# Toxicological profile for

## Lemon oils

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

## *1. Name of substance and physico-chemical properties*

### *1.1. IUPAC systematic name*

Not applicable.

### *1.2. Synonyms*

Lemon oil, terpeneless; Cedro oil; Lemon oil, terpeneless, sesquiterpeneless; Oil of petigrain lemon; Oils, lemon, terpene-free; Lemon oil, terpeneless (Citrus limon (L.) Burm. F.); FEMA No. 2626; (ChemIDplus; CoE, 2000)

### *1.3. Molecular formula*

Unspecified (ChemIDplus).

### *1.4. Structural Formula*

Not applicable.

### *1.5. Molecular weight (g/mol)*

Not applicable.

### *1.6. CAS registration number*

68648-39-5

### *1.7. Properties*

#### *1.7.1. Melting point*

(°C): 153 (EPISuite, 2017)

#### *1.7.2. Boiling point*

(°C): 407.16 (estimated) (EPISuite, 2017)

#### *1.7.3. Solubility*

1000 or 383 g/L at 25°C (EPISuite, 2017)

#### *1.7.4. pKa*

No data available to us at this time.

#### *1.7.5. Flashpoint*

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

#### *1.7.6. Flammability limits (vol/vol%)*

No data available to us at this time.

*1.7.7. (Auto)ignition temperature*

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

*1.7.8. Decomposition temperature*

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

*1.7.9. Stability*

No data available to us at this time.

*1.7.10. Vapor pressure*

1.66 E-08 mmHg at 25°C (EPISuite, 2017)

*1.7.11. log Kow*

-1.64 (EPISuite, 2017)

## *2. General information*

### *2.1. Exposure*

| |
|---|
| Cosmetics : Yes (Cosmetics Bench Ref, 1993). |
| Food : Yes (Burdock, 2010). |
| Environment : No evidence. |
| Pharmaceuticals : Yes (Martindale, 1993). |

Lemon oil, terpeneless is found in a variety of foods. Burdock (2010) lists an individual's daily consumption as being 0.1066 mg/kg bw/day. The FEMA PADI (possible average daily intake) is 7.047 mg.

"Lemon oil and terpeneless lemon oil are extensively used as flavor ingredients in most food products, including alcoholic (bitters, vermouths, sweet liqueurs, etc.) and nonalcoholic beverages (soft drinks, drink mixes, etc.), frozen dairy desserts, candy, baked goods, gelatins and puddings, meat and meat products, breakfast cereals, and fats and oils, among others."

As taken from Khan and Abourashed, 2010.

Reported used as a flavouring in:

| Food category | Usual (ppm) | Max (ppm) | Food category | Usual (ppm) | Max (ppm) |
|---|---|---|---|---|---|
| Alcoholic beverages | 15.39 | 51.27 | Frozen dairy | 13.49 | 38.55 |
| Baked foods | 23.05 | 64.51 | Gelatins, puddings | 13.51 | 48.02 |
| Breakfast cereals | 40.0 | 40.0 | Hard candy | 12.65 | 19.25 |
| Chewing gum | 1.53 | 14.85 | Meat products | 3.43 | 5.71 |

| | | | | | |
|---|---|---|---|---|---|
| Condiments, relishes | 9.00 | 10.0 | Nonalcoholic beverages | 12.94 | 14.86 |
| Confection, frosting | 25.0 | 75.0 | Soft candy | 13.65 | 31.90 |
| Fats, oils | 10.0 | 20.0 | | | |

As taken from Burdock, 2010.

| | average usual ppm | average maximum ppm |
|---|---|---|
| beverages: | - | 13 |
| ice cream, ices, etc.: | - | 25 |
| candy: | - | 68 |
| baked goods: | - | 50 |
| gelatins and puddings: | - | 80 |
| chewing gum: | 110 | 670 |
| toppings: | - | 1000 |

As taken from Hall and Oser, 1965.

Oils, lemon, terpene-free (US EPA InertFinder Database) and lemon oil, terpeneless (IFRA) (both CAS RN 68648-39-5) are listed as fragrance ingredients.

### *2.2. Combustion products*

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

| **Ingredient Name & CAS Number** | **Max. cig. appln. level (ppm)** | **Composition of pyrolysate (Compound, %)** | **Max. level in smoke (µg)** |
|---|---|---|---|
| Lemon oil, terpeneless<br>CAS 68648-39-5 | 10 | E-Citral 30.6 Z-Citral 17.1 Geranryl acetate 11.2 Genaryl isobutyrate 7.0 Limonene 0.5 | 2<br>0.9<br>0.6<br>0.4<br>0.3 |

### *2.3. Ingredient(s) from which it originates*

“From the normal essential oil, the terpeneless and sesquiterpeneless oils are obtained by vacuum rectification, washing with dilute alcohol or by column chromatography” (Burdock, 2010).

Source: Citrus limon (L.) Burm. f. (syn. C. limonum Risso) (Family Rutaceae).

As taken from Khan and Abourashed, 2010.

## *3. Status in legislation and other official guidance*

A website reports that the US Government has approved the use of lemon oil and extract as a tobacco additive (Anon, undated).

**ADI**

No ADI identified for lemon oils.

Lemon, oil, terpeneless (Citrus limon (l.) Burm. F.) (CAS RN 68648-39-5) is included on the FDA's list of Substances Added to Food (formerly EAFUS) as a flavoring agent or adjuvant, and is generally recognized as safe (GRAS) under 21 CFR section 182.20 (Essential oils, oleoresins (solvent-free) and natural extractives (including distillates)) (FDA, 2024).

Terpeneless lemon oil (CAS RN 68648-39-5; FEMA no. 2626) has been designated as GRAS (generally recognized as safe) by FEMA (Hall and Oser, 1965).

Oils, lemon, terpene-free (CAS RN 68648-39-5) are not registered under REACH (ECHA).

Oils, lemon, terpene-free (CAS RN 68648-39-5), terpeneless lemon oil, lemon ext. terpeneless, and lemon oil terpeneless and sesquiterpeneless (no CAS RNs given) are not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2025).

d-Limonene is the major constituent (80-90%) of lemon oil (bibra, 1998). An ADI "not specified" was recommended by JECFA at its 41st meeting since it was believed that the total daily intake (from its use in food and background levels in food) would not represent a hazard to health (JECFA, 1993a).

Oils, lemon, terpene-free (CAS RN 68648-39-5) are listed in the US EPA InertFinder Database (2023) as approved for food, nonfood and fragrance use pesticide products.

Citrus oils and other furocoumarins containing essential oils:

Implementation dates: For new submissions*: February 10, 2021

For existing fragrance compounds*: February 10, 2021

*These dates apply to the supply of fragrance mixtures (formulas) only, not to the finished consumer products in the marketplace.

Recommendation: Restriction.

Restriction limits in the finished product (%):

| | | | |
|---|---|---|---|
| Category 1 | 0.0015 % (5-MOP) | Category 7A | No Restriction |
| Category 2 | 0.0015 % (5-MOP) | Category 7B | 0.0015 % (5-MOP) |
| Category 3 | 0.0015 % (5-MOP) | Category 8 | 0.0015 % (5-MOP) |
| Category 4 | 0.0015 % (5-MOP) | Category 9 | No Restriction |
| Category 5A | 0.0015 % (5-MOP) | Category 10A | No Restriction |
| Category 5B | 0.0015 % (5-MOP) | Category 10B | 0.0015 % (5-MOP) |
| Category 5C | 0.0015 % (5-MOP) | Category 11A | No Restriction |
| Category 5D | 0.0015 % (5-MOP) | Category 11B | 0.0015 % (5-MOP) |

| | | | |
|---|---|---|---|
| Category 6 | 0.0015 % (5-MOP) | Category 12 | No Restriction |

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

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

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

The sum of the concentrations of all furocoumarin-containing phototoxic fragrance ingredients (extracts), expressed in % of their recommended upper concentration level in the finished consumer product, shall not exceed 100. Restrictions for furocoumarin-containing fragrance ingredients (extracts) have been recommended for:

• Angelica root oil,

• Bergamot oil expressed,

• Bitter orange oil expressed,

• Cumin oil,

• Grapefruit oil expressed,

• Lemon oil cold pressed,

• Lime oil expressed,

• Rue oil.

The following essential oils contain small amounts of phototoxic furocoumarins (typical levels are provided in brackets):

• Petitgrain Mandarin oil (50 ppm),

• Tangerine oil cold pressed (50 ppm),

• Parsley leaf oil (20 ppm).

These levels are not high enough to require special restrictions if used alone, but if used in combination with one or the other furocoumarin-containing phototoxic fragrance ingredients (extracts), attention should be paid that the total level of Bergapten in the consumer product does not exceed 15 ppm.”

“Flavor Requirements: Due to the possible ingestion of small amounts of fragrance ingredients from their use in products in Categories 1 and 6, materials must not only comply with IFRA Standards but must also be recognized as safe as a flavoring ingredient as defined by the IOFI Code of Practice (www.iofi.org). For more details see chapter 1 of the Guidance for the use of IFRA Standards.”

“Intrinsic property driving risk management: Phototoxicity”

“RIFM summaries: These recommendations are based on the published phototoxic effects of Bergapten and the established dose-effect relationships (Young at al., J. Photochem. Photobiol. B,7, 231 (1990); Dubertret et al.ibid 7, 251 (1990), idem, ibid, 7, 362 (1990).”

“Expert Panel for Fragrance Safety rationale/conclusion: The Expert Panel for Fragrance Safety reviewed all the available data for Citrus oils and other furocoumarins containing essential oils and recommends the limits for the 12 different product categories, which are the acceptable use levels of Citrus oils and other furocoumarins containing essential oils in the various product categories.”

As taken from IFRA, 2020.

Oils, lemon, terpene-free (CAS RN 68648-39-5) are listed in the US EPA Toxic Substances Control Act (TSCA) inventory and 2024 CDR TSCA Inv.

US EPA Substance Registry Services (SRS) – TSCA and CDR lists.

## *4. Metabolism/Pharmacokinetics*

### *4.1. Metabolism/metabolites*

No data available to us at this time.

### *4.2. Absorption, distribution and excretion*

No data available to us at this time.

### *4.3. Interactions*

No data available to us at this time.

## *5. Toxicity*

### *5.1. Single dose toxicity*

Lemon petigrain oil acute oral LD50 rats and dermal rabbits: >5 g/kg bw (Levenstein, 1975).

Lemon oil expressed acute oral LD50 rats and dermal rabbits: >5 g/kg bw (Hart, 1971).

Mice exposed to air containing lemon oil had a shortened barbiturate-induced sleeping time. Exposure presumably lasted until the mice awoke. The concentration was not specified, and the mechanism of the effect uncertain [possibly indicative of CNS excitation (Tsuchiya et al., 1991).

### *5.2. Repeated dose toxicity*

No data available to us at this time.

### *5.3. Reproduction toxicity*

No data available to us at this time.

### *5.4. Mutagenicity*

**In vivo:**

No data available to us at this time.

**In vitro:**

| Test system | Test conditions | Endpoint | Activation | Result | References |
|---|---|---|---|---|---|
| Chinese hamster lung fibroblasts | Lemon oil tested at up to 0.125 mg/ml (the highest non-toxic concentration) in a 24-hr incubation, cells examined for chromosome aberrations and polyploidy. | Chromosome damage and changes in chromosome number | Without | -ve (a limited study, as not tested with activation) | Ishidate et al., 1984 |
| Chinese hamster lung fibroblasts | Lemon oil tested at up to 0.125 mg/ml (the highest non-toxic concentration) in a 48-hr incubation, cells examined for chromosome aberrations. | Chromosome damage | Without | -ve (a limited study, as not tested with activation) | Ishidate et al., 1988 |
| Salmonella typhimurium TA98, TA100 | Ames test was used to screen 2 essential oil extracts of lemon [as well as 2 soluble essences and 2 hydroalcoholic extracts; no further details]. Tested at up to toxic concentrations or to a maximum of 100 ul/plate. | Mutation | With and without S9 | -ve (a limited study, only tested in 2 strains) | Crebelli et al., 1990 |
| Bacillus subtilis strains H17 and M45 | Tested at up to 30 ul/plate in a rec assay measuring differential killing. Information from tabulated data in a paper in Japanese. | DNA damage (indicative test) | With and without S9 | -ve (without S9) No result (with S9) [no toxicity in either strain] | Kuroda et al., 1989 |
|  | Lemon oil has been recommended for photogenotoxicity testing by the NTP Interagency Committee for Chemical Evaluation and Coordination. |  |  |  | PHS & DHHS, 2000 |

“The photogenotoxicity and photocarcinogenicity of several linear furocoumarins (psoralens) have been well documented (reviewed by Saffran, 1988). 5-Methoxypsoralen, a component of lemon and lime oil, is photogenotoxic in both prokaryotic and eukaryotic test systems. In addition, 5-methoxy-psoralen has been found to be photocarcinogenic in an experimental model (Zajdela & Bisagni, 1981). Little is known about the photogenotoxicity and photocarcinogenicity of other furocoumarins found in lemon oil and lime oil” (NIEHS, 2000).

### *5.5. Cytotoxicity*

“In order to exploit novel anticaries agents, we investigated the effects of citrus lemon oil (CLO), a type of natural product, on growth and adherence of the primary oral cariogenic bacteria Streptococcus mutans (S. mutans). The growth inhibitory effect was explored with a micro-dilution assay. Adherence was analyzed by colony counts on the respective surfaces and the adherence inhibition rate (AIR). Real time-PCR was used to investigate the effects of CLO on transcription of

glucosyltransferase (Gtf) encoding genes, gtfB, C and D. Neson-Somogyi method was used to measure the effects of CLO on Gtf activity. The minimum inhibitory concentration of CLO against S. mutans was 4.5 mg/ml. The CLO effectively reduced the adherence of S. mutans on glass surface (the AIR were from 98.3 to 100 %, $P > 0.05$) and saliva-coated enamel surface (the AIR were from 54.8 to 79.2 %, $P < 0.05$). CLO effectively reduced the activity of Gtf and the transcription of gtfs in a dose dependent manner ($P < 0.05$). In conclusion, CLO can effectively inhibit the growth and the adherence to glass and saliva-coated enamel surfaces of S. mutans. It can also inhibit the transcription of gtfs, as well as the Gtf enzyme activity." As taken from Liu Y et al. 2013. World J. Microbiol. Biotechnol. 29(7), 1161-7. PubMed, 2014

"Alicyclobacillus acidoterrestris is considered to be one of the important target microorganisms in the quality control of acidic canned foods. There is an urgent need to develop a suitable method for inhibiting or controlling the germination and outgrowth of A.acidoterrestris in acidic drinks. The aim of this work was to evaluate the chemicals used in the lemon industry (sodium benzoate, potassium sorbate), and lemon essential oil as a natural compound, against a strain of A. acidoterrestris in MEB medium and in lemon juice concentrate. The results pointed out that sodium benzoate (500-1000-2000 ppm) and lemon essential oil (0.08-0.12-0.16%) completely inhibited the germination of A. acidoterrestris spores in MEB medium and LJC for 11 days. Potassium sorbate (600-1200 ppm) was more effective to inhibit the growth of the microbial target in lemon juice than in MEB medium. The effect of sodium benzoate, potassium sorbate and essential oil was sporostatic in MEB and LJC as they did not affect spore viability." As taken from Maldonado MC et al. 2014. Braz. J. Microbiol. 44(4), 1133-7. PubMed, 2014

"Candida yeasts are saprophytes naturally present in the environment and forming colonies on human mucous membranes and skin. They are opportunistic fungi that cause severe and even fatal infections in immunocompromised individuals. Several essential oils, including eucalyptus, pine, cinnamon and lemon, have been shown to be effective against Candida strains. This study addresses the chemical composition of some commercial lemon essential oils and their antifungal potential against selected Candida yeast strains. Antifungal potential and minimum inhibitory concentrations were determined for six commercial lemon essential oils against five Candida yeast strains (Candida albicans 31, Candida tropicalis 32, Candida glabrata 33, Candida glabrata 35 and Candida glabrata 38). On the basis of the GCMS analysis, it was found that the tested lemon essential oils had different chemical compositions, but mostly, they contained almost exclusively terpenes and oxygenated terpenes. The tests show that antifungal potential of lemon essential oils against Candida yeast strains was related to the high content of monoterpenoids and the type of Candida strains. From six tested commercial oils, only four (ETJA, Vera-Nord, Avicenna-Oil and Aromatic Art) shows antifungal potential against three Candida species (C. albicans, C. tropicalis and C. glabrata). Vera-Nord and Avicenna-Oil show the best activity and effectively inhibit the growth of the C. albicans strain across the full range of the concentrations used. Our study characterises lemon essential oils, which could be used as very effective natural remedies against candidiasis caused by C. albicans." As taken from Białoń M et al. 2014. Mycopathologia 177(1-2), 29-39. PubMed, 2014

### *5.6. Carcinogenicity*

| **Species** | **Test conditions** | **Evidence of carcinogenicity** | **Reference** |
|---|---|---|---|
| Mouse | Tumour promotion assay. Weekly application for 33 wk of 0.25 ml [ca. 12 g/kg bw/week] lemon oil to uncovered skin previously treated with a skin carcinogen | Some evidence of tumour promotion, as lemon oil increased the ability of the carcinogen to produce benign skin tumours | Roe & Peirce, 1960 |

| | | | |
|---|---|---|---|
| Rat & mouse, groups of 50 per sex per species | Though no carcinogenicity studies on lemon oils were identified, high quality studies have been carried out by the US NTP on d-limonene (at 80-90% the major constituent of lemon oil). This major component was given by gavage to rats and mice on 5 days/wk for 2 years. Comprehensive tissue examinations were carried out. | No evidence in mice or in female rats. Kidney tumours developed in male rats. It was JECFA's view that the mechanism of tumour induction was probably not relevant to humans (JECFA, 1993a). | NTP, 1990; JECFA, 1993b |
| | Lemon oil has been recommended by the NTP for photocarcinogenicity testing, pending the results of photogenotoxicity tests. | | PHS & DHHS, 2000 |

"The photogenotoxicity and photocarcinogenicity of several linear furocoumarins (psoralens) have been well documented (reviewed by Saffran, 1988). 5-Methoxypsoralen, a component of lemon and lime oil, is photogenotoxic in both prokaryotic and eukaryotic test systems. In addition, 5-methoxy-psoralen has been found to be photocarcinogenic in an experimental model (Zajdela & Bisagni, 1981). Little is known about the photogenotoxicity and photocarcinogenicity of other furocoumarins found in lemon oil and lime oil" (NIEHS, 2000).

### *5.7. Irritation/immunotoxicity*

Volunteers exposed to 450 mg/m3 d-limonene for 2 hours showed no signs of respiratory irritation (Von Burg, 1995).

"Lemon oil applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was moderately irritating (Hart, 1971)" (Opdyke, 1974a & b).

"Applied full strength to intact or abraded rabbit skin for 24 hr under occlusion, it [lemon petitgrain oil] was not irritating (Levenstein, 1975)" (Opdyke, 1978).

"Three samples of lemon oil … applied undiluted to the backs of hairless mice were mildly irritating (Urbach & Forbes, 1972), but three other samples of lemon oil … similarly applied … were not irritating (Urbach & Forbes, 1972)" (Opdyke, 1974a).

"Lemon oil distilled … applied undiluted to the backs of hairless mice was slightly irritating (Urbach & Forbes, 1974). Lemon oil FCF applied undiluted to the backs of hairless mice was not irritating (Urbach & Forbes, 1974)" (Opdyke, 1974b).

"Undiluted lemon petitgrain oil applied to the backs of hairless mice and swine was slightly irritating (Urbach & Forbes, 1972)" (Opdyke, 1978).

"Lemon oil tested at 10% in petrolatum produced no irritation after a 48-hr closed-patch test in 25 human subjects (Kligman 1971)" (Opdyke, 1974a & b).

"A patch test using full strength lemon oil for 24 hr produced no reactions in 24 human subjects (Katz, 1946)" (Opdyke, 1974a).

Tested at 10% in petrolatum, [lemon petitgrain oil] produced no irritation after a 48-hr closed-patch test on human subjects (Epstein, 1975)" (Opdyke, 1978).

**Skin and respiratory tract sensitization**

At 10% lemon oil and lemon petitgrain oil were not sensitising to human skin (Epstein, 1975; Kligman, 1971) (as taken from Opdyke, 1974a, b & 1978).

Contact dermatitis and eczema patients were patch tested with lemon oil at 2% in petrolatum. None of the 48 patients tested exhibited definite positive reactions (Guin, 2005).

Two patients with contact dermatitis, associated with the use of essential aromatherapy oils, were patch-tested with 2% lemon oil in petrolatum. One negative and one strong positive reaction were observed (Trattner et al., 2008).

The results of patch-tests on 15,682 dermatitis patients were analysed. Of these, 6467 patients had been patch-tested with lemon oil at 2% in petrolatum. 0.22% of individuals exhibited a weakly positive sensitisation reaction, with stronger reactions seen in 0.07% (Uter et al., 2010).

The literature contains a brief report of a woman who developed allergy-related cough, headache and sore throat symptoms following a 10-minute exposure to the vapour from an open cup containing 0.1% “citrus peel oils” in isopropanol. At 1%, symptoms were more severe and reduced lung function was noted (Block & Marney, 1988). [The source of the oils may have been orange peel, since the woman’s original symptoms (nasal and bronchial congestion) resulted from occupational exposure to an orange-scented room deodorizer.]

