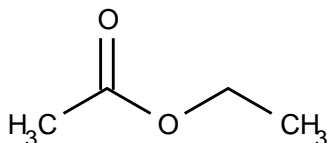


ETHYL ACETATE

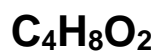
SYNONYMS

Acetic ether
Acetic acid
Ethyl ester

CHEMICAL STRUCTURE



CHEMICAL FORMULA



IDENTIFIER DETAILS

CAS Number	:	141-78-6
CoE Number	:	191
FEMA	:	2414
EINECS Number	:	205-500-4
E Number	:	-

CLP CLASSIFICATION

Ingredient CLP Classification: Yes

Endpoint	Classification	Category
Acute Oral Toxicity	conclusive but not sufficient for classification	-
Acute Dermal Toxicity	conclusive but not sufficient for classification	-
Acute Inhalation Toxicity	conclusive but not sufficient for classification	-
Skin Corrosive/irritant	conclusive but not sufficient for classification	-
Eye Damage/Irritation	conclusive but not sufficient for classification	-
Respiratory Sensitisation	conclusive but not sufficient for classification	-
Skin Sensitisation	conclusive but not sufficient for classification	-
Mutagenicity/Genotoxicity	conclusive but not sufficient for classification	-
Carcinogenicity	conclusive but not sufficient for classification	-
Reproductive Toxicity	conclusive but not sufficient for classification	-
Specific Target Organ Toxicity	H336- May cause drowsiness or dizziness Affected organs: Central Nervous System Route of exposure: Inhalation	3
Aspiration Toxicity	conclusive but not sufficient for classification	-

REACH

This ingredient has been registered under REACH. Under REACH, registrants have an obligation to provide information on substances they manufacture or import. This information includes data on hazardous properties (covering various toxicological endpoints), guidance on safe use and classification and labelling. The European Chemicals Agency (ECHA) makes this information publicly available on its website: <http://echa.europa.eu/>.

SPECIFICATIONS

Melting Point: -83.6°C

Boiling point: 77.1°C

PURPOSE

Flavouring substance.

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
-	-	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
0-25	JECFA	1967	No safety concern at current levels of intake when used as a flavouring agent

FDA Status:[CFR21]

Section Number	Comments
182.60	Synthetic flavoring substances and adjuvants

HUMAN EXPOSURE

Natural Occurrence: Although ethyl acetate has been reported present in some natural fruitial aromas and in some distillates [rum, rum ether], it has not been reported yet as a constituent of essential oils; it has also been identified in the petals of *Magnolia fuscata*, [Fenaroli, 1995]. Reported found in many foods including fresh and cooked apple, apricot, banana (169 ppm), sweet and sour cherry, citrus peel oils and juices, blueberry, cranberry, black currants, raspberry, blackberry, guava, passion fruit, melon, peaches. Papaya, pineapple, cabbage, onion, leek, potato, tomato (3-6 ppm), clove, ginger, vinegar, breads, cheeses (0.2-0.8 ppm), butter (2 ppm), yogurt, milk, meats, cognac, beer (4-64 ppm), whiskies, cider, sherry, grape wines, cocoa, coffee, tea, filbert, peanuts, popcorn, oats, honey, soybeans, coconut, olive oil (0.02 ppm) and olive [Fenaroli, 2006].

Reported Uses: Ethyl acetate is reportedly used in baked goods at 210.9 ppm, frozen dairy at 110.3 ppm, fruit juice at 15 ppm, meat products at 0.10 ppm, soft candy at 152.9 ppm, gelatin pudding at 122.8 ppm, non-alcoholic beverages at 61.02 ppm, alcoholic beverages at 17.24 ppm, gravies at 40 ppm, hard candy at 416.1 ppm and chewing gum at 2302 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 acetate at levels up to 515 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 acetate at 13 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 1h/day, 5 days/wk for 13 weeks to smoke at concentrations of 0.06, 0.2 or 0.8mg/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**

Route of Exposure	Species	LD₅₀ g/kg bw
Oral	Rat	5.6-10.2
	Mouse	4.1
	Guinea-pig	5.5
	Rabbit	4.9
	Rabbit	4.9
Dermal	Rabbit	>18
Intraperitoneal	Rat	2.1
	Mouse	0.709

[BIBRA Toxicity Profile, 1992]

