# ETHYL HEXANOATE

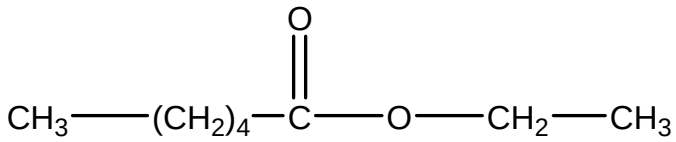
## SYNONYMS

Ethyl caproate
Ethyl capronate
Ethyl hexylate
Caproic acid ethyl ester
Ethyl butyl acetate
Ethyl hexanoate
Ethyl n –hexanoate
Ethyl n-hexanoat
Hexanoate d'éthyle
Hexanoic acid ethyl ester
Hexanoic acid, ethyl ester
Hexanoic acid, monoethyl ester
Acetic acid, butyl-, ethyl ester
Caproic acid ethylester
Capronic ether absolute
Ethyl caproate
ETHYL CAPRONATE
Ethyl ester of hexanoic acid
Ethyl hexoate
Ethylcaproate
Ethylhexanoate

## CHEMICAL FORMULA

$C_8H_{16}O_2$

## CHEMICAL STRUCTURE



## IDENTIFIER DETAILS

| | | |
|---|---|---|
| CAS Number | : | 123-66-0 |
| CoE Number | : | 310 |
| FEMA | : | 2439 |
| EINECS Number | : | 204-640-3 |
| E Number | : | - |

## CLP CLASSIFICATION

Ingredient CLP Classification: No

| Endpoint | Classification | Category |
|---|---|---|
| **Acute Oral Toxicity** | - | - |
| **Acute Dermal Toxicity** | - | - |
| **Acute Inhalation Toxicity** | - | - |
| **Skin Corrosive/irritant** | - | - |
| **Eye Damage/Irritation** | - | - |
| **Respiratory Sensitisation** | - | - |
| **Skin Sensitisation** | - | - |
| **Mutagenicity/Genotoxicity** | - | - |
| **Carcinogenicity** | - | - |
| **Reproductive Toxicity** | - | - |
| **Specific Target Organ Toxicity** | - | - |
| **Aspiration Toxicity** | - | - |

## SPECIFICATIONS

Melting Point: -67 to -68°C

Boiling point: 168°C

Smiles Code: C(C(OCC)=O)CCCC

## STATUS IN FOOD AND DRUG LAWS

**CoE limits:**

| Beverages (mg/kg) | Food (mg/kg) | Exceptions (mg/kg) |
|---|---|---|
| 5 | 40 | - |

**Acceptable Daily Intake:**

| ADI (mg/kg) | ADI Set by | Date Set | Comments |
|---|---|---|---|
| ACCEPTABLE | JECFA | 1996 | No safety concern at current levels of intake when used as a flavouring agent |

**FDA Status:** [CFR21]

| Section Number | Comments |
|---|---|
| 172.515 | Synthetic flavouring substances and adjuvants |

## HUMAN EXPOSURE

***Natural Occurrence:*** Ethyl hexanoate is reported to be found in the fruits of *Ananas sativus.*, apple, orange and grapefruit juice, guava, vitis vinifera, pineapple, strawberry jam, clove bud, cheeses, cognac, rum, whiskies, grape wines, cocoa, passion fruit juice, mango, fruit brandies, figs, corn oil, kiwifruit, mountain papaya, paw paw and mastic gum leaf oil [Fenaroli, 1995 and 2005].

***Reported Uses:*** Ethyl hexanoate is reportedly used in baked goods at 27.18 ppm, milk products at 20.0 ppm, cheese at 1.70 ppm, frozen dairy at 21.68 ppm, meat products at 0.20 ppm, soft candy at 17.55 ppm, gelatin pudding at 17.71 ppm, non-alcoholic beverages at 5.99 ppm, alcoholic beverages at 3.0 ppm, hard candy at 170.1 ppm, and chewing gum at 128.8 ppm [Fenaroli, 1995].

## TOXICITY DATA

Carmines (2002), Rustemeier *et al.,* (2002), Roemer *et al.,* (2002) and Vanscheeuwijck *et al.,* (2002) reported on a testing program designed to evaluate the potential effects of 333 ingredients added to typical commercial blended test cigarettes on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], determination of smoke chemical constituents and a 90-day rat inhalation study. Based on the findings of these studies, the authors concluded that the addition of the combined ingredients, including ethyl hexanoate at levels up to 26 ppm, “did not increase the overall toxicity of cigarette smoke” [Carmines, 2002].