### *5.8. All other relevant types of toxicity*

“This study sought to investigate the effects of essential oil from lemon (Citrus limoni) peels on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities in vitro. The essential oil was extracted by hydrodistillation, dried with anhydrous Na2SO4 and characterized using gas chromatography. Antioxidant properties of the oil and inhibition of pro-oxidant-induced lipid peroxidation in rats brain homogenate were also assessed. The essential oil inhibited AChE and BChE activities in a concentration-dependent manner. GC analysis revealed the presence of sabinene, limonene, α-pinene, β-pinene, neral, geranial, 1,8-cineole, linalool, borneol, α-terpineol, terpinen-4-ol, linalyl acetate and β-caryophyllene. Furthermore, the essential oil exhibited antioxidant activities as typified by ferric reducing property, Fe(2+)-chelation and radicals [DPPH, ABTS, OH, NO] scavenging abilities. The inhibition of AChE and BChE activities, inhibition of pro-oxidant induced lipid peroxidation and antioxidant activities could be possible mechanisms for the use of the essential oil in the management and prevention of oxidative stress-induced neurodegeneration.” As taken from Oboh G et al. 2014. J. Oleo. Sci. 63(4), 373-81. PubMed, 2014

Low-level to distinct phototoxic effects were reported with lemon oil, depending on the origin of the oil (Urbach & Forbes, 1972) (cited in Opdyke, 1974a).

No phototoxic effects were reported with lemon oil distilled (Urbach & Forbes, 1972), lemon oil FCF (Urbach & Forbes, 1974) or lemon petitgrain oil (Urbach & Forbes, 1972) (cited in Opdyke, 1974a & b, 1978).

“The aim of this study, linked-up with a previous study on bergamot oils, was the evaluation of phototoxic potential of essential oils (orange, lemon and Litsea cubeba), used as cosmetic ingredients. The applied tiered testing strategy included chemical analysis of the substances (by means of capillary gas chromatography/mass spectrometry), in vitro 3T3 NRU phototoxicity test and EpiDerm™ skin phototoxicity test. In order to clarify the situation in man, the highest non-phototoxic/non-cytotoxic concentrations and concentrations 10 x lower (safety factor 10) were tested xin vivo by means of human skin photopatch test in a limited group of human volunteers. The study revealed, that phototoxicity of the essential oils was dependent on the content of photoactive components and the solvent used. The highest non-phototoxic concentrations obtained by the skin model assay proved to be a useful starting point for subsequent confirmatory human photopatch test aimed to identify safe concentration for human use. However, the highest non-phototoxic concentration obtained in the skin model assay cannot be applied directly for human

practice (3 of 8 tested oils evoked a phototoxic reaction). A safety factor of 10 should be applied for extrapolation of experimental data from the skin model assay to man" (Kejlová et al., 2010).

"Lemon oil was diluted in ethanol (100% lemon oil, 50% lemon oil, and 20% lemon oil) and applied to backs of shaved, albino guinea pigs. Animals were then exposed to UVA radiation (320-400 nm, 13 J/cm2). Erythema was evaluated at 24, 48 and 72 hr after irradiation. Undiluted lemon oil and 50% lemon oil elicited phototoxicity for most of the samples tested. Lemon oil from Australia and Brazil elicited a weaker phototoxic than lemon oil from the Ivory Coast, Sicily, California, or Argentina. In an effort to identify the phototoxic components in lemon oil, these investigators fractionated samples by solvent extraction and subsequent phototoxicity testing of isolated components. Two phototoxic components were identified: oxypeucedanin and 5-methoxypsoralen" (NIEHS, 2000).

Lemon oil was fractionated and its phototoxicity was measured. The substances producing phototoxicity were identified as being oxypeucedanin and bergapten (Naganuma, 1985).

## *6. Functional effects on*

### *6.1. Broncho/pulmonary system*

The literature contains a brief report of a woman who developed allergy-related cough, headache and sore throat symptoms following a 10-minute exposure to the vapour from an open cup containing 0.1% "citrus peel oils" in isopropanol. At 1%, symptoms were more severe and reduced lung function was noted (Block & Marney, 1988). [The source of the oils may have been orange peel, since the woman's original symptoms (nasal and bronchial congestion) resulted from occupational exposure to an orange-scented room deodorizer.]

### *6.2. Cardiovascular system*

No data available to us at this time.

### *6.3. Nervous system*

Mice exposed to air containing lemon oil had a shortened barbiturate-induced sleeping time. Exposure presumably lasted until the mice awoke. The concentration was not specified, and the mechanism of the effect uncertain [possibly indicative of CNS excitation (Tsuchiya et al. 1991).

"BACKGROUND: Affections are thought to regulate pain perception through the descending pain inhibitory system in the central nervous system. In this study, we examined in mice the affective change by inhalation of the lemon oil, which is well used for aromatherapy, and the effect oflemonodor on pain sensation. We also examined the anterior cingulate cortex (ACC) and descending pain inhibitory system to such regulation of pain. RESULTS: In the elevated plus maze, the time spent in the open arms was increased by inhalation oflemon oil. The pain behavior induced by injection of formalin into the hind paw was decreased. By inhalation oflemon oil, the number of c-Fos expression by formalin injection was significantly increased in the ACC, periaqueductal grey (PAG), nucleu raphe magnus (NRM) and locus ceruleus, and decreased in the spinal dorsal horn (SDH). The destruction of the ACC with ibotenic acid led to prevent the decrease of formalin-evoked nocifensive behavior in mice exposed tolemon oil. In these mice, the change of formalin-induced c-Fos expression in the ACC, lateral PAG, NRM and SDH bylemonodor was also prevented. Antagonize of dopamine D1 receptor in the ACC prevented to the analgesic effect oflemon oil. CONCLUSIONS: These results suggest that the analgesic effect oflemon oilis induced by dopamine-related activation of ACC and the descending pain inhibitory system." As taken from Ikeda H et al. 2014. Mol. Pain 10, 14. PubMed, 2014

Prolonged exposure to lemon essential oil (150 µl added to bedding every three days for a total of 11-12 days) resulted in increased anxiety and gender-specific (female only) change to formalin-induced pain responses in Sprague Dawley rats (20 per gender) (Ceccarelli et al., 2004).

“The anti-dementia effects of s-limonene and s-perillyl alcohol were observed using the passive avoidance test (PA) and the open field habituation test (OFH). These lemon essential oils showed strong ability to improve memory impaired by scopolamine; however, s-perillyl alcohol relieved the deficit of associative memory in PA only, and did not improve non-associative memory significantly in OFH. Analysis of neurotransmitter concentration in some brain regions on the test day showed that dopamine concentration of the vehicle/scopolamine group was significantly lower than that of the vehicle/vehicle group, but this phenomenon was reversed when s-limonene or s-perillyl alcohol were administered before the injection of scopolamine. Simultaneously, we found that these two lemon essential oil components could inhibit acetylcholinesterase activity in vitro using the Ellman method” (Zhou et al., 2009).

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

“BACKGROUND: Nausea and vomiting of pregnancy are amongst the most common complaints that effects on both the physical and mental conditions of the pregnant women. Due to the increasing tendency of women to use herbal medications during pregnancy, the effect of lemon inhalation aromatherapy on nausea and vomiting of pregnancy was investigated in this study. OBJECTIVES: The aim of this study was to determine the effect oflemoninhalation aromatherapy on nausea and vomiting during pregnancy. MATERIALS AND METHODS: This was a randomized clinical trial in which 100 pregnant women with nausea and vomiting who had eligibility criteria were randomly divided into intervention and control groups based on four- and six-random block sampling method.Lemonessentialoiland placebo were given to the intervention and control groups, respectively, to inhale it as soon as they felt nausea. The nausea, vomiting, and retch intensity were investigated 24 hours before and during the four days of treatment by means of PUQE-24 (24-hour Pregnancy Unique Quantification of Emesis). RESULTS: There was a statistically significant difference between the two groups in the mean scores of nausea and vomiting on the second and fourth days (P = 0.017 and P = 0.039, respectively). The means of nausea and vomiting intensity in the second and fourth days in the intervention group were significantly lower than the control group. In addition, in intragroup comparison with ANOVA with repeated measures, the nausea and vomiting mean in the five intervals, showed a statistically significant difference in each group (P < 0.001 and P = 0.049, respectively). CONCLUSIONS:Lemonscent can be effective in reducing nausea and vomiting of pregnancy.” As taken from Yavari Kia P et al. 2014. Iran. Red Crescent Med. J. 16(3), e14360. PubMed, 2014

"Diabetic patients wound healing is slower than the healthy individuals. Three citrus peel extracts; Lemon (Citrus limon), Grapes fruits (Citrus paradise) and Orange (Citrus sinensis) promote wound healing in experimental animals. This study investigated the effect of oral treatment with citrus peel extracts on wound repair of the skin of diabetic rats. The extracts were estimated for vitamin C and total carotenoid contents prior to animal study. Diabetes mellitus was induced in rats by intraperitoneal injection of a single dose of streptozotocin (STZ, 75 mg kg(-1) b.wt.). One week after diabetes induction, full thickness excision wounds were made in hyperglycemic rats and were divided groups, each containing 6 rats. The different test group animals were treated with different citrus peel extract orally at the dose of 400 mg kg(-1) body weight daily for 12 days. The blood glucose, body weight and rate of wound closure of each rat were measured every 3rd day during the experimental period. At the end of experiment, granular tissues of wounds were removed and estimated for hydroxylproline and total protein content. The results showed significant reduction in blood glucose and time to wound closure. Tissue growth and collagen synthesis were significantly higher as determined by total protein and hydroxyl proline content. From our experimental data, we propose that oral administration of citrus peel extracts has a therapeutic potential in the treatment

of chronic wounds in diabetes." As taken from Ahmad M et al. 2013. Pak. J. Biol. Sci. 16(20), 1086-94. PubMed, 2014

## *7. Addiction*

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

## *8. Burnt ingredient toxicity*

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

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

| **Endpoint** | **Tested level (ppm)** | **Reference** |
|---|---|---|
| Smoke chemistry | <1 (oil terpenes, No CAS) | Carmines 2002 & Rustemeier et al., 2002 |
| | 12 (oil,terpeneless) | Baker et al., 2004a |
| In vitro genotoxicity | <1 (oil terpenes, No CAS) | Carmines 2002 & Roemer et al., 2002 |
| | 12 (oil,terpeneless) | Baker et al., 2004b |
| In vitro cytotoxicity | <1 (oil terpenes, No CAS) | Carmines 2002 & Roemer et al., 2002 |
| | 12 (oil,terpeneless) | Baker et al., 2004b |
| Inhalation study | <1 (oil terpenes, No CAS) | Carmines 2002 & Vanscheeuwijck et al., 2002 |
| | 12 (oil,terpeneless) | Baker et al., 2004b |

## *9. Heated/vapor emissions toxicity*

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

| **Endpoint** | **Tested level (ppm)** | **Reference** |
|---|---|---|
| Aerosol chemistry | 300 | Labstat International Inc. (2021) |

| | | |
|---|---|---|
| In vitro genotoxicity | 300 | Labstat International Inc. (2022) |
| In vitro cytotoxicity | 300 | Labstat International Inc. (2022) |

Aerosol from heated tobacco stick(s) containing Lemon oil was tested in aerosol chemistry and a battery of in vitro test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), the activity of the total particulate matter (TPM) and/or gas vapor phase (GVP) were not increased by the addition of this ingredient when compared to TPM and/or GVP from reference combustible cigarettes. The table below provides the highest tested level(s) and specific endpoint(s):

| **Endpoint** | **Tested level (mg/stick)** | **Reference** |
|---|---|---|
| Aerosol chemistry | 0.278 | JTI Heated Tobacco Stick Study Report(s) |
| In vitro genotoxicity | 0.278 | JTI Heated Tobacco Stick Study Report(s) |
| In vitro cytotoxicity | 0.278 | JTI Heated Tobacco Stick Study Report(s) |

## *10. Ecotoxicity*

### *10.1. Environmental fate*

EPISuite provides the following data:

**Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:**

| | |
|---|---|
| Bond Method : | 8.33E-018 atm-m3/mole (8.44E-013 Pa-m3/mole) |
| Group Method: | Incomplete |
| Exper Database: | 4.33E-14 atm-m3/mole (4.39E-009 Pa-m3/mole) |
| Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]: | HLC: 1.426E-015 atm-m3/mole (1.445E-010 Pa-m3/mole) VP: 5.64E-009 mm Hg (source: MPBPVP) WS: 1E+006 mg/L (source: WSKOWWIN) |

**Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:**

| | |
|---|---|
| Log Kow used: | -1.64 (exp database) |
| Log Kaw used: | -11.752 (exp database) |
| Log Koa (KOAWIN v1.10 estimate): | 10.112 |
| Log Koa (experimental database): | None |

**Probability of Rapid Biodegradation (BIOWIN v4.10):**

| | |
|---|---|
| Biowin1 (Linear Model): Biowin2 (Non-Linear Model) : Biowin3 (Ultimate Survey Model): Biowin4 (Primary Survey Model) : Biowin5 (MITI Linear Model) : Biowin6 (MITI Non-Linear Model): Biowin7 (Anaerobic Linear Model): | 0.6902 0.6193 3.6563 (days-weeks) 4.5738 (hours-days) 0.8505 0.8377 1.1142 |
| Ready Biodegradability Prediction: | YES |

**Hydrocarbon Biodegradation (BioHCwin v1.01):**

| |
|---|
| Structure incompatible with current estimation method! |

**Sorption to aerosols (25 Dec C) [AEROWIN v1.00]:**

| | |
|---|---|
| Vapor pressure (liquid/subcooled): | 4.08E-005 Pa (3.06E-007 mm Hg) |
| Log Koa (Koawin est): | 10.112 |
| Kp (particle/gas partition coef. (m3/ug)):<br>Mackay model:<br>Octanol/air (Koa) model: | 0.0735 0.00318 |

Fraction sorbed to airborne particulates (phi):

| | |
|---|---|
| Junge-Pankow model: | 0.726 |
| Mackay model: | 0.855 |
| Octanol/air (Koa) model: | 0.203 |

**Atmospheric Oxidation (25 deg C) [AopWin v1.92]:**

Hydroxyl Radicals Reaction:

| | |
|---|---|
| OVERALL OH Rate Constant = | 7.0238 E-12 cm3/molecule-sec |
| Half-Life = | 1.523 Days (12-hr day; 1.5E6 OH/cm3) |
| Half-Life = | 18.274 Hrs |
| Ozone Reaction: | No Ozone Reaction Estimation |
| Fraction sorbed to airborne particulates (phi): 0.791 (Junge-Pankow, Mackay avg) 0.203 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation | |

**Soil Adsorption Coefficient (KOCWIN v2.00):**

| | |
|---|---|
| Koc : | 10 L/kg (MCI method) |
| Log Koc: | 1.000 (MCI method) |
| Koc : | 0.06873 L/kg (Kow method) |
| Log Koc: | -1.163 (Kow method) |

**Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:**

Rate constants can NOT be estimated for this structure!

**Volatilization from Water:**

Henry LC: 4.33E-014 atm-m3/mole (Henry experimental database)

| | |
|---|---|
| Half-Life from Model River: | 1.874E+010 hours (7.809E+008 days) |
| Half-Life from Model Lake: | 2.045E+011 hours (8.519E+009 days) |

**Removal In Wastewater Treatment:**

| | |
|---|---|
| Total removal: | 1.85 percent |
| Total biodegradation: | 0.09 percent |
| Total sludge adsorption: | 1.75 percent |
| Total to Air: | 0.00 percent |

(using 10000 hr Bio P,A,S)

**Level III Fugacity Model:**

| | Mass Amount (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|---|
| Air | 9.95e-006 | 36.6 | 1000 |
| Water | 28.1 | 208 | 1000 |
| Soil | 71.8 | 416 | 1000 |
| Sediment | 0.0592 | 1.87e+003 | 0 |

Persistence Time: 414 hr

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oils, lemon, terpene-free are of uncertain persistence in the environment.

Data accessed June 2017 on the OECD website

### *10.2. Aquatic toxicity*

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oils, lemon, terpene-free are not inherently toxic to aquatic organisms and are of low ecotoxicological concern.

Data accessed June 2017 on the OECD website

“Tests on lemon peel oil extract as a mosquito larvicide were carried out. The oil was found to be toxic on larvae, pupae and eggs of Culex quinquefasciatus. The oil also fulfilled other required specifications like suitable specific gravity, spreading pressure and viscosity. It was also toxic at a wide pH range, stable to heat and light in terms of chemical change which could alter larvicidal action. However, it was volatile and did not form a permanent film on water surfaces for long periods. This affected the larvicidal action” (Mwaiko & Savaeli, (1994).

ECOSAR version 1.11 reports the following aquatic toxicity data for CAS RN 68648-39-5:

Values used to Generate ECOSAR Profile:

Log Kow: -1.673 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 1E+006 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations:

Not Related to an Existing ECOSAR Class Definition. Estimates provided below use the Neutral Organics QSAR equations which baseline toxicity potential (minimum toxicity) assuming a simple non-polar narcosis model. Without empirical data on structurally similar chemicals, it is uncertain if this substance will present significantly higher toxicity above baseline estimates.

| ECOSAR Class | Organism | Duration | End Pt | Predicted mg/L (ppm) |
|---|---|---|---|---|
| --> Acid moeity found: Predicted values multiplied by 10 | | | | |
| Neutral Organics-acid : | Fish | 96-hr | LC50 | 3.14e+006 * |
| Neutral Organics-acid : | Daphnid | 48-hr | LC50 | 1.27e+006 * |
| Neutral Organics-acid : | Green Algae | 96-hr | EC50 | 2.33e+005 |
| Neutral Organics-acid : | Fish | | ChV | 2.06e+005 |
| Neutral Organics-acid : | Daphnid | | ChV | 48229.512 |
| Neutral Organics-acid : | Green Algae | | ChV | 28660.252 |
| Neutral Organics-acid : | Fish (SW) | 96-hr | LC50 | 3.87e+006 * |
| Neutral Organics-acid : | Mysid | 96-hr | LC50 | 3.46e+007 * |
| Neutral Organics-acid : | Fish (SW) | | ChV | 44579.078 |
| Neutral Organics-acid : | Mysid (SW) | | ChV | 8.89e+006 * |

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

### *10.3. Sediment toxicity*

No data available to us at this time.

### *10.4. Terrestrial toxicity*

“The vine mealybug, Planococcus ficus (Signoret) (Hemiptera: Pseudococcidae), is a pest in grape vine growing areas worldwide. The essential oils from the following aromatic plants were tested for their insecticidal activity against P. ficus: peppermint, Mentha piperita L. (Lamiales: Lamiaceae), thyme-leaved savory, Satureja thymbra L., lavender, Lavandula angustifolia Mill, and basil, Ocimum basilicum L. Essential oils from peels of the following fruits were also tested: lemon, Citrus limon L. (Sapindales: Rutaceae), and orange, C. sinensis L. The reference product was paraffin oil. Bioassays were conducted in the laboratory by using spray applications on grape leaves bearing clusters of P. ficus of one size class, which mainly represented either 3rd instar nymphs or pre-ovipositing adult females. The LC50 values for each essential oil varied depending on the P. ficus life stage but did not significantly differ between 3(rd) instar nymphs and adult females. The LC50 values of the citrus, peppermint, and thyme-leaved savory essential oils ranged from 2.7 to 8.1 mg/mL, and the LC50 values of lavender and basil oil ranged from 19.8 to 22.5 and 44.1 to 46.8mg/mL, respectively. The essential oils from citrus, peppermint and thymeleaved savory were

more or equally toxic compared to the reference product, whereas the lavender and basil essential oils were less toxic than the paraffin oil. No phytotoxic symptoms were observed on grape leaves treated with the citrus essential oils, and low phytotoxicity was caused by the essential oils of lavender, thyme-leaved savory, and mint, whereas the highest phytotoxicity was observed when basil oil was used." As taken from Karamaouna F et al. 2013. J. Insect. Sci. 13(142), 1-3. PubMed, 2014

"Peel oils of lemon, grapefruit and navel orange were tested for insecticidal activities against larvae and adults of Culex pipiens and Musca domestica. Lemon peel oil was the most effective against larvae and adults of C. pipiens. Grapefruit peel oil was more toxic to adults of M. domestica while lemon oil, was more toxic Musca larvae. On the other hand, the orange peel oil was the least effective against larvae and adults of both species. The toxicity of oils applied to larval stages was extended to pupal and adult stages. C. pipiens adults appeared with paralyzed legs, while M domestica adults appeared normal. The weights of pupae treated as larvae were generally less than that of the control. All oils produced deleterious effects on fecundity of survivors of sublethal doses. The effect was obviously recorded in treated adults. Treatment of Culex & Musca with oils caused serious latent effect" (Shalaby et al. 1998).

"The larviciddal action of three ethanol extracts of peel oils of lemon, grapefruit and navel orange were tested against the early 4th instar larvae of Culex pipiens and the resulting pupae. The LC50 were 18.5, 20.3 and 26.5 and the slope functions were 2.9, 2.9 and 3.9 respectively. The action of the lemon extract extended to the pupae which resulted from larvae exposed to sublethal dose. Some of the pupae were unable to escape from the larval exuviae" (Al Dakhil & Morsy, 1999).