Route of Exposure	Species	Exposure Time (hr)	LC₅₀ mg/litre	Comments
Inhalation	Rat	8	5.86	
	Rat	Unspecified	200	
	Rats	4	-	57.6 mg/l killed all six rats in study
	Mouse	3	44	
	Mouse	30 minutes	-	2mg/l caused central nervous system depression
	Mouse	45 minutes	-	36mg/ml resulted in death
	Guinea-pig	5 minutes	-	Air saturated with ethyl acetate caused narcosis

	Rabbits	9 minutes	-	An unspecified concentration resulted in narcosis, followed by coma and death
	Cat	7.5-24	-	35mg/l produced vomiting, excessive salivation and coughing
	Cat	1-3	-	56mg/l caused death
	Dog	24	-	36mg/l induced vomiting

[BIBRA Toxicity Profile, 1992]

Carcinogenicity and mutagenicity

Groups of five mice [strain A/He which are genetically susceptible to lung tumours] were given 24 intraperitoneal injections over an 8-week period [3 injections per week] resulting in a total dose of diacetyl of 8.40 g/kg mouse. Twenty-four weeks after the first injection, the mice were sacrificed and no increase in the incidence of lung tumours was seen [Stoner, 1973].

Dermal toxicity

Similarly, a recent mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including ethyl acetate at less than 0.1 ppm. The authors concluded that the study “did not indicate any substantive effect of these ingredients on the tumorigenicity of cigarette smoke condensate” [Gaworski *et al.*, 1999]. [It should be noted that the cigarettes contained a typical American blend humectants and sugar component (*i.e.* glycerine \approx 20,000 ppm, propylene glycol at \approx 24,000 ppm, and brown invert sugar at \approx 24,000 ppm)] [Gaworski *et al.*, 1999].

Ethyl acetate was applied at a concentration of 10% in petrolatum in a 48h-closed patch test in 25 human subjects. No irritation was seen. Similarly, no irritation was observed following the application of ethyl acetate at a concentration of 16.5% in acetone in a semi-covered 48h patch test in 118 human subjects, or when applied at a concentration of 97% in a covered 23h patch test [the test material was applied daily for 3 weeks] in 10 human subjects [BIBRA Toxicity Profile, 1992].

When applied to the skin of rabbits for 24h neat ethyl acetate did not cause any irritation. In another study a 10% solution of ethyl acetate in acetone was applied to rabbit skin for 3 consecutive 24h periods, again no irritation effects were seen [BIBRA Toxicity Profile, 1992].

When 10 human volunteers were exposed to an atmosphere containing 1.44mg of ethyl acetate/litre for 3-5 minutes, eye irritation was observed. Instillation of 0.5ml or ‘one to two drops’ of the neat liquid produced mild to moderate irritation and corneal effects in rabbits [BIBRA Toxicity Profile, 1992].

Exposure to an atmosphere containing 29mg ethyl acetate/litre for 20 minutes resulted in eye irritation in cats [BIBRA Toxicity Profile, 1992].

A number of studies reported that ethyl acetate vapour [1.44mg/litre for 3-5 minutes] was also found to be irritating to the nose and throat of ten humans, and that it may cause inflammation of the gums [unspecified concentrations]. Prolonged or repeated exposure may lead to defatting of the skin leading to a reduction in the barrier properties [BIBRA Toxicity Profile, 1992].

When a 10% solution of ethyl acetate was applied to the outer ear of guinea pigs, local anaesthesia was induced within about 2 minutes [BIBRA Toxicity Profile, 1992].

High concentrations of ethyl acetate in man have been reported to cause irritation of the eyes, respiratory tract, central nervous system effects and possibly metabolic disturbances of the liver. There was reported to be virtually no information on exposure to ethyl acetate at moderate or low exposure levels. It was suggested that a level of 58 ppm might be uncomfortable but tolerated in man [Dutch Expert Committee for Occupational Standards, 1992].

A formulation of 97% ethyl acetate was applied in 5 consecutive 48h covered patches to a group of human volunteers. Ten days after the last patch test, the subjects were again challenged and no adverse skin effects were seen. An identical study in which a 10% ethyl acetate solution in petrolatum was applied also gave negative results in 25 volunteers [BIBRA Toxicity Profile].

The ethyl acetate threshold limit value has been set at 400 ppm at which point it produces nose and throat irritation and has a mild narcotic action. The American Conference of Industrial Hygienists set this level in 1973 [Opdyke, 1974].