Renne *et* al., (2006) evaluated the effects of tobacco flavouring and casing ingredients on both mutagenicity, and a number of physiological parameters in Sprague-Dawley (SD) rats. Test cigarettes containing a mixture of either 165 low-uses or eight high-use flavouring ingredients which included ethyl hexanoate at 10.4 ppm, were compared to a typical commercial tobacco blend without flavouring ingredients. The Ames assay (TA 98, 100,102, 1535 and 1537 ± S9) did not show any increase in mutagenicity from “low” or “high” cigarette smoke condensate compared to the control. SD rats were exposed by nose-only inhalation for 1 h/day, 5 days/wk for 13 weeks to smoke at concentrations of 0.06, 0.2 or 0.8 mg/L from the test or reference cigarettes, or to air only. Plasma nicotine, COHb and respiratory parameters were measured periodically. Rats were necropsied after 13wk of exposure or following 13 wk of recovery from smoke exposure. Biological endpoints assessed included; clinical appearance, body weight, organ weights, and lesions (both gross and microscopic). The results of these studies did not indicate any consistent differences in toxicological effects between smoke from cigarettes containing the flavouring or casing ingredients and reference cigarettes.

### *In Vivo* Toxicity Status

| Test Type | Route | Species | Reported Dosage |
|---|---|---|---|
| $LD_{50}$ | Oral | Rat | >5g/kg |
| $LD_{50}$ | Dermal | Rabbit | >5g/kg |

[Opdyke, 1976]

Opdyke (1976) in his monograph on ethyl hexanoate describes a study in which rats were fed 1000, 2500 and 10,000 ppm of ethyl hexanoate for 17 weeks. No pathological changes were observed. In a later study, the no observed effect level (NOEL) for rats fed ethyl hexanoate for 1 year was established as 2500 ppm [Opdyke, 1976].

**Dermal toxicity**

The RTECS database has classified ethyl hexanoate as a primary irritant based on the following data. When ethyl hexanoate at a 500mg dose was applied to rabbit skin for 24h moderate irritation was seen [RTECS 24/01/02].

Although moderately irritating to rabbit skin, when applied at 4% in petrolatum ethyl hexanoate was neither an irritant nor a sensitizer of the skin of human volunteers [Opdyke, 1976].

**Inhalation toxicity**

When tested at <0.1 ppm in cigarettes, in a 13-week inhalation study, the presence of ethyl hexanoate "…had no discernible effect on the character of extent of the biologic responses normally associated with inhalation of mainstream cigarette smoke in rats"[Gaworski *et al.,* 1998]. However, it should be noted that the cigarettes had been spiked with a number of flavour ingredients in combination prior to smoking, and they contained a typical American blend humectant and sugar component [*i.e.* glycerine ≈ 20,000 ppm, propylene glycol at ≈ 24,000 ppm, and brown invert sugar at ≈ 24,000 ppm].

A recent study investigated the effect of cigarettes, containing various additives in three combinations, in a 90-day nose-only smoke inhalation study in rats. These ingredients included ethyl hexanoate at 26 ppm, a level described as a multiple of its typical use in a US cigarette. The data from this study along with that from a number of other biological and chemical studies indicate that the addition of the combined ingredients "did not increase the inhalation toxicity of the smoke, even at the exaggerated levels used" [Vanscheeuwijck *et al.,* 2002].

The addition of ethyl hexanoate at 64 ppm to reference cigarettes, used in a 90 day-sub-chronic inhalation exposure in rats, led to a series of pathological changes to smoke exposure that were indistinguishable from those changes caused by the control cigarettes. This indicated that addition of ethyl hexanoate to a reference cigarette had no discernable effect upon the type or

severity of the treatment related pathological changes associated with tobacco smoke exposure [Baker *et al.,* 2004].

Roemer (2014) and Schramke (2014) reported on a testing program designed to evaluate the potential effects of 350 ingredients added to an experimental kretek cigarette on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], Mouse Lymphoma assay, determination of smoke chemical constituents, a 4-day in vivo micronucleus assay and a 90-day rat inhalation study. Based on the results of these studies, the authors concluded that the addition of ingredients commonly used in the manufacture of kretek cigarettes, including Ethyl hexanoate at levels up to 24 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

**Behavioural data:**

No data identified

**Other relevant studies**

Aliphatic esters including ethyl hexanoate are thought to be readily hydrolysed to the corresponding alcohol and acid and are then further metabolised by beta-oxidation [Opdyke, 1976].

The partition coefficient (n-octanol/water) Log $K_{ow}$ for ethyl hexanoate was reported to be 2.83 [Teratox, 2002].

A study in Sprague Dawley rats was performed to investigate the inhibitory effects of alkyl esters on carboxylesterase in the intestine. Carboxylesterase activity was measured by measuring the inhibition of adefoir dipivoxil metabolism. After isolation and homogenisation of the rat intestinal mucosa, preparations were treated with adefoir dipivoxil and various alkyl esters for 15 minutes at 37°C. Ethyl caproate was found to have an $IC_{50}$ of 26.4 ± 4.3 mM [Li *et al.,* (2010].