ECOSAR version 1.11 reports the following terrestrial toxicity data for CAS RN 68648-39-5:

Values used to Generate ECOSAR Profile:

Log Kow: -1.673 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 1E+006 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations:

Not Related to an Existing ECOSAR Class Definition. Estimates provided below use the Neutral Organics QSAR equations which baseline toxicity potential (minimum toxicity) assuming a simple non-polar narcosis model. Without empirical data on structurally similar chemicals, it is uncertain if this substance will present significantly higher toxicity above baseline estimates.

| **ECOSAR Class** | **Organism** | **Duration** | **End Pt** | **Predicted mg/L (ppm)** |
|---|---|---|---|---|
| --> Acid moeity found: Predicted values multiplied by 10 | | | | |
| Neutral Organics-acid : | Earthworm | 14-day | LC50 | 8030.058 |

"The toxicity of essential oils from the citrus peel has been proposed as the major resistance mechanism offered by citrus to fruit fly infestation. We evaluated the insecticidal activity of the ether extracts from the lemon (Citrus limon [L.] Burm.) and grapefruit (C. paradisi Macfadyen) peel as well as from limonene and citral against Anastrepha fraterculus (Wiedemann) and Ceratitis capitata (Wiedemann) immature stages. We also evaluated the toxicity of the extracts at two ripening stages. Extracts proved toxic to A. fraterculus egg and larvae. The lemon and grapefruit extracts showed the same toxicity in both fruit fly species. For A. fraterculus eggs, citral was more toxic than limonene; for larvae, they showed equal toxicity. Anastrepha fraterculus eggs were more sensitive than C. capitata eggs. In conclusion, we provide evidence of chemical resistance mechanisms that could account for the nonhost condition of lemon for A. fraterculus." As taken from Ruiz MJ et al. 2014. J. Agric. Food Chem. 62(41), 10084-10091. PubMed, 2015

“The fumigant, contact, and repellent activities of four essential oils extracted from Citrus limonum (Sapindales: Rutaceae), Litsea cubeba (Laurales: Lauraceae), Cinnamomum cassia, and Allium sativum L. (Asparagales: Alliaceae) against 6th instars and adults of the darkling beetle, Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae), one of the main pests of materials and products of Juncus effuses L. (Poales: Juncaceae) during the storage period, were assayed, and chemical ingredients were analyzed with gas chromatography-mass spectrometry in this study. While the major ingredients found in C. limonum and C. cassia were limonene and (E)-cinnamaldehyde, the main constituents of L. cubea were D-limonene, (E)-3,7-dimethyl-,2,6-octadienal, (Z)-3,7-dimethyl,2 ,6-octadienal, and diallyl disulphide (18.20%), while the main constituents of and A. sativum were di-2-propenyl trisulfide and di-2-propenyl tetrasulfide. The fumigation activities of A. sativum and C. limonum on A. diaperinus adults were better than those of the other two essential oilss. The toxicities of A. sativum and C. limonum were almost equitoxic at 96 hr after treatment. Essential oils from Allium sativum and L. cubeba also showed good contact activities from 24 hr to 48 hr, and toxicities were almost equitoxic 48 hr posttreatment. The repellent activities of A. sativum and L. cubeba oils on 6th instars were also observed, showing repellence indexes of 90.4% and 88.9% at 12 hr after treatment, respectively. The effects of A. sativum on AChE activity of 6th instars of A. diaperinus were strongest compared to the other essential oils, followed by C. limonum, L. cubeba, and C. cassia. These results suggest that the essential oils of C. limonum and A. sativum could serve as effective control agents of A. diaperinus.” As taken from Wang X et al. 2014. J. Insect Sci. 14, 75. PubMed, 2015

### *10.5. All other relevant types of ecotoxicity*

EPISuite provides the following data:

**Bioaccumulation Estimates (BCFBAF v3.01):**

| | |
|---|---|
| Log BCF from regression-based method: | 0.500 (BCF = 3.162 L/kg wet-wt) |
| Log Biotransformation Half-life (HL): | -1.6429 days (HL = 0.02275 days) |
| Log BCF Arnot-Gobas method (upper trophic): | -0.049 (BCF = 0.8942) |
| Log BAF Arnot-Gobas method (upper trophic): | -0.049 (BAF = 0.8942) |
| log Kow used: | -1.64 (expkow database) |

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oils, lemon, terpene-free are of uncertain bioaccumulative potential in the environment.

Data accessed June 2017 on the OECD website

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Review

# The FEMA GRAS assessment of benzyl derivatives used as flavor ingredients

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**Abstract**

This publication is the eighth in a series of safety evaluations performed by the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA). In 1993, the panel initiated a comprehensive program to re-evaluate the safety of more than 1700 GRAS flavoring substances under conditions of intended use. Elements that are fundamental to the safety evaluation of flavor ingredients include exposure, structural analogy, metabolism, pharmacokinetics and toxicology. Flavor ingredients are evaluated individually and in the context of the available scientific information on the group of structurally related substances. Scientific data relevant to the safety evaluation of the use of benzyl derivatives as flavoring ingredients is evaluated. The group of benzyl derivatives was reaffirmed as GRAS (GRASr) based, in part, on their self-limiting properties as flavoring substances in food; their rapid absorption, metabolic detoxication, and excretion in humans and other animals, their low level of flavor use, the wide margins of safety between the conservative estimates of intake and the no-observed-adverse effect levels determined from subchronic and chronic studies and the lack of significant genotoxic and mutagenic potential. This evidence of safety is supported by the fact that the intake of benzyl derivatives as natural components of traditional foods is greater than their intake as intentionally added flavoring substances.


*Abbreviations*: ABS, chromosomal aberration; AMS, Ames assay; *B. subtilis*, *Bacillus subtilis*; bw, body weight; CHO, Chinese hamster ovary; CoA, coenzyme A; DNA, deoxyribonucleic acid; *E. coli*, *Escherichia coli*; F, Female; FDA, United States Food and Drug Administration; FEMA, The Flavor and Extract Manufacturers Association; GRAS, generally recognized as safe; GRASa, GRAS affirmed; GRASr, GRAS reaffirmed; im, intramuscular; ip, intraperitoneal; iv, intravenous; $LD_{50}$, median lethal dose; M, Male; MLA, mouse lymphoma cell assay; NAS, National Academy of Science; NCI, National Cancer Institute; NOAEL, No-observed-adverse effect level; NR, not reported; NTP = National Toxicology Program; ppm = parts per million; SMVCE, sperm morphology and vaginal cytology examinations; *S. typhimurium*, *Salmonella typhimurium*; SCE, sister chromatid exchanges; SLR, scientific literature review; UDS, unscheduled DNA synthesis.

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*Keywords:* Benzyl derivatives; Flavoring ingredients; FEMA GRAS

**Contents**



## 1. Chemical identity

This summary presents the key data relevant to the safety evaluation of benzyl alcohol, benzaldehyde, or benzoic acid and 34 structurally related substances for their intended use as flavoring ingredients (see Table 1). All members of this group are aromatic primary alcohols, aldehydes, carboxylic acids, or their corresponding esters or acetals. The structural features common to this group of substances include an oxygenated functional group bonded directly to a benzene ring that may be ring-substituted with alkyl substituents. The group contains the parent saturated alcohol, benzyl alcohol (No. 1), and related benzyl esters (No. 2–12); the corresponding aldehyde, benzaldehyde (No. 13), and related acetals (No. 14–17); the corresponding parent carboxylic acid, benzoic acid (No. 18), and related benzoate esters (No. 19–30). This group also includes 7 additional benzyl derivatives (No. 31–37) containing ring alkyl substituents.

The substances in this group were selected based on the criteria that: (1) all members contain a benzene ring substituted with a reactive primary oxygenated functional group or can be hydrolyzed to such a functional group, (2) the major pathway of metabolic detoxication involves hydrolysis and oxidation to yield the corresponding benzoic acid derivative which is excreted either as the free acid or the glycine conjugate, (3) they show a consistent pattern of toxicity in both short- and long-term studies, and (4) they exhibit no evidence of genotoxicity in standardized batteries of in vitro and in vivo assays. Metabolism studies in humans and animals and four 2-year bioassays performed on parent substances in this group provide the basis for a comprehensive metabolic and toxicological assessment of the group.

## 2. Exposure

### *2.1. Flavor use and natural occurrence*

The total annual volume of the 37 benzyl derivatives is approximately 464,110 kg in USA (NAS, 1970, 1982,

Table 1
Identity and exposure data for benzyl derivatives used as flavor ingredients

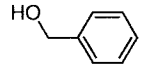
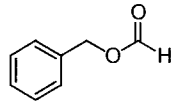
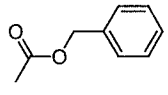
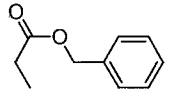
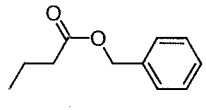
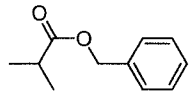
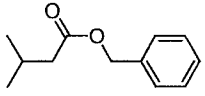
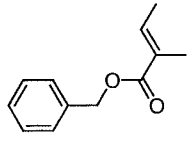
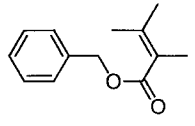
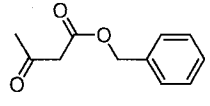
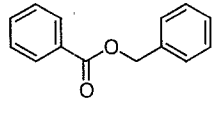
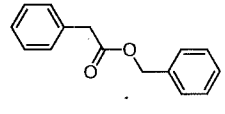
| Flavoring ingredient | FEMA no. | CAS no. and structure | Most recent annual volume (kg)[a] | Daily per capita intake ("eaters only")[b] | | Annual volume in naturally occurring foods (kg)[c] | Consumption ratio[d] |
|---|---|---|---|---|---|---|---|
| | | | | μg/d | μg/kg bw/d | | |
| 1. Benzyl alcohol | 2137 | 100-51-6  | 130,635 | 17,207 | 287 | 7099 | 0.05 |
| 2. Benzyl formate | 2145 | 104-57-4  | 386 | 51 | 1 | 12 | 0.03 |
| 3. Benzyl acetate | 2135 | 140-11-4  | 6486 | 854 | 14 | 3453 | 0.5 |
| 4. Benzyl propionate | 2150 | 122-63-4  | 748 | 99 | 2 | + | NA |
| 5. Benzyl butyrate | 2140 | 103-37-7  | 2209 | 291 | 5 | + | NA |
| 6. Benzyl isobutyrate | 2141 | 103-28-6  | 163 | 21 | 0.4 | + | NA |

Table 1 (*continued*)

| Flavoring ingredient | FEMA no. | CAS no. and structure | Most recent annual volume (kg)[a] | Daily per capita intake ("eaters only")[b] | | Annual volume in naturally occurring foods (kg)[c] | Consumption ratio[d] |
|---|---|---|---|---|---|---|---|
| | | | | μg/d | μg/kg bw/d | | |
| 7. Benzyl isovalerate | 2152 | 103-38-8  | 95 | 14 | 0.2 | + | NA |
| 8. Benzyl *trans*-2-methyl-2-butenoate | 3330 | 37526-88-8  | 0.2 | 0.03 | 0.0004 | + | NA |
| 9. Benzyl 2,3-dimethyl crotonate | 2143 | 7492-69-5  | 9[e] | 2 | 0.03 | – | NA |
| 10. Benzyl acetoacetate | 2136 | 5396-89-4  | 0.5[f] | 0.08 | 0.001 | + | NA |
| 11. Benzyl benzoate | 2138 | 120-51-4  | 31,797 | 4188 | 70 | 108 | 0.003 |
| 12. Benzyl phenylacetate | 2149 | 102-16-9  | 435 | 57 | 1 | + | NA |

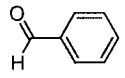
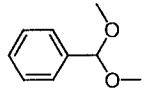
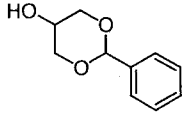
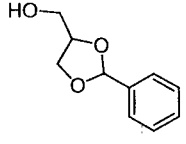
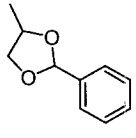
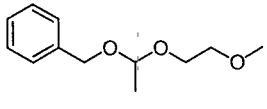
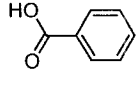
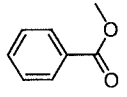
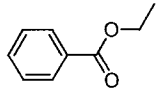
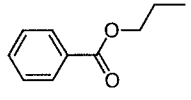
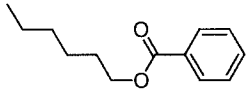
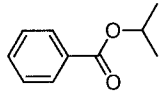
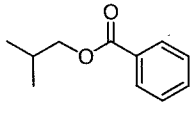
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 13. Benzaldehyde | 2127 | 100-52-7  | 273,516 | 36,027 | 600 | 57,956 | 0.2 |
| 14. Benzaldehyde dimethyl acetal | 2128 | 1125-88-8  | 2[g] | 0.3 | 0.005 | + | NA |
| 15. Benzaldehyde glyceryl acetal | 2129 | 1319-88-6  or  | 2254 | 297 | 5 | – | NA |
| 16. Benzaldehyde propylene glycol acetal | 2130 | 2568-25-4  | 821 | 108 | 2 | + | NA |
| 17. Benzyl methoxyethyl acetal | 2148 | 7492-39-9  | 10[h] | 2 | 0.03 | – | NA |
| 18. Benzoic acid | 2131 | 65-85-0  | 2604 | 343 | 6 | + | NA |

Table 1 (*continued*)

| Flavoring ingredient | FEMA no. | CAS no. and structure | Most recent annual volume (kg)[a] | Daily per capita intake ("eaters only")[b] | | Annual volume in naturally occurring foods (kg)[c] | Consumption ratio[d] |
|---|---|---|---|---|---|---|---|
| | | | | μg/d | μg/kg bw/d | | |
| 19. Methyl benzoate | 2683 | 93-58-3  | 1719 | 226 | 4 | 26 | 0.02 |
| 20. Ethyl benzoate | 2422 | 93-89-0  | 794 | 105 | 2 | 1070 | 1 |
| 21. Propyl benzoate | 2931 | 2315-68-6  | 1[e] | 0.2 | 0.004 | + | NA |
| 22. Hexyl benzoate | 3691 | 6789-88-4  | 1 | 0.2 | 0.004 | 1711 | 1711 |
| 23. Isopropyl benzoate | 2932 | 939-48-0  | 1 | 0.2 | 0.004 | + | NA |
| 24. Isobutyl benzoate | 2185 | 120-50-3  | 6 | 1 | 0.01 | + | NA |

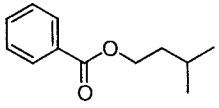
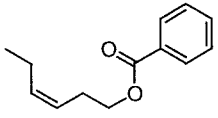
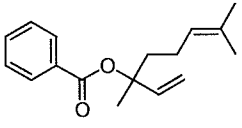
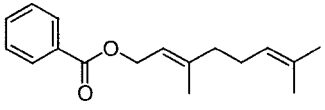
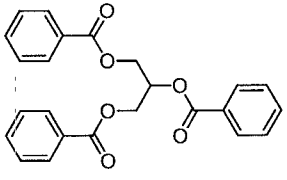
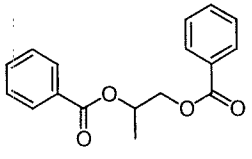
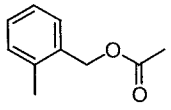
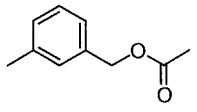
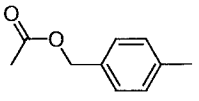
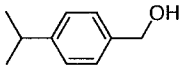
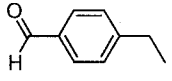
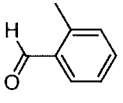
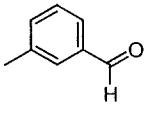
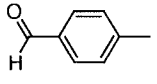
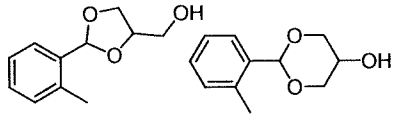
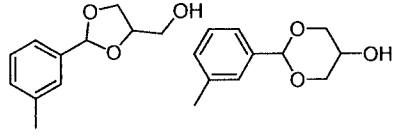
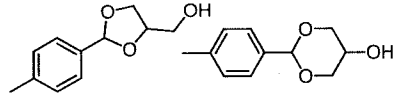
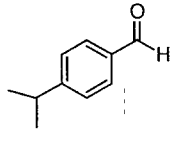
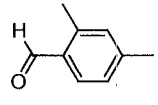
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 25. Isoamyl benzoate | 2058 | 94-46-2  | 250 | 33 | 1 | 216 | 1 |
| 26. *cis*-3-hexenyl benzoate | 3688 | 25152-85-6  | 1[e] | 0.1 | 0.002 | 138 | 138 |
| 27. Linalyl benzoate | 2638 | 126-64-7  | 14[e] | 2 | 0.03 | + | NA |
| 28. Geranyl benzoate | 2511 | 94-48-4  | 0.2 | 0.03 | 0.0004 | – | NA |
| 29. Glyceryl tribenzoate | 3398 | 614-33-5  | 370[f] | 65 | 1 | – | NA |
| 30. Propylene glycol dibenzoate | 3419 | 19224-26-1  | 104[f] | 18 | 0.3 | – | NA |

Table 1 (*continued*)

| Flavoring ingredient | FEMA no. | CAS no. and structure | Most recent annual volume (kg)[a] | Daily per capita intake ("eaters only")[b] | | Annual volume in naturally occurring foods (kg)[c] | Consumption ratio[d] |
|---|---|---|---|---|---|---|---|
| | | | | μg/d | μg/kg bw/d | | |
| 31. Methylbenzyl acetate (mixed *o*, *m*, *p*) | 3702 | 17373-93-2  17369-57-2  2216-45-7  | 0.5[g] | 0.08 | 0.001 | + | NA |
| 32. *p*-Isopropylbenzyl alcohol | 2933 | 536-60-7  | 2 | 0.3 | 0.004 | + | NA |
| 33. 4-Ethylbenzaldehyde | 3756 | 4748-78-1  | 45[h] | 8 | 0.1 | + | NA |
| 34. Tolualdehydes (mixed *o*, *m*, *p*) | 3068 | 1334-78-7 (mixture) 529-20-4  620-23-5  104-87-0  | 8618 | 1135 | 19 | + | NA |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 35. Tolualdehyde glyceryl acetal (mixed *o*, *m*, *p*) | 3067 | 1333-09-1  1333-11-5  4757-23-7  | 5 | 1 | 0.01 | – | NA |
| 36. Cuminaldehyde | 2341 | 122-03-2  | 7 | 1 | 0.02 | + | NA |
| 37. 2,4-Dimethylbenzaldehyde | 3427 | 15764-16-6  | 1 | 0.1 | 0.002 | + | NA |

[a] From Lucas et al. (1999) or NAS (1970, 1982, 1987).

[b] Intake (μg/person/day) calculated as follows: [[(annual volume (kg)) × ($1 \times 10^9$ μg/kg)]/[population × survey correction factor × 365 days]], where population (10%, "eaters only") = $26 \times 10^6$ for USA; where correction factor = 0.6 for NAS surveys and 0.8 for the Lucas et al. USA survey representing the assumption that only 60% and 80% of the annual flavor volume, respectively, was reported in the poundage surveys (Lucas et al., 1999; NAS, 1970, 1982, 1987). Intake (μg/kg bw/d) calculated as follows: [(μg/person per day)/body weight], where body weight = 60 kg. Slight variations may occur from rounding.

[c] Quantitative data for United States reported by Stofberg and Grundschober (1987).

[d] The consumption ratio is calculated as follows: (annual consumption via food, kg)/(most recent reported volume as a flavoring substance, kg); NA = data not available.

[e] NAS (1970).

[f] NAS (1982).

[g] NAS (1987).

[h] The volume cited is the anticipated annual volume, which was the maximum amount of flavor ingredient estimated to be used annually by the manufacturer at the time the material was proposed for flavor use.

1987; Lucas et al., 1999) (see Table 1). Approximately 94% of the total annual volume in USA is accounted for by three substances in the group [benzyl alcohol (No. 1), benzaldehyde (No. 13), and benzyl benzoate (No. 11)]. Approximately 59% of the total annual volume in USA is accounted for by benzaldehyde. The daily *per capita* intake [1] of each agent is reported in Table 1.

In addition to exposure through food and flavor sources, benzoic acid has been found endogenously in the human body. Endogeneus benzoic acid is formed through the phenylalanine–tyrosine pathway (JECFA, 1980, 1997).

Thirty (30) of the 37 substances have been reported to occur naturally in foods. They have been detected in a wide variety of fruits (apple, avocado, blackberry, blueberry, cherry, cranberry, melon, papaya, plum, raspberry, strawberry), vegetables (artichokes, asparagus, beans, cabbage, corn, leek, mushroom, potato, tomato), meats (cooked beef, cooked and roasted chicken, cured pork, shell fish), cheeses (gruyere, cheddar, mozzarella, parmesan), teas, and wines (Maarse et al., 1999).

Quantitative natural occurrence data is reported for 10 substances in the group. Five of these substances show that human oral exposure occurs predominantly in food (i.e., consumption ratio greater than or equal to 1), while the other five substances have consumption ratio less than 1 (See Table 1) (Stofberg and Kirschman, 1985; Stofberg and Grundschober, 1987).

## 3. Hydrolysis, absorption, distribution, excretion and metabolism

### 3.1. Hydrolysis

In general, aromatic esters are hydrolyzed in vivo through the catalytic activity of carboxylesterases or esterases (Heymann, 1980), the most important of which are the A-esterases. These enzymes are found throughout mammalian tissues (Anders, 1989; Heymann, 1980), but predominate in hepatocytes (Heymann, 1980).

Hydrolysis of benzyl and benzoate esters to yield the corresponding alcohols and carboxylic acids and hydrolysis of acetals to yield benzaldehyde and simple aliphatic alcohols have been reported in several in vitro experiments (see Fig. 1).

Benzyl acetate (No. 3) when incubated with rat plasma in vitro was rapidly hydrolyzed to benzyl alcohol (No. 1) with peak alcohol concentration after 4 minutes. No plasma benzyl alcohol was observed in vivo following gavage or feed administration of benzyl acetate to rats; however high plasma levels of hippuric acid and benzoic acid were detected. The absence of benzyl acetate in plasma is evidence that benzyl acetate is rapidly hydrolyzed to benzyl alcohol, which is then rapidly oxidized to benzoic acid in vivo (Yuan et al., 1995).

