of two different samples of verbena oil both exceeded 5 g/kg based on 0/10 and 1/10 deaths (Moreno, 1976, 1977). The dermal LD50 in guinea pigs exceeded 5 g/kg based on 0/10 deaths (Moreno, 1976). In a dermal LD50 on rabbits, a dose of 1.25 g/kg produced 0/2 deaths, while 5 g/kg produced 2/2 deaths (Moreno, 1976); however, Moreno (1977) reported that the dermal LD50 in rabbits exceeded 5 g/kg based on 0/5 deaths at that dose.
Irritation : As part of a dermal LD50 study, doses of 1.25 or 5.0 g/kg of the undiluted material produced moderate to marked oedema and erythema on rabbits after an occluded application for 24 hr (Moreno, 1976 and 1977). Similarly, a dose of 5.0 g/kg produced slight to moderate oedema and erythema on guinea pigs (Moreno, 1976).
A 48hr closed patch test with 12% in petrolatum using six different samples of verbena oil on the backs or forearms of a total of 159 volunteers produced two irritation reactions (Epstein, 1976, 1978 and 1980; Kligman, 1976, 1977 and 1979).
Sensitization : Maximization tests (Kligman, 1966; Kligman and Epstein, 1975) were carried out at 12% in petrolatum on numerous panels (averaging 25 human subjects each) using different samples of verbena oil prepared by various methods : sample no. 75-141NU (verbena boufarik) produced 0/30 sensitization reactions and one irritant reaction (Epstein, 1976); sample no. 75-141 (verbena oil, French) produced 13/25 sensitization reactions (Kligman, 1976); sample no. 76-313RBD (verbena oil, pure) produced 18/25 sensitization reactions (Kligman, 1977); sample no. 77-205RD (verbena oil, pure) produced 2/28 sensitization reactions and one irritant reaction (Epstein, 1978); sample no. 78-12-ROB (verbena de Grasse, pure) produced 15/25 sensitization reactions (Kligman, 1979); And sample no. 80-29 (Verbena oil, maroc) produced 4/26 sensitization reactions (Epstein, 1980). This test concentration was based on a reported maximum concentration of 1.2% in consumer products.
Phototoxicity : 'A commercially available plant extract' of verbena was tested in an in vitro yeast test. The material was applied to plates seeded with Candida albicans and grown under blacklight fluorescent lamps. No phototoxic effects were produced (vehicle and concentration not specified) (Daniels, 1965). In an in vitro phototoxicity assay with Saccharomyces cerevisiae, the undiluted material produced positive effects (Forbes et al., 1978); 5% in methanol produced no effects (Tenenbaum et al., 1984).

Six different samples of verbena oil were tested for phototoxic effects; no phototoxic effects were produced when three different samples of verbena oil (samples no. 75-141NU, no. 76-313RBD, no. 77-205RD) were applied to the skin of hairless mice or miniature swine followed by UVA irradiation from fluorescent blacklight or xenon lamp (Forbes et al., 1977 and 1978); undiluted verbena oil, French (sample no. 75-141) applied to the skin of hairless mice or miniature swine followed by UVA irradiation from fluorescent blacklight or xenon lamp produced phototoxic effects (Forbes et al., 1977a); verbena oil (sample no. 78-12-ROB) applied undiluted to the skin of hairless mice followed by UVA irradiation from fluorescent blacklight or solar simulators was also phototoxic, at 12.5% in methanol it produced no phototoxic effects (Forbes and Davies, 1979); verbena oil, maroc (sample no. 80-29) applied undiluted to the skin of hairless mice followed by UVA irradiation from fluorescent blacklight or xenon lamp produced irritation and phototoxicity (Forbes and Davies, 1980); 50% in methanol was irritating but not phototoxic.

References : Monographs on Fragrance Raw Materials, Food and Chemical Toxicology, Volume 30 Supplement, 1992, p. 137S

Entry n° 36 : VERBENA ABSOLUTE

CAS n° : : 8024-12-2

Acute toxicity : The oral LD50 in rats exceeded 5 g/kg based on 0/10 deaths at dose, and the dermal LD50 in rabbits exceeded 5 g/kg based on 0/ 10 deaths (Moreno, 1980).

Irritation : As part of a dermal LD50 study, 5 g/kg of the undiluted material produced slight to moderate irritation on rabbits after an occluded application for 24 hr (Moreno, 1980).

A 48hr closed patch test with 12% in petrolatum on the backs of 52 volunteers (Epstein, 1979 and 1980), or 2% in petrolatum on the backs of 27 volunteers (Epstein, 1987), produced no irritation.

A mixture of verbena absolute-d-limonene (80:20) tested at a concentration of 15% in petrolatum in a 48hr closed patch test on the backs of 28 volunteers produced no irritation (Epstein, 1986).

Sensitization : Three maximization tests (Kligman, 1966; Kligman and Epstein, 1975) were carried out on 79 volunteers; sample no. 79-9188 at 12% in petrolatum produced 2/26 sensitization reactions (Epstein, 1979); sample no. 80-28 at 12% in petrolatum produced one irritation reaction in 26 subjects (Epstein, 1980); sample no. 1616-2 at 2% in petrolatum produced no sensitization reactions in 27 subjects (Epstein, 1987).

A mixture of verbena absolute-d-limonene (80:20) was tested in a maximization test (Kligman, 1966; Kligman and Epstein, 1975) at a concentration of 15% in petrolatum on 28 volunteers. One sensitization reaction was produced (Epstein, 1986).

Phototoxicity : Two different samples of verbena absolute applied undiluted to the skin of hairless mice followed by UVA irradiation from fluorescent blacklight or xenon arc solar simulators produced no phototoxic effects (Forbes and Davies, 1980).

References : Monographs on Fragrance Raw Materials, Food and Chemical Toxicology, Volume 30 Supplement, 1992, p. 135S