***In Vitro* Toxicity Status**

**Carcinogenicity and mutagenicity**

Roemer *et al.,* (2002) reported on a study in which cigarettes containing various additives in three different combinations were produced. Smoke condensates prepared from these cigarettes were then tested in two different *in vitro* assays. The mutagenicity of the smoke condensate was assayed in the *Salmonella* plate incorporation [Ames] assay with tester strains TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of an S9 metabolic activation system. The cytotoxicity of the gas/vapour phase and the particulate phase was determined in the neutral red uptake assay with mouse embryo BALB/c 3T3 cells. The authors concluded that the *in vitro*

mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients, which included ethyl hexanoate at levels up to 26 ppm [a multiple of its typical use in a US cigarette] [Roemer *et al.,* 2002].

Baker *et al.,* [2004]; examined the effects of the addition of 482 tobacco ingredients upon the biological activity and chemistry of mainstream smoke. The ingredients, essentially different groups of flavourings and casings, were added in different combinations to reference cigarettes. The addition of ethyl hexanoate at 64 ppm was determined not to have affected the mutagenicity of the total particulate matter (TPM) of the smoke in either the Ames, *in vitro* micronucleus assay or the neutral red assay when compared with that of the control cigarettes [Baker *et al.,* 2004].

The mutagenicity of the smoke condensate was assayed in the *Salmonella* plate incorporation [Ames] assay with the tester strain TA98 in the presence of an S9 metabolic activation system. The cytotoxicity of the cigarette condensate was determined in the neutral red uptake assay and the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium, inner salt assay (MTS assay) with the human hepatocellular liver carcinoma cell line, HEP-G2. It was concluded that the *in vitro* mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients, which included ethyl hexanoate at levels up to 42 ppm.

Ethyl hexanoate was negative in the Ames assay tested in the presence and absence of metabolic activation in TA98 and TA100 strains, and in *E. Coli* pKM101 strain [NTP, 2007].

The US National Library of Medicine database CCRIS lists information on the mutagenicity of ethyl hexanoate. Ethyl hexanoate was found to be negative in two strains of *Salmonella typhimurium* [TA97 and TA102] at concentrations up to 0.1 mg/plate, with and without metabolic activation [CCRIS, 2002].

A study which looked at the genotoxicity of 33 synthetic flavouring agents used the spore rec-assay with *B. subtilis* strains [M45 rec negative and H17 rec positive] which is a method for detecting DNA damaging activity by differences in growth inhibition. Ethyl hexanoate at a maximum dose of 20 μl/disk was considered to be negative in this assay as it had little or no toxic effects [Yoo *et al.,* 1986].

A similar study also using the rec-assay for detecting DNA damage found ethyl hexanoate to be negative in this assay when applied at a maximum dose of 17 μl/disk [Oda *et al.,* 1978].

Additional information concerning the *in vitro* mutagenicity of this material may be found in “An Interim report on data originating from Imperial Tobacco Limited’s Genotoxicity testing programme September 2003” or “An updated report on data originating from Imperial Tobacco Limited’s external Genotoxicity testing programme – Round 2 August 2007”.

A total of 95 ingredients were tested individually through addition at different concentrations to the tobacco of experimental cigarettes. Mainstream cigarette smoke chemistry analysis, bacterial mutagenicity testing, and cytotoxicity testing were conducted. The authors concluded that these ingredients, which included ethyl hexanoate applied at levels up to 10,000 ppm on cigarettes produced minimal changes in the overall toxicity profile of mainstream cigarette smoke, and in some cases, the addition of high levels of an ingredient caused a small reduction in toxicity findings, probably due to displacement of burning tobacco [Gaworski *et al*., 2011].

Roemer (2014) and Schramke (2014) reported on a testing program designed to evaluate the potential effects of 350 ingredients added to an experimental kretek cigarette on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], Mouse Lymphoma assay, determination of smoke chemical constituents, a 4-day in vivo micronucleus assay and a 90-day rat inhalation study. Based on the results of these studies, the authors concluded that the addition of ingredients commonly used in the manufacture of kretek cigarettes, including Ethyl hexanoate at levels up to 24 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

## PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of diacetyl is detailed in the Report on Thermochemical Properties of Ingredients document. In the aforementioned document, the term 'pyrolysis' means the heating of an ingredient in isolation under controlled conditions in an analytical device to examine its degradation potential. The expression 'transfer data' on the other hand is used to describe the fate of an ingredient in qualitative and quantitative terms following the smoking of a tobacco product to which it has been applied.

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