Benzyl acetate was readily hydrolyzed in pig liver homogenate (Heymann, 1980). The plasma half-lives ($t_{1/2}$) for the in vitro hydrolysis of a series of four alkyl benzoates [including methyl benzoate (No. 19), ethyl benzoate (No. 20) and propyl benzoate (No. 21)] and two aryl benzoates in 80% human blood plasma decreased from 210 min for ethyl benzoate to 24 min for butyl benzoate and 19 and 15 min for phenyl benzoate and benzyl benzoate, respectively (Nielson and Bundgaard, 1987).

An in vitro hydrolysis study found that benzyl phenylacetate (No. 12) was 90% hydrolyzed within 1 h and completely hydrolyzed within 2 h of incubation with a 2% pancreatin solution (Leegwater and van Straten, 1974).

Benzaldehyde related acetals readily hydrolyze to their component alcohols and benzaldehyde under acidic conditions. Benzaldehyde propylene glycol acetal (No. 16) was 97% hydrolyzed after incubation for 5 h with simulated gastric juice and intestinal fluid in vitro compared to blank incubation (Morgareidge, 1962).

### 3.2. Absorption, distribution and excretion

The benzyl derivatives are rapidly absorbed through the gut, metabolized primarily in the liver, and excreted in the urine as glycine conjugates of benzoic acid derivatives (Davison, 1971; Abdo et al., 1985; Temellini, 1993). Recent studies on the effect of dose, species, sex and mode of administration on the absorption, distribution and excretion of these substances are described in detail below.

Groups of 50 male Fischer 344 rats and 50 male B6C3F$_1$ mice were administered $^{14}$C-benzyl acetate (No. 3) orally at levels of 5–500 mg/kg bw for rats and 10–1000 mg/kg bw for mice for five days per week for a period of two weeks. Urine and feces were collected separately over a period of 24 h following dosing. Carbon dioxide ($CO_2$) and other volatile substances were collected at 2, 4, 5, and 24 h following each dose. At termination, blood, liver, muscle, adipose, skin, lung, kidney, and stomach were collected and analyzed for

[1] Intake (μg/kg) calculated as follows: [[(annual volume, kg) × ($1 \times 10^9$ μg/kg)]/[population × correction factor × 365 days]], where population (10%, "eaters only") = $26 \times 10^6$ for USA; where correction factor = 0.8 for the Lucas et al. and 0.6 for NAS USA surveys, and represents the assumption that only 80% and 60%, respectively, of the flavor volume was reported in the surveys (Lucas et al., 1999; NAS, 1970, 1982, 1987). For agents that were not surveyed the anticipated volume was used with a correction factor of 0.6. Intake (μg/kg bw per day) calculated as follows: [(μg per day)/body weight], where body weight = 60 kg. Slight variations may occur from rounding off.

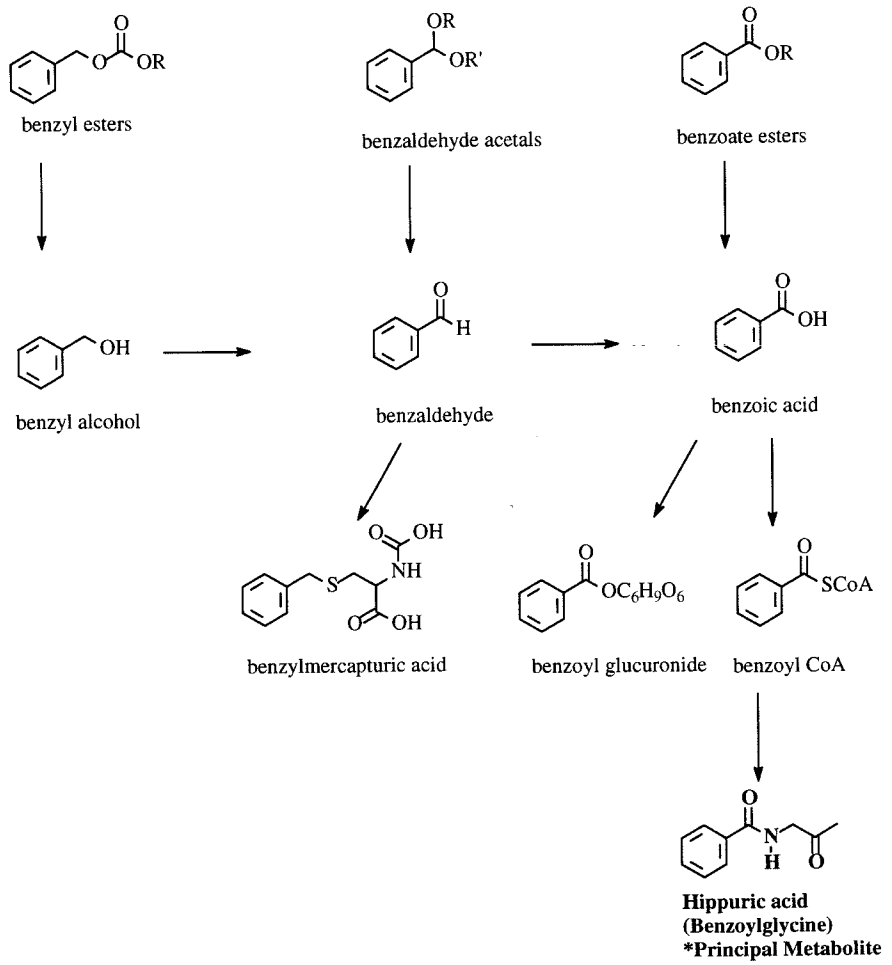



Fig. 1. Metabolism of benzyl derivatives.

radioactivity. The compound was readily absorbed from the gastrointestinal tract of both species, and approximately 90% and 0.3–1.3% of the total dose was recovered as hippuric acid in the urine and feces, respectively, within 24 h. No benzyl acetate-derived radioactivity was detected in any tissue analyzed at 24 h. The clearance pattern was not affected by repeated dosing 5 days/week for 2 weeks at 500 mg/kg bw in rats or 1000 mg/kg bw in mice. Such complete clearance indicates that benzyl acetate is readily absorbed and excreted (Abdo et al., 1985).

Following gavage administration of $^{14}$C-benzyl acetate to groups of three or more male Fischer 344 rats at doses of 5, 250 or 500 mg/kg bw as the substance alone, in corn oil, or propylene glycol, 70–89% of the dose was excreted in the urine within 24 h. Approximately 4% radioactivity was detected in the feces after 72 h. The elimination of benzyl acetate and metabolites, regardless of vehicle, was largely complete after 3 days. Only negligible residues were found in tissues. Although no benzyl acetate was detected in the plasma or urine, small amounts of benzyl alcohol were detected in the plasma. At 500 mg/kg bw, unconjugated benzoic acid was the major plasma metabolite while at 5 mg/kg, hippuric acid was the major urinary metabolite. The proportion of the glucuronic acid conjugate of benzoic acid increased with increasing dose, while low levels (1.0–3.6%) of benzoic acid and benzylmercapturic acid were not affected by dose or vehicle (Chidgey and Caldwell, 1986).

Fischer 344 rats and C57Bl/6N mice were given a single oral dose of $^{14}$C benzyl acetate at doses of 5 or 500 mg/kg bw (rats) or 10 mg/kg bw (mice) to determine the effects of age on disposition of benzyl acetate (McMahon et al., 1989). Age groups studied were 3–4, 9, and 25-month-old rats and 2, 13, and 25-month-old mice. In rats, approximately 80% of radioactivity was recovered in the urine in the first 24 h for all age groups. The major urinary metabolite was hippuric acid and a minor urinary metabolite was benzylmercapturic acid in rats. There was no age difference in the percentage of $^{14}$C benzyl acetate excreted as hippuric acid, but the amount of excreted benzylmercapturic acid increased slightly in the 25-month-old rats. The percentage of

radioactivity excreted in the feces was slightly decreased in the 25-month-old group.

In mice, hippuric acid was the major urinary metabolite, constituting 93–96% of the total dose. Less radioactivity was excreted in the urine of 25-month-old mice than in the younger groups. Fecal excretion was a minor route and the amount was similar for all age groups. The authors concluded that formation of hippuric acid is not affected by age, but aging does affect the minor routes of metabolism and excretion of benzyl acetate in rats and mice (McMahon et al., 1989).

In a study of the influence of gavage and dietary administration on the toxicokinetics of benzyl acetate, plasma levels of benzyl alcohol, benzoic acid and hippuric acid were measured at 24 h intervals after groups of 6 F344 rats were given 500 mg/kg bw of benzyl acetate by gavage in corn oil, and groups of 12 B6C3F$_1$ mice were given 1,000 mg/kg bw of benzyl acetate by the same route. Groups of 10 rats and 10 mice were fed diets containing 2700 ppm and 10,800 ppm, respectively, of benzyl acetate for 7 days. The calculated daily dose levels were reported to be approximately 648 mg/kg bw in rats and 900 mg/kg bw in mice. Benzyl acetate was undetectable in the plasma after gavage or dietary administration. After gavage administration, benzyl alcohol could not be detected in the plasma after 5 min in mice and 10 min in rats. Peak plasma levels of benzoic acid and hippuric acid were reached within 3 h of gavage administration. Compared to the gavage mode of administration, peak plasma concentrations of benzoic acid were 40-fold less in rats and 300-fold less in mice after dietary administration. Plasma concentrations of hippuric acid were similar regardless of the mode of administration (Yuan et al., 1995).

Following administration of 375 mg $^{14}$C benzoic acid (No. 18)/kg bw to rats (oral) and mice (ip), 88–89% of the radioactivity was recovered in the urine within 24 h and 91–94% after 72 h, and only 1–6% was present in the feces (Nutley, 1990).

In a study on the effects of benzyl alcohol in neonates, 4 full-term and 9 pre-term infants received intravenous (iv) or intramuscular (im) doses of 0.007–0.222 μmol benzyl alcohol (No. 1)/kg bw in medication. Maximum serum concentration levels of benzoic acid in pre-term infants were approximately 10 times those in full-term infants. Larger percentages of benzyl alcohol doses were found as benzoic acid {75.2% (iv) and 83.8%(im) vs. 38.1%(iv) and 57.7%(im)} and less as hippuric acid {64.2%(iv) and 26.3%(im) vs. 82.3%(iv) and 85.2%(im)} in pre-term infants compared to term infants. The study indicates that glycine conjugation is deficient in pre-term compared to full-term infants (LeBel et al., 1988).

After administration of oral doses of 40, 80, and 160 mg/kg bw of sodium benzoate to humans, the clearance of benzoic acid increased disproportionately to the dose while the clearance for hippuric acid was proportional to dose. Peak plasma concentrations of benzoic acid increased with increasing dose, while peak hippuric acid concentrations did not change. The data suggest that the conjugation with glycine to form hippuric acid is a saturable process in humans (Kubota et al., 1988; Kubota and Ishizaki, 1991).

Based on these studies, benzyl derivatives are expected to be rapidly absorbed, and rapidly metabolized to benzoic acid. The acid is then conjugated with glycine and excreted mainly in the urine. At high dose levels, the glycine conjugation pathway may be saturated; in which case, free benzoic acid is excreted unchanged.

### *3.3. Metabolism*

#### *3.3.1. Metabolism of benzyl alcohol, benzaldehyde, benzoic acid and related esters*

Benzyl esters and acetals are hydrolyzed to benzyl alcohol and benzaldehyde, respectively (see Fig. 1). The alcohol and aldehyde are rapidly oxidized to benzoic acid while benzoate esters are hydrolyzed to benzoic acid. The benzoic acid derivatives are excreted primarily as the glycine conjugate. Benzoic acid is readily conjugated with glycine in liver (321 nmol/min/g) and kidney homogenate (254 nmol/min/g) (Temellini, 1993). At high dose levels formation of the glycine conjugate is glycine limited. When glycine is depleted, free benzoic acid may be excreted unchanged or as the glucuronic acid conjugate (Bray et al., 1951; Diack and Lewis, 1928; Williams, 1959).

Following oral, subcutaneous, or ip administration of $^{14}$C benzyl acetate to mice (10–1000 mg/kg bw) and rats (5–500 mg/kg bw), hippuric acid was the major urinary metabolite. Minor amounts of benzyl alcohol, benzoic acid and benzylmercapturic acid were also detected in the urine (Abdo et al., 1985; Chidgey and Caldwell, 1986; Yuan et al., 1995; Clapp and Young, 1970; McMahon et al., 1989).

Using specific enzyme inhibitors, a pathway was proposed for the metabolic conversion of benzyl acetate to benzylmercapturic acid in male Fischer rats. Five hundred (500) mg/kg bw of $^{14}$C benzyl acetate was administered to groups of 3–5 male Fischer rats by gavage, alone or in conjunction with ip injections of 200 mg/kg bw of pyrazole (an alcohol dehydrogenase inhibitor), 10 mg/kg bw of pentachlorophenol (a sulfotransferase inhibitor) or both. Within 24 h of administration of benzyl acetate alone, 92% of the dose was recovered in the urine, 3% in the feces and <1% in the carcasses. The combination treatment of benzyl acetate and inhibitor delayed the excretion of $^{14}$C in urine compared to treatment with benzyl acetate alone. The pyrazole only group had an 11-fold increase in the amount of benzylmercapturic acid excreted in the first 24 h with a halving of the percentage of benzoyl glucuronide eliminated in the

urine. The elimination of hippuric acid was unaffected in the pyrazole treated group as compared to controls. The amount of benzylmercapturic acid was significantly increased in the combination group, but not to the same levels as the pyrazole only group. Benzyl sulphate has been shown to be converted to benzylmercapturic acid in rats (Clapp and Young, 1970) and S-benzylglutathione is formed in the presence of rat liver cytosol (Gillam, 1971). Benzyl alcohol is converted to benzylmercapturic acid in rats involving an obligatory benzylsulphate intermediate (Van Doorn et al., 1981). In the case of benzyl acetate, these results suggest that benzylmercapturic acid forms via the sulfate ester of benzyl alcohol (Chidgey et al., 1986).

Less than 5 min after single intraperitoneal doses of 770–1100 mg/kg bw of benzyl alcohol given to CD1 mice, benzyl alcohol and benzaldehyde were detected in the plasma. Animals pretreated with an alcohol dehydrogenase inhibitor (pyrazole) resulted in a 200% increase in plasma benzyl alcohol levels, while pretreatment with an aldehyde inhibitor (disulfiram) resulted in a 368% increase in plasma benzaldehyde levels. These data suggest that benzaldehyde and benzyl alcohol are interconvertible in the liver (McCloskey et al., 1986).

The principal pathway of metabolism of benzaldehyde (No. 13) includes oxidation to yield benzoic acid, which is subsequently conjugated with glycine and excreted as hippuric acid (Bray et al., 1951; NTP, 1990a,b). In the rabbit, approximately 83% of single doses of 350 or 750 mg/kg bw of benzaldehyde is excreted in the urine of both dose groups. The aldehyde is oxidized mainly to benzoic acid and excreted predominantly as hippuric acid (~68%). Other urinary metabolites detected are benzoylglucuronic acid (10%), benzoyl glucuronide (3%), free benzoic acid (1.5%), and trace amounts of benzylmercapturic acid (Laham et al., 1988).

To a minor extent, benzaldehyde is reduced to benzyl alcohol, which as the sulfate conjugate may react with glutathione to form benzylmercapturic acid (Laham and Potvin, 1987). Groups of Sprague–Dawley rats (5/group/sex) were given single oral doses of 400, 750, or 1000 mg/kg bw of pure benzaldehyde by gavage daily for 13 consecutive days. Twenty-four (24) h urinary excretion of benzylmercaputric acid was measured after the 2nd, 8th, and 13th dose. Although females in the mid- and high-dose groups exhibited a slight decrease in excretion of benzylmercapturic acid after the 8th dose, all groups showed increased urinary levels of the conjugated acid after 13 doses. An increase in dose from 400 to 1000 mg/kg bw per day resulted in a 7–8-fold increase in excreted benzylmercapturic acid.

Administration of 375 mg $^{14}$C- benzoic acid/kg bw to mice (ip) and rats (oral) resulted in excretion of hippuric acid (70.2–84.2%), benzoyl glucuronide (0.7–1.8%), benzoic acid (0.4–12.8%), and 3-hydroxy-3-phenyl propionic acid (0.1–0.2%) (Nutley, 1990).

$^{14}$C-Benzoic acid was administered orally at doses in the range from 1 to 400 mg/kg bw to various species including primates, pigs, rabbits, rodents, cats, dogs, hedgehogs, bats, birds, and reptiles. Hippuric acid was the primary urinary metabolite in most species. The ornithine conjugate of benzoic acid, ornithic acid, was the major urinary metabolite excreted within 24 h in chickens and reptiles. Benzoyl glucuronide was predominant in the fruit bat. In humans, >99% $^{14}$C was excreted as hippuric acid within 24 h (Bridges et al., 1970).

Male volunteers were given oral doses of 2000–5000 mg sodium benzoate. The 5000 mg dose group was given a 5000 mg dose of glycinel h later and 2000 mg doses every 2 h thereafter. Benzoate was excreted mainly as hippuric acid. No free benzoic acid was detected. Minor amounts of benzoylglucuronide were detected, with more formed at the 5000 mg dose than at the 2000 mg dose. Co-administration of glycine with benzoate increased the rate of hippuric acid excretion, indicating that at high dose levels, glycine is rate limiting for formation of hippuric acid (Amsel and Levy, 1969).

### *3.3.2. Metabolism of substituted benzyl alcohols, benzaldehydes and benzoic acid derivatives*

Aromatic ring substitution has little or no influence on the principal pathway of metabolism. Oxidation of the alcohol or aldehyde to the benzoic acid derivative may be accompanied by minor amounts of side-chain oxidation or *O*-dealkylation of a ring alkoxy group.

Large doses (2 g) of cuminaldehyde (*p*-isopropylbenzaldehyde, No. 37) were administered orally to male rabbits. Urine was collected for 3 days post-treatment. Cuminaldehyde undergoes a combination of oxidation of the aldehyde function and the oxidation of the alkyl-side chain to yield 9-hydroxycuminic acid, 8-hydroxycuminic acid. Cumyl alcohol and 2-carboxyphenylpropionic acid were minor urinary metabolites (Ishida et al., 1989).

### *3.3.3. Summary of metabolic data*

In summary, benzyl and benzoate esters and benzaldehyde acetals are readily hydrolyzed to the corresponding parent alcohol, aldehyde, or acid. Following hydrolysis, benzyl alcohol is sequentially oxidized to benzaldehyde and then benzoic acid, followed by excretion as hippuric acid. To a minor extent, benzyl alcohol may conjugate with glutathione, benzaldehyde may be reduced to benzyl alcohol, and benzoic acid may conjugate with glucuronic acid. The latter conjugation pathway may become more important as high levels of benzoic acid saturate the glycine (hippurate) conjugation pathway. At very high levels of exposure, free

benzoic acid may sequester significant quantities of acetyl CoA.

## 4. Toxicological studies

### 4.1. Acute toxicity

Oral $LD_{50}$ values have been reported for 30 of the 37 substances in this group (see Table 2). $LD_{50}$ values in rats are in the range from 1020 mg/kg bw for *p*-isopropylbenzyl alcohol (No. 32) to 12,300 mg/kg bw for hexyl benzoate (No. 22), indicating that the oral acute toxicity of the benzyl derivatives is very low (Graham and Kuizenga, 1945; Draize et al., 1948; Smyth et al., 1951, 1954; Jenner et al., 1964; Taylor et al., 1964; Sporn et al., 1967; Kravets-Bekker and Ivanova, 1970; Owen and Meyer, 1971; Shelanski and Moldovan, 1971; Weir and Wong, 1971; Levenstein, 1973, 1974; Moreno, 1973, 1974, 1977, 1979, 1980; DeGroot et al., 1974; Lewis and Palanker, 1979; Costello and Moore, 1984; Ciba-Geigy, 1991; Proctor and Gamble, 1992). In mice the oral $LD_{50}$ values are in the range from 1580 mg/kg bw for benzyl alcohol (No. 1) to 9408 mg/kg bw for linalyl benzoate (No. 27) indicating that the acute oral toxicity of benzyl derivatives is very low (Jenner et al., 1964; Hoffmann-LaRoche, 1967; Kravets-Bekker and Ivanova, 1970; Sado, 1973; Shell, 1982; Schafer and Bowles, 1985). In rabbits, guinea pigs and cats the oral LD50 values are in the range from 1000 mg/kg bw for benzaldehyde (No. 13) to greater than 5000 mg/kg bw for linalyl benzoate (No. 27) indicating that the acute oral toxicity of benzyl derivatives is very low (Graham and Kuizenga, 1945; Draize et al., 1948; Jenner et al., 1964; Kravets-Bekker and Ivanova, 1970; Moreno, 1973).

### 4.2. Short-term and long-term studies of toxicity

Short-term toxicological studies have been reported for nine (9) representative benzyl derivatives. Short-term studies for benzyl alcohol, one benzyl ester, three benzoate esters, and two alkyl-substituted benzaldehyde derivatives are summarized in Table 2 and described in detail below.

#### 4.2.1. Mice

*4.2.1.1. Benzyl alcohol (No. 1).* Groups of 10 male and 10 female B6C3F$_1$ mice were administered 0 (control), 50, 100, 200, 400, or 800 mg/kg bw benzyl alcohol daily by gavage 5 days per week for a period of 13 weeks. No compound related histopathology was observed. Deaths occurred in most groups, however, all but one was attributed to the gavage procedure. Mean body weights of males and females were reduced by 5% and 8%, respectively, at the 800 mg/kg bw per day dose level and body weights of females were reduced 5% at the 400 mg/kg bw per day. At the highest dose level, staggering was seen in both male and female mice during the first 2 weeks of the study. The dose level of 100 mg/kg bw per day produced no adverse effects (NTP, 1989).

*4.2.1.2. Benzyl acetate (No. 3).* Benzyl acetate was administered to groups of 10 male B6C3F$_1$ mice in corn oil daily by gavage at doses of 0 (control), 62.5, 125, 250, 500, or 1,000 mg/kg bw 5 days per week for a period of 13 weeks. Groups of 10 female mice were administered benzyl acetate by gavage at doses of 0 (control), 125, 250, 500, 1,000, or 2,000 mg/kg bw on the same schedule. Eight of the 10 female mice that received 2,000 mg/kg bw died; however, one of these deaths was due to gavage error. Compound-related clinical signs, including trembling, inactivity, labored breathing, and depressed body temperature, were reported in the high-dose mice. Gross examination after necropsy and histopathological examination failed to reveal any effects related to administration of the test substance (NTP, 1986). Subsequent microscopic examination revealed hippocampal necrosis in one female at the 1000 mg/kg bw per day dose (NTP, 1993a). No adverse effects were observed at dose levels less than or equal to 500 mg/kg bw per day (NTP, 1986).

For a period of 13 weeks, 5 days per week, groups of 10 male and 10 female B6C3F$_1$ mice were maintained on diets containing 0, 3130, 6250, 12,500, 25,000, or 50,000 ppm benzyl acetate calculated to provide an average daily intake of 0 (control), 425, 1000, 2000, 3700, or 7900 mg benzyl acetate/kg bw for males and 650, 1280, 2980, 4300, or 9400 mg/kg bw for females (FDA, 1993). At all dose levels, statistically significant ($P < 0.01$) decreases in body weights were reported compared to the control group in both sexes. Absolute and relative organ weights were affected by lower terminal body weights. A non-statistically significant decrease in feed consumption was reported at all dose levels, which may have been due to poor palatability. Hematological examination and clinical chemistry determinations show normal values. In the 50,000 ppm group, brain lesions, consisting of hippocampal necrosis and cerebellar hemorrhage, were reported in 4 mice (1 male, 3 females). Hepatocellular necrosis also occurred in the male mouse with brain lesions (NTP, 1993a).

*4.2.1.3. Benzaldehyde (No. 13).* Groups of 10 male and 10 female B6C3F$_1$ mice were administered doses of 0 (control), 75, 150, 300, 600 or 1200 mg benzaldehyde/kg bw by gavage daily in corn oil 5 days per week for a period of 13 weeks. At the 1200 mg/kg bw per day dose, 9/10 males and 1/10 females died during the first week of the study. Mean body weight of males at the 600 mg/kg bw per day level were 9% less than that of controls. Mild to moderate renal tubule degeneration

Table 2
Acute, short- and long-term toxicity studies for benzyl derivatives used as flavor ingredients

| Flavoring ingredient | Oral acute studies | Reference | Short- and long-term studies | Time (days)/ route | NOAEL (mg/kg bw) | Reference |
|---|---|---|---|---|---|---|
| | Oral $LD_{50}$ mg/kg bw (Species) | | Species, sex[a] | | | |
| Benzylalcohol | 1040 (Rabbit) | Graham and Kuizenga (1945) | Rat, MF | 91 | 100 | NTP (1989) |
| Benzylalcohol | 2979 (Rat) | Ciba-Geigy (1991) | Rat, MF | 16 | 125 | NTP (1989) |
| Benzylalcohol | 2080 (Rat) | Graham and Kuizenga (1945) | Rat, MF | 721 | <200 | NTP (1989) |
| Benzylalcohol | 1230 (Rat) | Jenner et al. (1964) | Mouse, MF | 91 | 100 | NTP (1989) |
| Benzylalcohol | 1570 (Rat) | Proctor and Gamble (1992) | Mouse, MF | 16 | 250 | NTP (1989) |
| Benzylalcohol | 3100 (Rat) | Smyth et al. (1951) | Mouse, MF | 721 | 200 | NTP (1989) |
| Benzylalcohol | 1580 (Mouse) | Jenner et al. (1964) | | | | |
| Benzyl formate | <5000 (Rat) | Shelanski and Moldovan (1971) | Rat, MF | 14 | 500 | NTP (1986) |
| Benzyl acetate | 2640 (Rabbit) | Graham and Kuizenga (1945) | Rat, MF | 91 | 250 | NTP (1986) |
| Benzyl acetate | 2490 (Rat) | Jenner et al. (1964) | Rat, MF | 91 | 460 | NTP (1993a) |
| Benzyl acetate | 3690 (Rat) | Graham and Kuizenga (1945) | Rat, MF | 721 | <130 | NTP (1993a) |
| Benzyl acetate | | | Rat, MF | 721 | 250 | NTP (1986) |
| Benzyl acetate | | | Mouse, MF | 14 | 1000 | NTP (1986) |
| Benzyl acetate | | | Mouse, M | 91 | 500 | NTP (1986) |
| Benzyl acetate | | | Mouse, MF | 721 | <35 | NTP (1993a) |
| Benzyl acetate | | | Mouse, MF | 721 | <500 | NTP (1986) |
| Benzyl propionate | 3300 (Rat) | Moreno (1973) | | | | |
| Benzyl butyrate | 1850 (Rat) | Moreno (1973) | | | | |
| Benzyl butyrate | 2330 (Rat) | Jenner et al. (1964) | | | | |
| Benzyl isobutyrate | 2850 (Rat) | Owen and Meyer (1971) | | | | |
| Benzyl isovalerate | <5000 (Rat) | Moreno (1974) | | | | |
| Benzyl *trans*-2-methyl-2-butenoate | >5000 (Rat) | Moreno (1979) | | | | |
| Benzyl benzoate | 2240 (Cat) | Graham and Kuizenga (1945) | | | | |
| Benzyl benzoate | 2016 (Rabbit) | Draize et al. (1948) | | | | |
| Benzyl benzoate | 1680 (Rabbit) | Graham and Kuizenga (1945) | | | | |
| Benzyl benzoate | 1120 (Guinea pig) | Draize et al. (1948) | | | | |
| Benzyl benzoate | 1904 (Rat) | Draize et al. (1948) | | | | |
| Benzyl benzoate | 2800 (Rat) | Graham and Kuizenga (1945) | | | | |
| Benzyl benzoate | 1568 (Mouse) | Draize et al. (1948) | | | | |
| Benzyl phenylacetate | >5000 (Rat) | Owen and Meyer (1971) | | | | |
| Benzaldehyde | 1000 (Guinea pig) | Jenner et al. (1964) | Rat, MF | 91 | 200 | Kluwe et al. (1983) |
| Benzaldehyde | 1300 (Rat) | Jenner et al. (1964) | Rat, MF | 91 | 200 | NTP (1990a, 1990b) |
| Benzaldehyde | 2850 (Rat) | Sporn et al. (1967) | Rat, MF | 189-196 | >50 | Hagan et al. (1967) |
| Benzaldehyde | 1300 (Rat) | Taylor et al. (1964) | Rat, MF | 721 | <200 | NTP (1990a, 1990b) |
| Benzaldehyde | 1250 (Mouse) | Schafer and Bowles (1985) | Mouse, MF | 91 | 300 | Kluwe et al. (1983) |
| Benzaldehyde | | | Mouse, MF | 91 | 300 | NTP (1990a, 1990b) |
| Benzaldehyde | | | Mouse, MF | 728 | <200 | NTP (1990a, 1990b) |
| Benzaldehyde dimethyl acetal | 1220 (Rat) | Moreno (1977) | | | | |
| Benzaldehyde glyceryl acetal | 3749 (Rat) | Levenstein (1974) | | | | |

(*continued on next page*)

Table 2 (*continued*)

| Flavoring ingredient | Oral acute studies | Reference | Short- and long-term studies | Time (days)/ route | NOAEL (mg/kg bw) | Reference |
|---|---|---|---|---|---|---|
| | Oral $LD_{50}$ mg/kg bw (Species) | | Species, sex[a] | | | |
| Benzaldehyde glyceryl acetal | 2750 (Rat) | Moreno (1980) | | | | |
| Benzaldehyde propylene glycol acetal | 3000 (Rat) | Lewis and Palanker (1979) | | | | |
| Benzoic acid | 1250 (Mouse) | Schafer and Bowles (1985) | Rat, MF | 540-730 | <370 | Sodemoto and Enomoto (1980) |
| Benzoic acid | 1996 (Mouse) | Sado (1973) | Mouse, MF | 90 | 80 | Shtenberg and Ignat'ev (1970) |
| Benzoic acid | 1950 (Mouse) | Shell (1982) | Mouse, MF | 510 | 40 | Shtenberg and Ignat'ev (1970) |
| Methyl benzoate | 2170 (Rabbit) | Graham and Kuizenga (1945) | Rat | 183 | 0.005 | Kravets-Bekker and Ivanova (1970) |
| Methyl benzoate | 4100 (Guinea pig) | Kravets-Bekker and Ivanova (1970) | | | | |
| Methyl benzoate | 2170 (Rat) | Graham and Kuizenga (1945) | | | | |
| Methyl benzoate | 1350 (Rat) | Jenner et al. (1964) | | | | |
| Methyl benzoate | 3500 (Rat) | Kravets-Bekker and Ivanova (1970) | | | | |
| Methyl benzoate | 3420 (Rat) | Smyth et al. (1954) | | | | |
| Methyl benzoate | 3330 (Mouse) | Jenner et al. (1964) | | | | |
| Methyl benzoate | 3000 (Mouse) | Kravets-Bekker and Ivanova (1970) | | | | |
| Ethyl benzoate | 2630 (Rabbit) | Graham and Kuizenga (1945) | | | | |
| Ethyl benzoate | 2100 (Rat) | Graham and Kuizenga (1945) | | | | |
| Ethyl benzoate | 6480 (Rat) | Smyth et al. (1954) | | | | |
| Hexyl benzoate | 12,300 (Rat) | Smyth et al. (1951) | | | | |
| Isopropyl benzoate | 3730 (Rat) | Smyth et al. (1951) | | | | |
| Isobutyl benzoate | 3685 (Rat) | Levenstein (1973) | | | | |
| Isoamyl benzoate | 6330 (Rat) | Weir and Wong (1971) | | | | |
| *cis*-3-Hexenyl benzoate | >5000 (Rat) | Moreno (1976) | | | | |
| Linalyl benzoate | >5000 (Rabbit) | Moreno (1973) | | | | |
| Linalyl benzoate | >5000 (Rat) | Moreno (1973) | | | | |
| Linalyl benzoate | 9408 (Mouse) | Hoffmann-LaRoche (1967) | | | | |
| Geranyl benzoate | >5000 (Rat) | Moreno (1973) | | | | |
| Glyceryltribenzoate | | | Rat, MF | 90 | 604 | Carson (1972a) |
| Propylene glycol dibenzoate | | | Rat, MF | 90 | 2541 | Carson (1972b) |
| *p*-Isopropylbenzyl alcohol | 1020 (Rat) | Moreno (1973) | | | | |
| 4-Ethylbenzaldehyde | 1970 (Rat) | Costello and Moore (1984) | | | | |
| Tolualdehydes (mixed *o*, *m*, *p*) | 2250 (Rat) | Moreno (1973) | Rat, MF | 90 | 36.1 | Oser et al. (1965) |
| Tolualdehydes (mixed *o*, *m*, *p*) | | | Rat, MF | 91 | 250 | Brantom et al. (1972) |
| Tolualdehyde glyceryl acetal | 3400 (Rat) | Moreno (1972) | | | | |
| Cuminaldehyde | 1390 (Rat) | Jenner et al. (1964) | | | | |
| 2,4-Dimethyl-benzaldehyde | <5000 (Rat) | DeGroot et al. (1974) | Rat, MF | 14 | 1.75 | DeGroot et al. (1974) |

[a] M = Male; F = Female. If not listed, sex was not specified in the report.

was reported in all males at the 1200 mg/kg bw per day dose level and 1 male at the 600 mg/kg bw per day dose level. These lesions were not observed in lower dose levels in males or any dose level in females. The no adverse

effect level was reported to be 300 mg/kg bw per day (NTP, 1990a).

*4.2.1.4. Benzoic acid (No. 18).* Groups of 50 male and 50 female crossbred white mice (strain not specified) were administered 80 mg benzoic acid/kg bw by oral intubation daily for 3 months. Weight gain of the test animals was reduced compared to control animals. This was not due to reduced feed intake, but it may have been due to stress factors such as food restriction, low temperature, swimming test, centrifugation, carbon tetrachloride detoxication test or kidney function testing (Shtenberg and Ignat'ev, 1970). Benzoic acid was administered orally to groups of 25 male and 25 female mice at 40 mg/kg bw daily for 17 months. Survival at 2.5 months was greater for the benzoic acid group (68%) than that for the control group of males (60%) or female rats (62%) (Shtenberg and Ignat'ev, 1970).

*4.2.2. Rats*

*4.2.2.1. Benzyl alcohol (No. 1).* Benzyl alcohol was administered to groups of 10 male and 10 female F344/N rats at doses of 0 (control), 50, 100, 200, 400 or 800 mg/kg bw in corn oil daily by gavage five days per week for a period of 13 weeks. After dosing, staggering, lethargy and labored breathing occurred in both sexes at 800 mg/kg bw. Eight (8) males and 2 females at 800 mg/kg bw per day dose died after treatment, with the deaths of 4 males and one female being attributed to gavage errors. Reduction in the relative weight gain of female rats in the 200 and the 400 mg/kg bw per day groups was observed. At the end of the study, body weights of males and females at the 800 mg/kg bw level were reduced by 7% and 5%, respectively. At the 800 mg/kg bw per day level, histopathologic examination revealed necrosis of the dentate gyrus of the hippocampus in all surviving male (9) and female (7) rats, skeletal muscle necrosis in 5 males, kidney nephrosis in 6 males, consisting of degeneration of the tubular epithelium. The renal lesions were non-specific and similar to age related renal disease. No adverse effects were reported at 100 mg/kg bw per day (NTP, 1989).

*4.2.2.2. Benzaldehyde (No. 13).* Groups of 10 male and 10 female F344/N rats were administered benzaldehyde five days per week for a period of 13 weeks by corn oil gavage at doses of 0 (control), 50, 100, 200, 400 or 800 mg/kg bw. Prior to the end of the study, 6/10 males and 3/10 females at 800 mg/kg bw per day, 1/10 female at the 400 mg/kg bw per day and one control female died. Surviving male rats at 800 mg/kg bw per day had body weights 26% lower than those of controls. At the highest dose, there was necrosis of the cerebellum and neurons of the hippocampus, forestomach hyperplasia and hyperkeratosis, liver degeneration (males only), and kidney tubular epithelial degeneration. No adverse effects were observed at dose levels of 200 mg/kg (NTP, 1990a,b).

Groups of 5 male and 5 female Osborne–Mendel rats were maintained on a diet calculated to provide an average daily intake of 50 mg benzaldehyde/kg of food for a period of 27–28 weeks (Hagan et al., 1967). Weekly measurement of body weight and food intake revealed no significant difference between test animals and controls. At termination, hematological examinations revealed normal values. At necropsy, no differences were reported between major organ weights of test and control animals. Gross examination and histopathological examinations failed to reveal any changes related to the administration of the test substance. No adverse effects were seen at the dietary level examined (50 mg/kg of food) which was calculated to be 600 μg benzaldehyde/kg bw per day (FDA, 1993).

*4.2.2.3. Benzoic acid (No. 18).* Male and female Fischer 344 rats were fed 0, 1, or 2% sodium benzoate in the diet (approximately 0, 370, or 735 mg/kg bw per day for males and 0, 445, or 880 mg/kg bw per day for females) for a period of 18–24 months (Sodemoto and Enomoto, 1980). Mortality of all groups was affected by hemorrhagic pneumonia; however, there was no reported difference in mortality, growth, or food intake between treated and control rats. There was also no significant difference in tumor response in treated as compared to control rats.

*4.2.2.4. Benzyl acetate (No. 3).* Groups of F344 male rats were maintained on diets providing 0 (control), 20,000, 35,000, or 50,000 ppm of benzyl acetate 5 days a week for 103 weeks. These dietary levels correspond to an average daily intakes of 0, 1000, 1750, 2500 mg/kg bw of benzyl acetate (FDA, 1993). No effects on mortality or body weights of rats were reported at dietary concentrations of 20,000 ppm. Compound-related neuronal necrosis was seen in the groups receiving 35,000 or 50,000 ppm benzyl acetate. When a glycine supplement was fed in conjunction with the highest dose, mortality and signs of toxicity were significantly reduced. The authors concluded that adequate levels of glycine were instrumental in reducing the toxicity of benzyl acetate (Abdo et al., 1998).

Groups of 10 male and 10 female F344/N rats were administered 0 (control), 62.5, 125, 250, 500 or 1000 mg benzyl acetate/kg bw in corn oil daily by gavage 5 days per week for a period of 13 weeks. Male and female rats receiving 1000 mg/kg bw per day and females receiving 500 mg/kg bw per day showed signs of ataxia, trembling and sluggishness. On day 86, 2 males and one female died at the highest dose. At 1000 mg/kg bw per day, males showed depressed mean body weight (12%). At necropsy, thickened stomach walls were reported in 2 of 8 surviving males and 4 of 9 surviving

females. No adverse effects were observed at 250 mg/kg bw per day (NTP, 1986). At 1000 mg/kg bw per day, histopathologic reexamination of brains revealed hippocampal necrosis in 8 males and 4 females, the severity being greater in males (NTP, 1993a).

For a period of 13 weeks, groups of 10 male and 10 female F344/N rats were maintained on diets containing 0, 3130, 6250, 12500, 25,000, or 50000 ppm of benzyl acetate. These dietary levels were calculated to provide an average daily intake of 0 (control), 230, 460, 900, 1750, or 3900 mg benzyl acetate/kg bw for males and 0 (control), 240, 480, 930, 1870, or 4500 mg benzyl acetate/kg bw for females (FDA, 1993). At the highest dose level, 9/10 rats of each sex died before completion of the study. Final mean body weights of males and females at the highest dietary level were less than half that of the control group. Males at 1750 mg/kg bw per day showed a 10% decrease in mean body weight compared to that of controls. Food consumption in the males at 1750 and 3900 mg/kg bw and females at 4500 mg/kg bw per day was reduced compared to controls. Serum cholesterol was significantly decreased in females at the three highest dose levels. Substance-related histopathology in males and females at the highest dose level included necrosis of neurons and glial cells in the cerebellum and hippocampus, renal tubule degeneration and regeneration, and degeneration and hyperplasia of the sarcolemmal nuclei in the skeletal muscles of the thigh and in the tongue. Indirect treatment-related effects secondary to the poor condition of the animals included bone marrow depletion, thymus atrophy, degeneration of splenic lymphoid follicles, zymogen granule depletion, and increased basophilia of the pancreatic acinar cells, erosion of the glandular stomach, secretory depletion in of the seminal vesicles and immature/hypoplastic uterus. At termination, examination of females of the control, 1870 and 4500 mg/kg bw per day dose groups showed an increase in the volume, surface, and numerical density of hepatic peroxisomes in treated groups compared to those of controls. At 900 mg/kg bw per day and higher dose levels, testicular changes, including aspermatogenesis and atrophy of the seminiferous tubules were seen. No adverse effects were observed at dosages of 480 mg/kg bw per day (NTP, 1993a).

In an earlier 4-month study, no pancreatic tumors were reported in male F344 and Lewis rats administered 500 mg/kg bw per day of benzyl acetate by gavage for 5 days a week (Longnecker et al., 1986).

*4.2.2.5. Benzyl butyrate (No. 5).* For a period of 12 weeks, rats (unspecified strain, 12/sex/group) were maintained on a diet containing a mixture of six aromatic esters at levels calculated to provide an average daily target intake of 0 or100 mg/kg bw per day. Six aromatic esters commonly used in foods (ethyl benzoate, 0.15 ppm; isobutyl benzoate, 25 ppm: benzyl acetate, 18.7 ppm; benzyl butyrate, 25 ppm; ethyl methylphenylglycidate, 25 ppm; and glycidate M-116, 25 ppm) were blended and incorporated in the diet in the ratio of practical use levels in foods. The blend was fed in a nutritionally adequate diet to weanling rats for the 12-week period at a level computed to be 100 times the assumed flavor intake for man (on a per kilogram of body weight basis). Throughout the experimental period the group receiving this aromatic ester blend exhibited normal body weight gain, food consumption, efficiency of food utilization, appearance, and behavior. At the end of the feeding period, blood and urine samples were taken from 3 males and 3 females. There was no significant difference between blood hemoglobin and urine glucose levels in test and control groups. Traces of albumin present in urine specimens from the control as well as the test group are not regarded as significant. At autopsy no abnormalities were observed relating to the test materials and the weights of livers and kidneys were within normal limits for both groups. These experimental data indicate that no toxic effect was manifest in rats as a result of feeding a blend of six aromatic esters in common use as flavoring agents for a 12-week period at a daily dosage level calculated to be 126 mg/kg bw per day (Oser, 1957).

*4.2.2.6. Tolualdehydes—mixed o, m, p (No. 34).* For a period of 90 days, groups of 15 male and 15 female weanling Sprague–Dawley rats, individually housed, were provided with a diet containing tolualdehyde at levels calculated to provide an average daily intake of 36.1 mg/kg bw. Substances were dissolved in acetone and blended into a basal laboratory diet to yield the required daily intake for the test substance. Control diet was the basal laboratory diet admixed with acetone. Dietary acetone was evaporated before presentation to the animals. Samples of the treatment diet were taken weekly for assessment of stability and concentration of the test material. Daily observations of appearance, behavior, appetite, elimination, gross signs of toxic effects, and mortality were similar among test and control animals. Weekly measurement of body weights and food consumption revealed no significant differences between test and control animals. Hematological examinations, blood chemical determinations, and urine analysis performed during weeks 6 and 12 on 8 males and 8 females revealed normal values. At necropsy, the weights of the liver and kidneys were recorded. Tissues from major organs of 8 male and 8 female rats were subsequently preserved in formalin. In addition, the livers and kidneys of the remaining 7 animals were examined for histopathological changes. There was no difference in absolute or relative organ weights between test and control animals or any evidence of gross pathology or histopathology. The dietary intake of

36.1 mg/kg bw per day produced no changes attributable to tolualdehyde (Oser et al., 1965) is greater than 1,000 times the daily *per capita* intake ("eaters only") of 19 μg tolualdehydes/kg bw from use as a flavoring agent in USA.

Groups of 15 male and 15 female CFE rats were administered 0 (control), 50, 250, or 500 mg tolualdehyde/kg bw in corn oil daily by oral intubation for a period of 13 weeks. Additional groups of 5 rats per sex were given 0, 250, or 500 mg/kg bw daily for 2 or 6 weeks by the same method. Weekly measurement of body weights and food and water intake revealed that the female group at 500 mg/kg bw per day exhibited significantly lower body weight after 2 weeks. No body weight changes were observed in any other group regardless of the study duration (2, 6, or 13 weeks). Hematological examination, blood chemical determinations, and urine analysis performed at 2, 6, or 13 weeks revealed a transient increase in erythrocyte count, hematocrit, and hemoglobin concentration in males, but only at 2 weeks. At necropsy at week 13, measurement of organ weights revealed a significant decrease (9–18%) in absolute or relative small intestine weight in all treatment groups and decreased relative pituitary weights in females at 500 mg/kg bw per day. The magnitude of the change in mean small intestine weights was not dose related. In a second study, groups of 30 female rats were administered 0 or 500 mg/kg bw in corn oil by gavage daily for 13 weeks. There was no significant difference in absolute or relative mean small intestine weights between the control and test group in the second study or between the control group in the second study and female dosed group in the first 13-week study. The authors noted that, for some unknown reason, the small intestine weights of the control animals in the first 13-week study were abnormally high. The organ weight changes were not associated with any evidence of gross and histopathological abnormalities. The authors reported a no observable adverse effect level of 250 mg/kg bw per day (Brantom et al., 1972).

*4.2.2.7. Glyceryl tribenzoate (No. 29) and Propylene glycol dibenzoate (No. 30).* For a period of 90 days, 4 groups each consisting of 15 male and 15 female weanling FDRL-weanling rats, individually housed, were maintained on a diet containing glyceryl tribenzoate or propylene glycol dibenzoate at levels calculated to provide an average daily intake of 120, 604 or 2571 mg/kg bw or 126, 633, or 2,541 mg/kg bw, respectively. Daily observations of appearance, behavior, appetite, elimination, gross signs of toxic effects, and mortality were similar among test and control animals. Weekly measurement of body weights and food consumption revealed a depressed growth rate and efficiency of food utilization in high dose males in the glyceryl tribenzoate study. No significant differences in these parameters were observed in the propylene glycol dibenzoate study. In either study, hematological examinations, blood chemical determinations, and urine analysis performed during weeks 6 and 12 on 8 males and 8 females from each group revealed normal values. At necropsy, the weights of the liver and kidneys were recorded. Tissues from major organs of 8 male and 8 female rats were subsequently preserved in formalin. Histopathologic examinations were made on hematoxylin and eosin-stained sections. In addition, the livers and kidneys of the remaining seven animals were examined for histopathological changes. There was no difference in absolute or relative organ weights between test and control animals or any evidence of gross pathology or histopathology in either study. Based on the depressed weight gain in high dose males, the no observable adverse effect level for glyceryl tribenzoate was reported to be 604 mg/kg bw per day (Carson, 1972a). This dose is greater than 100 times the daily *per capita* intake of 1 μg/kg bw from use of glyceryl tribenzoate as a flavoring substance in the USA. The NOAEL of 2541 mg/kg bw per day is greater than 100,000 times the daily *per capita* intake ("eaters only") of 0.2 μg propylene glycol dibenzoate/kg bw from use as a flavoring ingredient in the USA.

*4.2.2.8. 2,4-Dimethylbenzaldehyde (No. 37).* Groups of 5 male and 5 female rats were administered 0 (control), 0.175 or 1.75 mg 2,4-dimethylbenzaldehyde/kg bw by stomach tube daily, 6 days per week for a period of 14 days. At 1.75 mg/kg bw per day, male rats showed an increased relative liver weight. Female rats showed no significant difference in relative liver weight at the same dose level. Histopathological examination of the liver and kidneys revealed no abnormalities associated with the administration of dimethylbenzaldehyde (DeGroot et al., 1974).

### *4.3. Long-term studies of toxicity and carcinogenicity*

The results of six long-term toxicity and carcinogenicity studies with 4 representative benzyl derivatives are summarized in Table 2 and described below.

#### *4.3.1. National toxicology program studies*

*4.3.1.1. Benzyl alcohol (No. 1), benzaldehyde (No. 13), benzyl acetate (No. 3).* The National Toxicology Program (NTP) conducted four long-term toxicity and carcinogenicity studies on three members of this group: benzyl alcohol, benzaldehyde and benzyl acetate (a gavage and a microencapsulation study). Based on extensive metabolic data that benzyl acetate is readily hydrolyzed to benzyl alcohol in vivo, and benzyl alcohol is rapidly oxidized to benzaldehyde in vivo, the results of all four 2-year bioassay studies are reviewed together.

Each of the three test compounds were administered in corn oil gavage five days per week to groups of 50 F344/N rats and B6C3F$_1$ mice for a period of two years at two dose levels. An additional study was conducted in which test groups of 60 rats or mice were maintained on diets containing benzyl acetate. Animals were observed once weekly for 12–13 weeks and at least monthly thereafter. All animals were subject to necropsy after death, or at the end of the study. Throughout these studies, mean body weights were comparable among all groups, except for rats and mice maintained on the high dose level of benzyl acetate in the feed, which was reduced (5–16%) due to decreased feed consumption. Increased mortality seen in some of the groups was attributable to the gavage procedure, reflux and aspiration of the gavage material into the lungs, or administration errors resulting in direct disposition of material into the lungs (NTP, 1986, 1989, 1990a,b, 1993b).

*4.3.1.2. Results and discussion*

*4.3.1.2.1. Benzyl alcohol (No. 1) and benzaldehyde (No. 13).* According to the NTP, "under conditions of the 2 year gavage study, there was no evidence of carcinogenic activity of benzyl alcohol in male or female F344/N rats or B6C3F1 mice receiving either 200 or 400 mg/kg bw."

NTP also concluded "under conditions of the 2 year gavage studies, there was no evidence of carcinogenic activity for male or female F344/N rats receiving 200 or 400 mg/kg bw/d benzaldehyde. However, there was some evidence of carcinogenic activity of benzaldehyde in B6C3F$_1$ mice at 300 or 600 mg/kg bw, as indicated by increased incidences of squamous cell papillomas and hyperplasia of the forestomach." (NTP, 1989, 1990a).

The occurrence of squamous cell papillomas and forestomach hyperplasia in rodents is common in NTP bioassay gavage studies in which a high concentration of an irritating material in corn oil is delivered daily by tube into the forestomach for two years. High concentrations of aldehydes (i.e. malonaldehyde, furfural, and benzaldehyde) (NTP, 1988, 1990a,b) and other irritating substances (i.e. dihydrocoumarin and coumarin) (NTP, 1992, 1993b) delivered in corn oil by gavage are consistently associated with these phenomena in the forestomach of rodents. Squamous cell papillomas are benign lesions of surfaces covered with squamous epithelium. A majority of papillomas arise as a result of chronic irritation, or less frequently from infection from some strains of viruses (Smith and Ford, 1993). Additionally, forestomach hyperplasia and papillary proliferation in these studies did not progress to squamous cell carcinomas.

The relevance of the appearance of forestomach tumors in rodents to potential carcinogenic targets in humans has been the subject of much investigation (Grice, 1988; Wester and Kroes, 1988; Clayson et al., 1990). Although it has been suggested that the mucosa of the rodent forestomach is similar to that of the human esophagus, this is clearly not the case. The rodent forestomach has a storage function and contains mucosa of keratinizing squamous epithelium that is constantly exposed to the strong acid medium of the gastric contents. Conversely, the esophagus has no storage capacity and contains non-keratinizing squamous epithelium that is extremely sensitive to the adverse effects of strong acid medium. The esophagus has no significant contact with food contents in that it is a muscle that exerts a motive action on food contents propelling them from the pharynx to the stomach.

Apparently, the combination of daily introduction of a dosing tube into the forestomach and delivery of high concentrations of an irritating test material in corn oil, which itself is a mild irritant and mitogen, was the likely source of the papillomas in the rodent forestomach. This conclusion is supported by the observation that the occurrence of squamous cell papillomas and forestomach hyperplasia in gavage administration of a test material in corn oil for 2 years (NTP, 1986) disappear when the same substance is administered at similar intake levels in the diet (NTP, 1993a). Therefore, the appearance of these benign lesions in the 2-year rodent bioassay has no relevance to humans, given that human exposure occurs when low levels of benzaldehyde are consumed in the diet.

*4.3.1.2.2. Benzyl acetate (No. 3).* In the first 2-year bioassay, F344/N rats and B6C3F1 mice received benzyl acetate in corn oil by gavage daily, five days per week, for a period of 103 weeks at doses of 0 (control), 250, or 500 mg/kg bw and 0 (control), 500 or 1000 mg/kg bw, respectively. The NTP concluded: "Under conditions of the gavage studies, benzyl acetate administration was associated with increased incidence of acinar cell adenoma of the exocrine pancreas in male F344/N rats. No evidence of carcinogenicity was found for female F344/N rats. For male and female B6C3F1 mice there was evidence of carcinogenicity, in that benzyl acetate caused an increased incidence of hepatocellular neoplasms particularly adenoma, and squamous cell neoplasms of the forestomach." (NTP, 1986).

In the subsequent benzyl acetate dietary study, F344/N rats and B6C3F1 mice were maintained on diets containing 0 (control), 3000, 6000, or 12,000 ppm and 0 (control), 330, 1000, or 3000 ppm, respectively, for 103 weeks. This corresponds to 0, 130, 260, or 510 mg/kg bw and 0, 145, 290, or 575 mg/kg bw for male and female rats, respectively, and 0, 35, 110 or 345 mg/kg bw and 0, 40, 130 or 375 mg/kg bw for male and female mice, respectively. In this study NTP concluded: "under conditions of these 2-year feed studies, there was no evidence of carcinogenic activity of benzyl acetate in male or female F344/N rats receiving 3000, 6000, or 12,000 ppm. There was no evidence of carcinogenic

activity of benzyl acetate in male or female B6C3F1 mice receiving 330, 1000, or 3000 ppm." (NTP, 1993a).

Upon completion of the second study, the NTP concluded that the increased incidence of pancreatic acinar cell neoplasms specific to male rats reported in the earlier study was probably due to the use of corn oil as a vehicle in the gavage study. Administration of high levels of fat to experimental animals has been shown to enhance the development of spontaneous and chemical-induced neoplasms (NTP, 1994a,b). More specifically, administration of corn oil and other high-fat vehicles by gavage daily at doses of 2.5, 5, or 10 ml/kg bw for 2 years was associated with an increased incidence of proliferative lesions of the exocrine pancreas (NTP, 1994a).

The increased incidence of hepatocellular adenomas observed in the gavage study was not observed in the dietary study. The NTP proposed that the difference was due to the different mode of administration and the resulting higher plasma levels of benzyl acetate metabolites (benzoic acid) in the gavage study (NTP, 1993a). In a comparative toxicokinetic study of gavage and dietary mode of administration discussed earlier (Yuan et al., 1995), peak plasma concentrations of benzoic acid were higher in the gavage study than those in the dietary study. Although daily dietary intake level and gavage dose levels were similar, gavage administration saturated the benzoic acid elimination pathway. Hippuric acid plasma concentrations were similar indicating depletion of the glycine pool in the gavage study with concomitant increases in free plasma benzoic acid. The authors suggested that higher plasma levels of benzyl acetate and free benzoic acid in the gavage study may be, in part, associated with the different toxicological outcomes of the gavage and dietary studies. The conclusion that high levels of free benzoic acid is associated with hepatocellular adenomas is highly unlikely, given the fact that chronic administration of high dietary levels of benzoic acid (greater than 1% in the diet) failed to induce hepatocellular neoplasms in lifetime mice studies (Toth, 1984). However, there is evidence that the incidence of hepatocellular adenomas in the gavage study were the result of chronic exposure to high levels of the corn oil vehicle (Haseman et al., 1985).

Statistical analysis of liver adenomas is suspect because the vehicle control animals had an aberrantly low incidence when compared to historical controls. Control values for liver adenomas in NTP bioassays vary from 20% to as high as 42%. In this study, the liver adenoma rates were zero. If these historical controls are adopted for comparison, the statistical significance of the adenoma finding in the high dose animals disappears or becomes marginal ($p = 0.038$ for females, and $p = 0.078$ for males). Procedural concerns include the fact that the diets were not tested for contaminants and there are interpretive problems with corn oil gavage and tumor occurrence (NTP, 1986; Bernard, 1983).

In conclusion, evidence of carcinogenicity in the gavage benzaldehyde and benzyl acetate studies are associated with the repeated gavage administration of high dose levels of test substance in a corn oil vehicle or a statistical anomaly. The above observations strongly suggest that the tumors found in the gavage NTP studies have no relevance to human carcinogenic risk.

### 4.4. Genotoxicity studies

Genotoxicity testing has been performed on 13 representative compounds in this group. The vast majority of standardized in vitro genotoxicity assays Ames (AMS), Mouse Lymphoma (MLA), Sister Chromatid Exchange (SCE), Chromosomal Aberration (ABS), and Unscheduled DNA Synthesis (UDS) show no evidence of genotoxicity. Equivocal results have been reported mainly for aromatic aldehydes in the MLA and ABS assays. None of the 13 in vivo assays produced any evidence of genotoxicity for these benzyl derivatives. The results of these tests are presented in Table 3 and are summarized below.

#### 4.4.1. In vitro

The benzyl derivatives were non-mutagenic in all standard plate incorporation and/or pre-incubation Ames assays using *Salmonella typhimurium* strains TA92, TA94, TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, TA1538, and TA2637, when tested at concentrations ranging up to the level of cytotoxicity or at ICH/OECD-recommended maximum test concentrations, both in the absence and presence of metabolic activation (S9 fraction) (FDA, 1975; McCann et al., 1975; Milvy and Garro, 1976; Cotruvo et al., 1977; Anderson and Styles, 1978; Sasaki and Endo, 1978; Rockwell and Raw, 1979; Florin et al., 1980; Rapson et al., 1980; Kasamaki et al., 1982; Haworth et al., 1983; Ball et al., 1984; Ishidate et al., 1984; Marnett et al., 1985; Nohmi et al., 1985; Mortelmans et al., 1986; Rogan et al., 1986; Schunk et al., 1986; Zeiger et al., 1988; Aeschbacher et al., 1989; Heck et al., 1989; NTP, 1989, 1990a,b; Vamvakas et al., 1989; Dillon et al., 1992; Zeiger et al., 1992;). Additional Ames tests on metabolites isolated from the urine of rats administered benzaldehyde (No. 13), isopropylbenzyl alcohol (No. 32), or cuminaldehyde (No. 36) by oral gavage also were negative in *S. typhimurium* strains TA98 and TA100, both with and without metabolic activation (Rockwell and Raw, 1979). A mutation assay in *S. typhimurium* strain TA1535/pSK1002, using *umu* gene expression as an endpoint, produced negative results with benzoic acid (No. 18) (Nakamura et al., 1987). Mutation or DNA repair assays using *Escherichia coli* strains WP2 *uvrA*, PQ37, or Sd-4-73

Table 3

| # | Substance name | Test system in vitro | Test object | Maximum concentration of substance | Result | Reference |
|---|---|---|---|---|---|---|
| *In vitro genotoxicity studies on benzyl derivatives* | | | | | | |
| 1 | Benzyl alcohol | Ames test[1] | *Salmonella typhimurium* TA92, TA94, TA98, TA100, TA1535, and TA1537 | 10,000 μg/plate | Negative[2] | Ishidate et al. (1984) |
| 1 | Benzyl alcohol | Ames test[3] | *S. typhimurium* TA100 | 1,000 μg/plate | Negative[4] | Ball et al. (1984) |
| 1 | Benzyl alcohol | Ames test[3] | *S. typhimurium* TA98, TA100 | Dose not reported | Negative[4] | Rogan et al. (1986) |
| 1 | Benzyl alcohol | Ames test[1] | *S. typhimurium* TA98, TA100, TA1535, TA1537 | 6,666 μg/plate | Negative[2] | Mortelmans et al. (1986) |
| 1 | Benzyl alcohol | Ames test[3] | *S. typhimurium* TA98, TA100, TA1535, and TA1537 | 3 μmole/plate | Negative[2] | Florin et al. (1980) |
| 1 | Benzyl alcohol | Ames test[3] | *S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 | 50,000 μg/plate | Negative[2] | Heck et al. (1989) |
| 1 | Benzyl alcohol | Ames test[3] | *S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 | 5 μl/plate | Negative[4] | Milvy and Garro (1976) |
| 1 | Benzyl alcohol | Ames test[1] | *S. typhimurium* TA98, TA100, TA1535, and TA1537 | 6,666 g/plate | Negative[2,5] | NTP (1989) |
| 1 | Benzyl alcohol | Mutation | *Escherichia coli* WP2 uvrA | 8.0 mg/plate | Negative[6,7] | Yoo (1986) |
| 1 | Benzyl alcohol | Rec assay | *B. subtilis* H17 and M45 | 21 μg/disk | Negative[6] | Oda et al. (1979) |
| 1 | Benzyl alcohol | Rec assay | *B. subtilis* H17 and M45 | 10 μg/disk | Positive[6,8] | Kuroda et al. (1984a) |
| 1 | Benzyl alcohol | Rec assay | *B. subtilis* H17 and M45 | 20 μl/disk | Positive[6,7] | Yoo (1986) |
| 1 | Benzyl alcohol | Chromosome aberration | Chinese hamster fibroblast cells | 1.0 mg/ml | Negative[4,9] | Ishidate et al. (1984) |
| 1 | Benzyl alcohol | Chromosome aberration | Chinese hamster ovary cells | 5,000 μg/ml | Equivocal[2,10,11] | Anderson et al. (1990) |
| 1 | Benzyl alcohol | Chromosome aberration | Chinese hamster ovary cells | 5,000 μg/ml | Positive[2,12] | NTP (1989) |
| 1 | Benzyl alcohol | Sister chromatid exchange (SCE) | Chinese hamster ovary cells | 5,000 μg/ml | Weakly Positive[13] | NTP (1989) |
| 1 | Benzyl alcohol | SCE | Chinese hamster ovary cells | 5,000 μg/ml | Weak Positive[2,11,14] | Anderson et al. (1990) |
| 1 | Benzyl alcohol | Mutation | L5178Y mouse lymphoma cells | 5,000 g/ml | Questionable[15,11] | McGregor et al. (1988); Myhr et al., 1990 |
| 1 | Benzyl alcohol | Mutation | L5178Y Mouse Lymphoma Cells | 4,500 μg/ml | Positive[2,16] | NTP (1989) |
| 1 | Benzyl alcohol | Mutation | *E. coli* WP2 uvrA | Dose not reported | Negative[17] | Kuroda et al. (1984b) |
| 1 | Benzyl alcohol | Cytotoxicity | Human alveolar tumor cells | 0.5 mM | Negative | Waters et al. (1982) |
| 1 | Benzyl alcohol | DNA damage | Human alveolar tumor cells | 0.5 mM | Negative | Waters et al. (1982) |
| 1 | Benzyl alcohol | DNA damage | Rat hepatocytes | 10 mM | Negative[18] | Storer et al. (1996) |
| 1 | Benzyl alcohol | DNA damage | *E. coli* P3478 | 50 μl/disk | Negative[2] | Fluck et al. (1976) |
| 2 | Benzyl formate | Rec assay | *B. subtilis* H17 and M45 | 20 μl/disk | Positive[6,7] | Yoo (1986) |
| 2 | Benzyl formate | Mutation | *E. coli* WP2 uvrA | 4.0 mg/plate | Negative[6,7] | Yoo (1986) |
| 3 | Benzyl acetate | Ames test[1] | *S. typhimurium* TA98, TA100, TA1535, and TA1537 | 10 mg/plate | Negative[2] | Mortelmans et al. (1986) |
| 3 | Benzyl acetate | Ames test[1,3] | *S. typhimurium* TA98, TA100 | 5,000 μg/plate | Negative[2,19] | Schunk et al. (1986) |
| 3 | Benzyl acetate | Ames test[20] | *S. typhimurium* TA98, TA100, TA1535, and TA1537 | 3 μmol/plate | Negative[2] | Florin et al. (1980) |
| 3 | Benzyl acetate | Rec assay | *B. subtilis* H17 and M45 | 21 μg/disk | Negative[2,6] | Oda et al. (1979) |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3 | Benzyl acetate | Rec assay | *B. subtilis* H17 and M45 | 20 µl/disk | Positive[6,7] | Yoo (1986) |
| 3 | Benzyl acetate | Mutation | *E. coli* WP2 uvrA | 2.0 mg/plate | Negative[6,7] | Yoo (1986) |
| 3 | Benzyl acetate | Mutation | Mouse lymphoma L5178Y cells and Human lymphoblast TK6 cells | Mouse cells = 500 µg/ml Human cells = 1,500 µg/ml | Positive[2,12] | Caspary et al. (1988) |
| 3 | Benzyl acetate | Mutation | Mouse lymphoma L5178Y cells | 1,600 µL/mL | Positive[2,18] | McGregor et al. (1988) |
| 3 | Benzyl acetate | Mutation | Mouse lymphoma L5178Y cells | Not reported | Positive[2,12] | Rudd et al. (1983) |
| 3 | Benzyl acetate | Chromosome aberration | Chinese hamster ovary cells | 5,000 µg/ml | Negative[2] | Galloway et al. (1987) |
| 3 | Benzyl acetate | Chromosome aberration | Chinese hamster lung fibroblast cells | 2.4 mg/ml | Negative[2,18] | Matsouka et al. (1996) |
| 3 | Benzyl acetate | SCE | Chinese hamster ovary cells | 5,000 µg/ml | Negative[2] | Galloway et al. (1987) |
| 3 | Benzyl acetate | Unscheduled DNA synthesis | Rat hepatocytes | Not reported | Negative[17] | Mirsalis et al. (1983) |
| 4 | Benzyl propionate | Rec assay | *B. subtilis* H17 and M45 | 21 µg/disk | Negative[4,6] | Oda et al. (1979) |
| 11 | Benzyl benzoate | Ames test[20] | *S. typhimurium* TA98, TA100, TA1535, and TA1537 | 3 µmol/plate | Negative[2] | Florin et al. (1980) |
| 11 | Benzyl benzoate | Ames test[1,3] | *S. typhimurium* TA98, TA100 | 5,000 µg/plate | Negative[2,19] | Schunk et al. (1986) |
| 13 | Benzaldehyde | Ames test[3] | *S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 | 37,500 µg/plate | Negative [2] | Heck et al. (1989) |
| 13 | Benzaldehyde | Ames test[3] | *S. typhimurium* TA98, TA100 | 300 µl/plate | Negative[2,21] | Rockwell and Raw (1979) |
| 13 | Benzaldehyde | Ames assay[3] | *S. typhimurium* TA98 and TA100 | 100 µl/plate | Negative[22] | Rockwell and Raw (1979) |
| 13 | Benzaldehyde | Ames test[20] | *S. typhimurium* TA98, TA100, TA2637 | 2.0 mg/plte | Negative[2,6] | Nohmi et al. (1985) |
| 13 | Benzaldehyde | Ames test[20] | *S. typhimurium* TA98, TA100, TA1535, and TA1537 | 3.0 µmol/plate | Negative[2] | Florin et al. (1980) |
| 13 | Benzaldehyde | Ames test[1] | *S. typhimurium* TA98, TA100, TA1535, and TA1537 | 1,000 µg/plate | Negative[2] | Haworth et al. (1983) |
| 13 | Benzaldehyde | Ames test[20] | *S. typhimurium* TA100, TA102 and TA104 | 3,333 µg/plate | Negative[2] | NTP (1990a, 1990b) |
| 13 | Benzaldehyde | Ames test[20] | *S. typhimurium* TA100 | 1.0 mg/plate | Negative[7] | Rapson et al. (1980) |
| 13 | Benzaldehyde | Ames test[1] | *S. typhimurium* TA98, TA100 | Not reported | Negative[2] | Sasaki and Endo (1978) |
| 13 | Benzaldehyde | Ames test[1] | *S. typhimurium* TA100, TA102 and TA104 | Not reported | Negative[2] | Dillon et al. (1992) |
| 13 | Benzaldehyde | Ames test[1] | *S. typhimurium* TA100 | 2,000 nmol/plate | Negative[2] | Vamvakas et al. (1989) |
| 13 | Benzaldehyde | Ames test[20] | *S. typhimurium* TA98, TA100 | 500 µg/plate | Negative[2] | Kasamaki et al. (1982) |
| 13 | Benzaldehyde | Rec assay | *B. subtilis* H17 and M45 | 21 µg/disk | Negative[6,7] | Oda et al. (1979) |
| 13 | Benzaldehyde | Rec assay | *B. subtilis* H17 and M45 | Not reported | Positive[2,12] | Matsui et al. (1989) |
| 13 | Benzaldehyde | Unscheduled DNA synthesis (UDS) | Rat hepatocytes | 251 µg/ml | Negative[2] | Heck et al. (1989) |
| 13 | Benzaldehyde | Mutation | Mouse L5178Y lymphoma cells | 600 µg/ml | Positive[12] | Heck et al. (1989) |
| 13 | Benzaldehyde | Mutation | Mouse L5178Y lymphoma cells | 800 µg/ml | Positive[4,23] | McGregor et al. (1991) |
| 13 | Benzaldehyde | Chromosomal aberrations | Chinese hamster cells | 1.2 mg/ml | Positive[6,16,24,25] | Sofuni et al. (1985) |

Table 3 (*continued*)

| # | Substance name | Test system in vitro | Test object | Maximum concentration of substance | Result | Reference |
|---|---|---|---|---|---|---|
| *In vitro genotoxicity studies on benzyl derivatives* | | | | | | |
| 13 | Benzaldehyde | Chromosomal aberrations | Chinese hamster ovary cells | 1,600 μg/ml | Negative[2] | Galloway et al. (1987) |
| 13 | Benzaldehyde | Chromosomal aberrations | Chinese hamster cells | 50 nM | Positive[2] | Kasamaki et al. (1982) |
| 13 | Benzaldehyde | SCE | Chinese hamster ovary cells | 1,600 μg/ml | Positive[2] | Galloway et al. (1987) |
| 13 | Benzaldehyde | SCE | Chinese hamster ovary cells | 1,000 μM | Negative[2,18] | Sasaki et al. (1989) |
| 13 | Benzaldehyde | SCE | Human lymphocytes | 2.0 mM | Positive[4] | Jansson et al. (1988) |
| 18 | Benzoic acid | Ames test[3] | *S. typhimurium* TA98, TA100, TA1535, and TA1538 | 2,500 μg/plate | Negative[2] | Anderson and Styles (1978) |
| 18 | Benzoic acid | Ames test[20] | *S. typhimurium* TA98, TA100, TA1535, and TA1536 | 3.6 μg/plate | Negative[2] | Cotruvo et al. (1977) |
| 18 | Benzoic acid | Ames test[1] | *S. typhimurium* TA97; TA98, TA100, TA1535, and TA1537 | 10 mg/plate | Negative[2] | Zeiger et al. (1988) |
| 18 | Benzoic acid | Ames test[20] | *S. typhimurium* TA100 | 1.0 mg/plate | Negative[7] | Rapson et al. (1980) |
| 18 | Benzoic acid | Ames test[3] | *S. typhimurium* TA98, TA100, TA1535, and TA1537 | 1.0 mg/plate | Negative[22] | McCann et al. (1975) |
| 18 | Benzoic acid | Ames test[1] | *S. typhimurium* TA92, TA94, TA98, TA100, TA1535, and TA1537 | 10.0 mg/plate | Negative[2] | Ishidate et al. (1984) |
| 18 | Benzoic acid | Ames test[3] | *S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 | 100 μg/plate | Negative[4] | Milvy and Garro (1976) |
| 18 | Benzoic acid | Ames test[3] | *S. typhimurium* TA1535, TA1537, and TA1538 | 0.5% | Negative[2] | FDA (1975) |
| 18 | Benzoic acid | Mutation | *S. typhimurium* TA1535/pSK1002 | 1.67 mg/ml | Negative[2,26] | Nakamura et al. (1987) |
| 18 | Benzoic acid | Rec assay | *B. subtilis* H17 and H45 | Not reported | Positive[17] | Nonaka (1989) |
| 18 | Benzoic acid | Mutation | Saccharomyces cerevisiae D3 | 0.18% | Negative[2] | Cotruvo et al. (1977) |
| 18 | Benzoic acid | Mutation | S. cerevisiae D4 | 0.15% | Negative[2] | FDA (1975) |
| 18 | Benzoic acid | Indirect DNA repair | *E. coli* PQ37 | 400 μg/ml | Negative[27] | Glosnicka and Dziadziuszko (1986) |
| 18 | Benzoic acid | Chromosome aberration test | Chinese hamster fibroblast cells | 1.5 mg/ml | Weak Positive[4,28] | Ishidate et al. (1984) |
| 18 | Benzoic acid | SCE | Human lymphocytes | 2.0 mM | Negative[4] | Jansson et al. (1988) |
| 19 | Methyl benzoate | Ames test[1] | *S. typhimurium* TA97, TA98; TA100, TA1535, and TA1537 | 6,666 μg/plate | Negative[2] | Zeiger et al. (1992) |
| 19 | Methyl benzoate | Mutation | *E. coli* Sd-4-73 | Dose not reported | Negative[4] | Szybalski (1958) |
| 25 | Isoamyl benzoate | Mutation | *E. coli* Sd-4-73 | Dose not reported | Negative[4] | Szybalski (1958) |
| 32 | Isopropylbenzyl alcohol | Ames assay[3] | *S. typhimurium* TA98 and TA100 | 100 μl/plate | Negative[22] | Rockwell and Raw (1979) |
| 32 | Isopropylbenzyl alcohol | Ames assay[3] | *S. typhimurium* TA98 and TA100 | 300 μl/plate | Negative[2,29] | Rockwell and Raw (1979) |
| 34 | Tolualdehydes (mixed *o*, *m*, *p*) | Ames test[1] | *S. typhimurium* TA104 | 0.8 μmoles/plate | Negative[2] | Marnett et al. (1985) |

| | | | | | | |
|---|---|---|---|---|---|---|
| 34 | Tolualdehydes (mixed *o*, *m*, *p*) | Ames test[20] | *S. typhimurium* TA98, TA100, TA1535, and TA1537 | 3 μmol/plate | Negative[2] | Florin et al. (1980) |
| 34 | Tolualdehydes (mixed *o*, *m*, *p*) | Ames test[3] | *S. typhimurium* TA98, TA100, TA1535, TA 1537, and TA1538 | 18,750 μg/plate | Negative[2] | Heck et al. (1989) |
| 34 | Tolualdehydes (mixed *o*, *m*, *p*) | Ames test[3] | *S. typhimurium* TA98, TA100 and TA102 | 0.8 mmol/plate | Negative[2] | Aeschbacher et al. (1989) |
| 34 | Tolualdehydes (mixed *o*, *m*, *p*) | Ames test[1] | *S. typhimurium* TA97, TA100, TA1535, and TA1537 | 666 μg/plate | Negative[2] | Zeiger et al. (1988) |
| 34 | Tolualdehydes (mixed *o*, *m*, *p*) | UDS | Rat hepatocytes | 1,000 μg/ml | Negative[2] | Heck et al. (1989) |
| 34 | Tolualdehydes (mixed *o*, *m*, *p*) | Mouse Lymphoma Mutation Assay | L5178Y mouse lymphoma cells | 300 g/ml | Negative[2] | Heck et al. (1989) |
| 36 | Cuminaldehyde | Ames assay[3] | *S. typhimurium* TA98 and TA100 | 100 μl/plate | Negative[22] | Rockwell and Raw (1979) |
| 36 | Cuminaldehyde | Ames assay[3] | *S. typhimurium* TA98 and TA100 | 300 μl/plate | Negative[2,30] | Rockwell and Raw (1979) |
| 36 | Cuminaldehyde | SCE | Chinese hamster ovary cells | 333 μM | | |
| *In vitro antimutagenicity studies on benzyl derivatives* | | | | | | |
| 1 | Benzyl alcohol | Antimutagenesis | *E. coli* WP2 uvrA | 2.5 mg/ml | Positive Antimutagenic Effect [6,31] | Yoo (1986) |
| 1 | Benzyl alcohol | Antimutagenesis | *E. coli* WP2 uvrA | 1,000 μg/ml | Negative Antimutagenic Effect[6,32] | Yoo (1986) |
| 1 | Benzyl alcohol | Antimutagenesis | *E. coli* WP2 uvrA | Dose not reported | Positive Antimutagenic Effect[31] | Kuroda et al. (1984b) |
| 2 | Benzaldehyde | Antimutagenesis | *E. coli* WP2 uvrA | 300 μg/ml | Negative[33] Antimutagenic Effect | (Ohta et al., 1983) |
| 2 | Benzyl formate | Antimutagenesis | *E. coli* WP2 uvrA | 8.0 mg/ml | Negative Antimutagenic Effect[6,31] | Yoo (1986) |
| 3 | Benzyl acetate | Antimutagenesis | *E. coli* WP2 uvrA | 8.0 mg/plate | Negative Antimutagenic Effect[6,31] | Yoo (1986) |
| 13 | Benzaldehyde | Antimutagenesis | *E. coli* PQ37 | 500 μg/ml | Negative[34] Antimutagenic effect | (Ohta et al., 1986a) |
| 20 | Ethyl benzoate | Antimutagenesis | *E. coli* PQ37 | 500 μg/ml | Negative Antimutagenic Effect[34] | (Ohta et al., 1986a) |
| 20 | Ethyl benzoate | Antimutagenesis | *S. typhimurium* TA98 | 200 μg/ml | Negative Antimutagenic Effect[35] | (Ohta et al., 1986b) |
| 20 | Ethyl benzoate | Antimutagenesis | *E. coli* WP2s | 200 μg/ml | Negative antimutagenic effect | (Ohta et al., 1986b) |

Table 3 (*continued*)

| # | Substance name | Test system in vitro | Test object | Maximum concentration of substance | Result | Reference |
|---|---|---|---|---|---|---|
| *In vitro genotoxicity studies on benzyl derivatives* | | | | | | |
| | | Test system in vivo | | | | |
| *In vivo genotoxicity studies on benzyl derivatives* | | | | | | |
| 1 | Benzyl alcohol | Sex-linked recessive lethal mutations (SLRL) | *Drosophila melanogaster* | 5000 ppm | Negative[36] | Foureman et al. (1994) |
| 1 | Benzyl alcohol | SLRL | *Drosophila melanogaster* | 8000 ppm | Negative[37] | Foureman et al. (1994) |
| 1 | Benzyl alcohol | Micronucleus test | Mouse bone marrow cells | 200 mg/kg bw | Negative[38] | Hayashi et al. (1988) |
| 1 | Benzyl alcohol | Replicative DNA synthesis test | Mouse hepatocytes | Not reported | Positive[39] | Yoshikawa (1996) |
| 3 | Benzyl acetate | SLRL | *Drosophila melanogaster* | 300 ppm | Negative[36] | NTP (1993a); Foureman et al. (1994) |
| 3 | Benzyl acetate | SLRL | *Drosophila melanogaster* | 20,000 ppm | Negative[37] | NTP (1993a); Foureman et al. (1994) |
| 3 | Benzyl acetate | SCE | Mouse bone marrow cells | 1,700 mg/kg bw | Negative[38] | NTP (1993a) |
| 3 | Benzyl acetate | Chromosome aberration | Mouse bone marrow cells | 1,700 mg/kg bw | Negative[38] | NTP (1993a) |
| 3 | Benzyl acetate | Micronucleus test | Mouse bone marrow cells | 1,250 mg/kg bw | Negative[38] | NTP (1993a); Shelby et al. (1993) |
| 3 | Benzyl acetate | Micronucleus test | Mouse erythrocytes | 50,000 ppm | Negative[38] | NTP (1993a) |
| 3 | Benzyl acetate | UDS | Rat hepatocytes | Not reported | Negative[17,40] | Mirsalis et al. (1983) |
| 3 | Benzyl acetate | UDS | Rat hepatocytes | 1,000 mg/kg bw | Negative[40] | Mirsalis et al. (1989) |
| 3 | Benzyl acetate | UDS | Rat pancreatic cells | 1,000 mg/kg bw | Negative[40] | Steinmetz and Mirsalis (1984) |
| 3 | Benzyl acetate | DNA damage | Rat pancreatic cells | 500 mg/kg bw | Negative[40] | Longnecker et al. (1990) |
| 3 | Benzyl acetate | DNA damage | Rat pancreatic cells | 0.9% | Negative[36] | Longnecker et al. (1990) |
| 13 | Benzaldehyde | SLRL | *Drosophila melanogaster* | 1,150 ppm | Negative[36] | Woodruff et al. (1985) |
| 13 | Benzaldehyde | SLRL | *Drosophila melanogaster* | 2,500 ppm | Negative[37] | Woodruff et al. (1985) |

[1] Preincubation method.
[2] Assay was performed with and without metabolic activation.
[3] Plate incorporation method.
[4] Assay was performed without metabolic activation.
[5] Cytotoxicity was reported at the highest concentration tested.
[6] Japanese article. English summary.
[7] Not reported if assay was performed with or without metabolic activation.
[8] Inhibition of growth was reported when assay was performed without metabolic activation.
[9] Cells were exposed for 48 h.[29] Assay of urine samples from rats given isopropylbenzyl alcohol by oral gavage.
[10] Positive results were not reproducible.
[11] No dose–response observed.
[12] Positive results reported only with metabolic activation.
[13] Dose response at concentrations from 500 to 1250 μg/ml (without metabolic activation).
[14] No dose response observed. Increase in SCE at single doses.
[15] Positive and negative responses could not be reproduced.
[16] Positive result reported only in absence of metabolic activation.
[17] Abstract; methods and test concentrations were not reported.
[18] Cytotoxicity was observed at the maximum dose tested.
[19] Cytotoxicity observed at 3 maximum concentrations.

[20] Mutagenesis induced by 2-amino-3-methyl-imidazo[3,4-*f*]quinoline (IQ), 2-amino-3, 4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3, 8-dimethylimidazo[4.5-*f*]quinoxaline HCl (MeIQx), 3-amino-4-dimethy-5 H-pyrido-[4,3-*b*]indole (Trp-P-1), 2-amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine HCl (PhIP).
[21] Assay of urine samples from rats given benzaldehyde by oral gavage.
[22] Assay was performed with metabolic activation.
[23] Significant increases in mutant fraction were close to toxic doses.
[24] Weak positive results were observed with metabolic activation.
[25] Cytotoxicity was observed at the two maximum concentrations tested.
[26] Assay measured induction of *umu* gene expression as indicator of genotoxicity.
[27] Genotoxicity measured as ability to induce β-galactosidase.
[28] Total incidence of cells with aberrations was 5.0–9.0%.
[29] Assay of urine samples from rats given isopropylbenzyl alcohol by oral gavage.
[30] Assay of urine samples from rats given cuminaldehyde by gavage.
[31] Mutagenesis induced by furylfuramide (AF-2).
[32] Mutagenesis induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG).
[33] Mutagenesis induced by 4-nitroquinoline 1-oxide and UV irradiation.
[34] Mutagenesis induced by UV irradiation.
[35] Mutagenesis induced by 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole or 2-amino-3-methyl-imidazo[4,5-*f*]quinoline.
[36] Feeding study.
[37] Dose administered by injection.
[38] Administered by intraperitoneal injection.
[39] Route of administration not reported.
[40] Administered by oral gavage.

with eight different benzyl derivatives (see Table 3) (Szybalski, 1958; Kuroda et al., 1984b; Glosnicka and Dziadziuszko, 1986; Yoo, 1986), and *Saccharomyces cerevisiae* strains D3 or D4 with benzoic acid (FDA, 1975; Cotruvo et al., 1977) also showed no evidence of genotoxicity. In a cytogenetics assay in *E. coli* strain P3478, benzyl alcohol did not show any activity (Fluck et al., 1976).

Equivocal results were obtained with the benzyl derivatives in the Rec DNA repair assay using *Bacillus subtilis* strains H17 and M45 (Oda et al., 1979; Sekizawa and Shibamoto, 1982; Kuroda et al., 1984a; Yoo, 1986; Matsui et al., 1989; Nonaka, 1989). Positive results only were reported for benzyl formate, while negative results were reported for benzyl propionate (No. 4) (Oda et al., 1979; Yoo, 1986). Positive and negative results for Rec assays using the same substance were apparently due to laboratory-specific factors. Yoo (1986) reported only positive findings and Oda et al. (1979) reported only negative results for the same compounds.

In vitro assays in isolated mammalian cells produced both negative and positive results. In addition to benzyl acetate, benzaldehyde exhibited evidence of mutagenicity in the forward mutation assay with L5178Y mouse lymphoma cells (MLA), both with and without metabolic activation (Rudd et al., 1983; Caspary et al., 1988; McGregor et al., 1988, 1991; Heck et al., 1989; Myhr et al., 1990). Questionable results were reported for benzyl alcohol in the MLA (McGregor et al., 1988). Tolualdehydes (mixed) (No. 34) were negative in the MLA (Heck et al., 1989).

In cytogenetic tests, equivocal results were reported for the benzyl derivatives in the ABS assay. In the ABS assay, performed in Chinese hamster ovary and fibroblast cell lines, both positive and negative results were reported with benzyl alcohol and benzaldehyde, with the positive result usually occurring at the higher culture concentration (Kasamaki et al., 1982; Ishidate et al., 1984; Sofuni et al., 1985; Galloway et al., 1987; Anderson et al., 1990). Negative results in two separate ABS assays were reported for benzyl acetate (Galloway et al., 1987; Matsouka et al., 1996). Weak positive results were reported for benzoic acid in the ABS assay (Ishidate et al., 1984). The positive results in the ABS assays were generally obtained independently of the presence or absence of metabolic activation.

The authors of the MLA and ABS assays (Heck et al., 1989) have emphasized that the positive results in the MLA and ABS assays may be artifacts resulting from changes in culture pH and osmolality. Treatment with high dose levels of substances (e.g., reactive aldehydes and carboxylic acids) with the potential to alter acidity or osmolality may induce a significant increase in mutant frequencies or aberrations in these assays. Often the results are inconsistent with the results of other genotoxicity assays (i.e., AMS and UDS) (Heck et al., 1989).

In the SCE assay, equivocal results were reported for benzyl alcohol and benzaldehyde, in Chinese hamster ovary cell lines and in human lymphocytes (Galloway et al., 1987; Sasaki et al., 1989; Jansson et al., 1988; NTP, 1989; Anderson et al., 1990). Negative results were obtained in this assay for benzyl acetate, benzoic acid, and cuminaldehyde (No. 36) (Galloway et al., 1987; Sasaki et al., 1989; Jansson et al., 1988).

DNA damage was not observed in either rat or human cells exposed to benzyl alcohol (Waters et al., 1982; Storer et al., 1996). No unscheduled DNA synthesis (UDS) was observed in rat hepatocytes exposed to benzaldehyde, benzyl acetate, or mixed tolualdehydes (Mirsalis et al., 1983; Heck et al., 1989).

The standardized in vitro genotoxicity assays {AMS, MLA, SCE, ABS, and UDS} show no evidence of genotoxicity. Equivocal results have been reported for mainly aromatic aldehydes in the MLA and ABS assays and may be due artifacts under experimental conditions.

*4.4.2. In vivo*

None of the benzyl derivatives showed any evidence of genotoxicity in well-recognized in vivo assays (mouse micronucleus, sex-linked recessive lethal, and in vivo–in vitro UDS assays). In mammals, compounds were administered orally, by gavage, or by ip injection at doses that were significant fractions of the reported lethal dose levels. Benzyl acetate was the most frequently tested compound, producing negative findings in SCE, CA, micronuclei, and UDS in both rats and mice (Mirsalis et al., 1983, 1989; NTP, 1993a,b; Shelby et al., 1993; Steinmetz and Mirsalis, 1984). The micronucleus test also was negative with benzyl alcohol (Hayashi et al., 1988). Positive results were reported for benzyl alcohol in mice (dose not specified) in the Replicative DNA Synthesis Assay (Yoshikawa, 1996).

In the (sub-mammalian) sex-linked recessive lethal mutation assay in fruit flies (*Drosophila melanogaster*), negative results were obtained with benzyl alcohol, benzaldehyde, and benzyl acetate, either after feeding or administration by injection (Woodruff et al., 1985; NTP, 1993a; Foureman et al., 1994).

*4.4.3. Conclusion*

Based on the mainly negative results in the standardized battery {i.e., AMS (−), UDS (−), SCE (±), ABS (±), and MLA (±)} of in vitro genotoxicity assays and the uniformly negative results in well-recognized in vivo genotoxicity assays, it is concluded that the group of benzyl derivatives is not genotoxic in vivo.

*4.5. Other relevant studies*

*4.5.1. Reproduction*

*4.5.1.1. Benzyl alcohol (No. 1).* Fifty female CD-1 mice were administered 550 mg/kg bw per day benzyl alcohol by gavage on days 6–15 of gestation. A control group of 50 mice received corn oil only. Body weight, clinical observations, and mortality were recorded daily throughout treatment and up to 3 days postpartum. All parameters tested, including gestation index, average number of live pups/litter, postnatal survival, and pup body weight, were statistically similar for the treated and control animals (York et al., 1986).

To examine the reproductive hazard of benzyl alcohol in CD-1 mice, 750 mg/kg bw per day of benzyl alcohol was administered by gavage to 50 mice on gestation days 6–13. A control group of 50 animals were given distilled water only. Maternal body weight gain and mortality, mating, gestation, numbers of live and dead pups per litter, total litter weight on days 1 and 2 postpartum, litter weight change between days 1 and 3 postpartum, and pup survival on days 1 and 3 postpartum were recorded. There was no significant difference in maternal body weight measured on days 4 and 7 of gestation between treated and control animals. However, statistically significant decreases were observed in treated females on gestation day 18 and day 3 postpartum. Maternal body weight gain during days 7–18 of gestation was also significantly lower than that of controls. Significant differences were also observed in pup body weight and weight gain, including mean pup weight per litter, mean litter weight change, and mean pup weight change (between day 1 and 3 postpartum). No differences were observed in the mating or gestation indices, the total number of resorptions, the number of live pups per litter, or in pup survival. Eighteen deaths were reported during the treatment period and they were all attributed to the treatment. One more death was reported the day after treatment was terminated (Hardin et al., 1987). It was reported in a review of this study that clinical signs of maternal toxicity were observed, including hunched posture, tremors, inactivity, prostration, hypothermia, ataxia, dyspnoea, swollen or cyanotic abdomen, and piloerection (York et al., 1986).

*4.5.2. Benzyl acetate (No. 3)*

Sperm morphology and vaginal cytology examinations (SMVCE) were developed by the National Toxicology Program, and used as a screen for reproductive toxicants. These examinations include evaluations of motility, concentration and head morphology of sperm from the caudia epididymis, and male reproductive organ weight data. At the end of the benzyl acetate 13-week dietary study (NTP, 1993a), 10 male and female mice from each dose group (0, 3, 130, 6250, 12,500, 25,000, and 50,000 ppm) were utilized for the SMVCE study. There was no effect on sperm motility, density or percent abnormality in any of the doses tested. There was no statistically significant change in the weights of the epididymis, the caudia epididymis, or the testis. The estrous cycle of the high dose female group was sig-

nificantly increased relative to the control group. There was also a significant decrease in body weight among animals in this group (Morrissey et al., 1988).

Morrissey et al. (1988) performed similar examinations on rats at the end of the benzyl acetate 13-week study (0, 3, 130, 6250, 12,500, 25,000, and 50,000 ppm) (NTP, 1993a). Benzyl acetate had no effect on any of the parameters measured.

*Benzaldehyde (No. 13).* Benzaldehyde (approximately 5 mg/kg bw) was administered by gavage to 10 breeding age rats every other day for a period of 32 weeks. Ten control animals received only the vehicle oil. Two pregnancies per rat were studied, one at 75 days and one at 180 days. The parameters examined included the number of pregnant females, number of off spring born, pup body weights at days 7 and 21 postpartum, and pup vitality. There was no statistically significant difference between treatment and control groups. It was reported that fewer females in the treated group became pregnant, however, no data or statistical analyses were performed, and the authors concluded that treatment did not cause a significant change in any of the parameters measured (Sporn et al., 1967).

### 4.6. Teratology

#### 4.6.1. Benzyl acetate (No. 3)

Groups of 20 or more pregnant Wistar rats were administered benzyl acetate by gastric intubation at doses of 0 (control), 10, 100, 500, or 1,000 mg/kg bw from day 6–15 of pregnancy. On day 20 of pregnancy (term), they were terminated and the fetuses were examined for intrauterine death, and internal, external and skeletal malformations. Maternal parameters were comparable for all groups. At the highest dose level, fetal body weight was decreased. In addition, skeletal and internal malformations were observed at 1000 mg/kg bw per day. The authors reported that the skeletal malformations may be related to the significant decrease in fetal body weight. No increase in intrauterine death or external variations was noted at any dose level. No adverse effects were seen at or below 500 mg/kg bw per day. The results of this study show no evidence of teratogenic effects for benzyl acetate (Ishiguro et al., 1993).

#### 4.6.2. Benzyl benzoate (No. 11)

Benzyl benzoate was fed to pregnant Wistar rats at concentrations of 0.4 and 1.0% during gestation days 0–21. These dietary levels correspond to the average daily intake of 200 and 500 mg/kg bw (FDA, 1993). There were no effects reported on the fetus relating to external, skeletal, or visceral anomalies (Morita et al., 1980).

#### 4.6.3. Benzoic acid (No. 18)

Sodium benzoate administered by oral intubation to pregnant rats or rabbits during gestation days 6–15 or 6–18 at doses reaching 175 mg/kg bw per day or 250 mg/kg bw per day, respectively, produced no effects on maternal or fetal rats (Morgareidge, 1972). In another teratology study, sodium benzoate fed during gestation only produced adverse effects on the fetus at maternally toxic concentrations in the diet of 4% (1600 mg/kg bw/d) or higher (Onodera et al., 1978).

## 5. Recognition of GRASr status

The group of benzyl derivatives discussed here was determined to be generally recognized as safe (GRAS) under conditions of intended use as flavor ingredients by the FEMA Expert Panel in 1965. In 1978, the Panel evaluated the available data and affirmed the GRAS status of these flavor ingredients (GRASa). In 1993, the Panel initiated a comprehensive program to reevaluate the status of all FEMA GRAS flavor ingredients concurrent with a systematic revision of the FEMA Scientific Literature Reviews (SLRs). The group of benzyl derivatives was reaffirmed as GRAS (GRASr) based, in part, on their self-limiting properties as flavoring substances in food; their rapid absorption, metabolic detoxication, and excretion in humans and other animals; their low level of flavor use; the wide margins of safety between the conservative estimates of intake and the no-observed-adverse effect levels determined from subchronic and chronic studies and the lack of significant genotoxic and mutagenic potential. This evidence of safety is supported by the fact that the intake of benzyl derivatives as natural components of traditional foods is greater than their intake as intentionally added flavoring substances.

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# SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.4
Revision Date 20.12.2023
Print Date 10.11.2025
GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

## SECTION 1: Identification of the substance/mixture and of the company/undertaking

### 1.1 Product identifiers

| | | |
|---|---|---|
| Product name | : | Lemon oil, terpeneless |
| Product Number | : | W262600 |
| Brand | : | Aldrich |
| REACH No. | : | A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline. |
| CAS-No. | : | 68648-39-5 |

### 1.2 Relevant identified uses of the substance or mixture and uses advised against

| | | |
|---|---|---|
| Identified uses | : | Laboratory chemicals, Manufacture of substances |

### 1.3 Details of the supplier of the safety data sheet

| | | |
|---|---|---|
| Company | : | Sigma-Aldrich Chemie GmbH<br>Industriestrasse 25<br>CH-9471 BUCHS |
| Telephone | : | +41 81 755 2511 |
| Fax | : | +41 81 756 5449 |
| E-mail address | : | technischerservice@merckgroup.com |

### 1.4 Emergency telephone

| | | |
|---|---|---|
| Emergency Phone # | : | +41 43-508-2011 (CHEMTREC)<br>+41 44-251-5151 (Tox-Zentrum)<br>145(Tox Info Suisse) |

## SECTION 2: Hazards identification

### 2.1 Classification of the substance or mixture

| | |
|---|---|
| Flammable liquids, (Category 3) | H226: Flammable liquid and vapor. |
| Skin irritation, (Category 2) | H315: Causes skin irritation. |
| Eye irritation, (Category 2) | H319: Causes serious eye irritation. |

| | |
|---|---|
| Skin sensitization, (Category 1) | H317: May cause an allergic skin reaction. |

## 2.2 Label elements

**Labelling according Regulation (EC) No 1272/2008**

Pictogram

| | |
|---|---|
| Signal Word | Warning |
| Hazard Statements | |
| H226 | Flammable liquid and vapor. |
| H315 | Causes skin irritation. |
| H317 | May cause an allergic skin reaction. |
| H319 | Causes serious eye irritation. |
| Precautionary Statements | |
| P210 | Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. |
| P280 | Wear protective gloves. |
| P302 + P352 | IF ON SKIN: Wash with plenty of water. |
| P305 + P351 + P338 | IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. |
| Supplemental Hazard Statements | none |

**Reduced Labeling (<= 125 ml)**

Pictogram

| | |
|---|---|
| Signal Word | Warning |
| Hazard Statements | |
| H317 | May cause an allergic skin reaction. |
| Precautionary Statements | |
| P280 | Wear protective gloves. |
| P302 + P352 | IF ON SKIN: Wash with plenty of water. |
| Supplemental Hazard Statements | none |

## 2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

# SECTION 3: Composition/information on ingredients

## 3.1 Substances

Synonyms : Lemon oil from Citrus limonum

CAS-No. : 68648-39-5

| Component | Classification | Concentration |
|---|---|---|
| **Oils, lemon, terpene-free** | | |
| CAS-No. 68648-39-5 | Flam. Liq. 3; Skin Irrit. 2; Eye Irrit. 2; Skin Sens. 1; H226, H315, H319, H317 | <= 100 % |

For the full text of the H-Statements mentioned in this Section, see Section 16.

## SECTION 4: First aid measures

### 4.1 Description of first-aid measures

**General advice**
Consult a physician. Show this material safety data sheet to the doctor in attendance.

**If inhaled**
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

**In case of skin contact**
Wash off with soap and plenty of water. Consult a physician.

**In case of eye contact**
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

**If swallowed**
Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

### 4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

### 4.3 Indication of any immediate medical attention and special treatment needed
No data available

## SECTION 5: Firefighting measures

### 5.1 Extinguishing media

**Suitable extinguishing media**
Dry powder Dry sand

**Unsuitable extinguishing media**
Do NOT use water jet.

### 5.2 Special hazards arising from the substance or mixture
Nature of decomposition products not known.

### 5.3 Advice for firefighters
Wear self-contained breathing apparatus for firefighting if necessary.

### 5.4 Further information
Use water spray to cool unopened containers.

## SECTION 6: Accidental release measures

### 6.1 Personal precautions, protective equipment and emergency procedures
Use personal protective equipment. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Beware of vapors accumulating to form explosive concentrations. Vapors can accumulate in low areas.
For personal protection see section 8.

### 6.2 Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

### 6.3 Methods and materials for containment and cleaning up
Contain spillage, and then collect with non-combustible absorbent material, (e.g. sand, earth, diatomaceous earth, vermiculite) and place in container for disposal according to local / national regulations (see section 13).

### 6.4 Reference to other sections
For disposal see section 13.

## SECTION 7: Handling and storage

### 7.1 Precautions for safe handling

**Advice on safe handling**
Avoid contact with skin and eyes. Avoid inhalation of vapor or mist.

**Advice on protection against fire and explosion**
Keep away from sources of ignition - No smoking.Take measures to prevent the build up of electrostatic charge.

**Hygiene measures**
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.
For precautions see section 2.2.

### 7.2 Conditions for safe storage, including any incompatibilities

**Storage conditions**
Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Store in cool place.

**Storage class**
Storage class (TRGS 510): 3: Flammable liquids

### 7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

## SECTION 8: Exposure controls/personal protection

### 8.1 Control parameters

**Ingredients with workplace control parameters**

### 8.2 Exposure controls

**Personal protective equipment**

**Eye/face protection**
Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

**Skin protection**
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

The selected protective gloves have to satisfy the specifications of Regulation (EU) 2016/425 and the standard EN 374 derived from it.

**Body Protection**
Complete suit protecting against chemicals, Flame retardant antistatic protective clothing., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

**Respiratory protection**
Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

**Control of environmental exposure**
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

## SECTION 9: Physical and chemical properties

### 9.1 Information on basic physical and chemical properties

| | | |
|---|---|---|
| a) | Physical state | clear, liquid |
| b) | Color | colorless, light yellow |
| c) | Odor | fruity, sweet |
| d) | Melting point/freezing point | No data available |
| e) | Initial boiling point and boiling range | No data available |

| | | |
|---|---|---|
| f) | Flammability (solid, gas) | No data available |
| g) | Upper/lower flammability or explosive limits | No data available |
| h) | Flash point | 49 °C - closed cup |
| i) | Autoignition temperature | No data available |
| j) | Decomposition temperature | No data available |
| k) | pH | No data available |
| l) | Viscosity | Viscosity, kinematic: No data available<br>Viscosity, dynamic: No data available |
| m) | Water solubility | No data available |
| n) | Partition coefficient: n-octanol/water | No data available |
| o) | Vapor pressure | No data available |
| p) | Density | 0,888 g/cm3 at 25 °C |
| | Relative density | No data available |
| q) | Relative vapor density | No data available |
| r) | Particle characteristics | No data available |
| s) | Explosive properties | No data available |
| t) | Oxidizing properties | No data available |

**9.2 Other safety information**
No data available

## SECTION 10: Stability and reactivity

**10.1 Reactivity**
No data available

**10.2 Chemical stability**
Stable under recommended storage conditions.

**10.3 Possibility of hazardous reactions**
No data available

**10.4 Conditions to avoid**
Heat, flames and sparks.

**10.5 Incompatible materials**
Strong oxidizing agents

### 10.6 Hazardous decomposition products

In the event of fire: see section 5

## SECTION 11: Toxicological information

### 11.1 Information on toxicological effects

**Acute toxicity**
Oral: No data available
Inhalation: No data available
Dermal: No data available

**Skin corrosion/irritation**
Remarks: No data available

**Serious eye damage/eye irritation**
Remarks: No data available

**Respiratory or skin sensitization**
No data available

**Germ cell mutagenicity**
No data available

**Carcinogenicity**
No data available

**Reproductive toxicity**
No data available

**Specific target organ toxicity - single exposure**
No data available

**Specific target organ toxicity - repeated exposure**
No data available

**Aspiration hazard**
No data available

### 11.2 Additional Information

sensitizing effects

## SECTION 12: Ecological information

### 12.1 Toxicity

No data available

### 12.2 Persistence and degradability

No data available

### 12.3 Bioaccumulative potential

No data available

### 12.4 Mobility in soil

No data available

### 12.5 Results of PBT and vPvB assessment

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

### 12.6 Endocrine disrupting properties

No data available

### 12.7 Other adverse effects

No data available

## SECTION 13: Disposal considerations

### 13.1 Waste treatment methods

**Product**

Offer surplus and non-recyclable solutions to a licensed disposal company. Waste material must be disposed of in accordance with the Directive on waste 2008/98/EC as well as other national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself.

**Contaminated packaging**

Dispose of as unused product.

## SECTION 14: Transport information

### 14.1 UN number

ADR/RID: 1993 IMDG: 1993 IATA: 1993

### 14.2 UN proper shipping name

ADR/RID: FLAMMABLE LIQUID, N.O.S. (Oils, lemon, terpene-free)
IMDG: FLAMMABLE LIQUID, N.O.S. (Oils, lemon, terpene-free)
IATA: Flammable liquid, n.o.s. (Oils, lemon, terpene-free)

### 14.3 Transport hazard class(es)

ADR/RID: 3 IMDG: 3 IATA: 3

### 14.4 Packaging group

ADR/RID: III IMDG: III IATA: III

### 14.5 Environmental hazards

ADR/RID: no IMDG Marine pollutant: no IATA: no

### 14.6 Special precautions for user

Tunnel restriction code : (D/E)

Further information : No data available

## SECTION 15: Regulatory information

### 15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

**National legislation**

| | | |
|---|---|---|
| Seveso III: Directive 2012/18/EU of the European Parliament and of the Council on the control of major-accident hazards involving dangerous substances. | P5c | FLAMMABLE LIQUIDS |

### 15.2 Chemical Safety Assessment

For this product a chemical safety assessment was not carried out

## SECTION 16: Other information

**Full text of H-Statements**

| | |
|---|---|
| H226 | Flammable liquid and vapor. |
| H315 | Causes skin irritation. |
| H317 | May cause an allergic skin reaction. |
| H319 | Causes serious eye irritation. |

**Full text of other abbreviations**

ADN - European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways; ADR - Agreement concerning the International Carriage of Dangerous Goods by Road; AIIC - Australian Inventory of Industrial Chemicals; ASTM - American Society for the Testing of Materials; bw - Body weight; CMR - Carcinogen, Mutagen or Reproductive Toxicant; DIN - Standard of the German Institute for Standardisation; DSL - Domestic Substances List (Canada); ECx - Concentration associated with x% response; ELx - Loading rate associated with x% response; EmS - Emergency Schedule; ENCS - Existing and New Chemical Substances (Japan); ErCx - Concentration associated with x% growth rate response; GHS - Globally Harmonized System; GLP - Good Laboratory Practice; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; IBC - International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk; IC50 - Half maximal inhibitory concentration; ICAO - International Civil Aviation Organization; IECSC - Inventory of Existing Chemical Substances in China; IMDG - International Maritime Dangerous Goods; IMO - International Maritime Organization; ISHL - Industrial Safety and Health Law (Japan); ISO - International Organisation for Standardization; KECI - Korea Existing Chemicals Inventory; LC50 - Lethal Concentration to 50 % of a test population; LD50 - Lethal Dose to 50% of a test population (Median Lethal Dose); MARPOL - International Convention for the Prevention of Pollution from Ships; n.o.s. - Not Otherwise Specified; NO(A)EC - No Observed (Adverse) Effect Concentration; NO(A)EL - No Observed (Adverse) Effect Level; NOELR - No Observable Effect Loading Rate; NZIoC - New Zealand Inventory of Chemicals; OECD - Organization for Economic Co-operation and Development; OPPTS - Office of Chemical Safety and Pollution Prevention; PBT - Persistent, Bioaccumulative and Toxic substance; PICCS - Philippines Inventory of Chemicals and Chemical Substances; (Q)SAR - (Quantitative) Structure Activity Relationship; REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals; RID - Regulations concerning the International Carriage of Dangerous Goods by Rail; SADT - Self-Accelerating Decomposition Temperature; SDS - Safety Data Sheet; TCSI - Taiwan Chemical Substance Inventory; TECI - Thailand Existing Chemicals Inventory; TSCA - Toxic Substances Control Act (United States); UN - United Nations; UNRTDG - United Nations Recommendations on the Transport of Dangerous Goods; vPvB - Very Persistent and Very Bioaccumulative

**Further information**



The information is believed to be correct but is not exhaustive and will be used solely as a guideline, which is based on current knowledge of the chemical substance or mixture and is applicable to appropriate safety precautions for the product. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

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