Reproductive and developmental toxicity:

Factory workers reportedly exposed to an atmosphere containing ethyl acetate at 0.54 mg/l [duration of exposure unspecified] did not have an altered sperm count [BIBRA Toxicity Profile, 1992].

The teratogenicity of 80 chemicals, including ethyl acetate, was assessed using the chicken embryo test. Ethyl acetate was injected into the yolk sac of fresh fertile chicken eggs at a concentration of 25mg/egg and was not found to be a teratogen [Verrett, 1980].

Inhalation toxicity

When tested at less than 0.1 ppm in cigarettes, in a 13-week inhalation study, the presence of ethyl acetate "...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 humectants and sugar component (*i.e.* glycerine \approx 20,000 ppm, propylene glycol at \approx 24,000 ppm, and brown invert sugar at \approx 24,000 ppm) [Gaworski *et al.*, 1998].

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 acetate at 515 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 acetate at 643 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 acetate 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]

A threshold limit value time weighted average [TLV-TWA] of 400 ppm was recommended for occupational exposure to ethyl acetate was recommended, it was however suggested that there was insufficient information to recommend a TLV short term exposure level [TLV-STEL] [Anonymous, 2001].

Exposure to 2.1-2.5 mg/litre ethyl acetate for 5-10 minutes caused a 50% decrease in the respiration rate of mice [BIBRA Toxicity Profile, 1992].

The metabolism of ethyl acetate was studied in the upper respiratory tracts [URT] of both Male Fischer 344 rats and Syrian golden hamsters with surgically removed URTs, at exposure concentrations up to 800 μ g/l. Deposition efficiencies of ethyl acetate were calculated to be 10-35% in rats and 32-71% in hamsters. Forty to 65% of the deposited ethyl acetate was metabolised in the rat URT and 63-90% in the hamster URT. The author concluded that URTs of rats and hamster were able to significantly metabolise ethyl acetate at a rate fast enough to generate high levels of metabolites in the blood stream. First pass metabolism of ethyl acetate or other simple ester vapours may significantly decrease the amount of parent compound available for absorption into the blood stream [Morris, 1990].

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 Acetate at levels up to 1065 ppm, did not change the overall *in vivo/vitro* toxicity profile of the mainstream smoke.

Other relevant studies

Rabbits exposed to 16 mg/l ethyl acetate for 1hr/day for a total of 40 days developed changes in blood chemistry, fatty degeneration, swelling and increased blood flow in various organs. Rats injected with 0.9g/kg bw/day for 8 days displayed altered blood chemistry and decreased liver glycogen levels [BIBRA Toxicity Profile, 1992].

The US EPA sponsored a 90-day sub chronic study in rats with ethyl acetate. Four groups of rats [30/sex/group] were gavaged daily with 0, 300, 900 and 3600 mg/kg/day. Six weeks after the initial dosing 10 rats/sex were subjected to interim sacrifice while the remaining rats were sacrificed after 90 days. During this study data was gathered on weekly body weights, food consumption, clinical signs of toxicity, ophthalmological evaluations, blood and urine chemistry and gross and histopathological evaluations of target organs. Evaluation of the data indicated that male rats exposed to the high concentration of ethyl acetate [3600mg/kg/day] showed significant toxic effects whilst female rats exposed to this dose did not show significant toxicity. The NOEL established from this study for ethyl acetate is 900 mg/kg/day. An uncertainty factor of 1000 was applied to this figure resulting in a final recommended NOEL of 0.9 mg/kg/day for humans [US EPA, 1986].

A group of 40 hamsters were fed either a single dose of 2.5g/kg bw ethyl acetate or given an intraperitoneal dose of 0.473 g/kg bw in both cases no damage to the bone marrow chromosomes was detected. A similar negative result was seen when groups of six mice were given an intraperitoneal injection of 0.473 g/kg bw [BIBRA Toxicity Profile, 1992].

Ethyl acetate is rapidly biotransformed being hydrolysed into ethanol and acetic acid. It was hydrolysed by human whole blood *in vitro* at 37°C at a rate of 20% in 8 hours. The half life in rat blood is 65-70 minutes [Lundberg, 1991].

Behavioural data

In a recent study rats were exposed to 0, 350, 750 or 1500 ppm of ethyl acetate by inhalation for 6h per day, 5 days per week for 13 weeks. Functional observational battery (FOB) and motor activity tests occurred on non-exposure days during weeks 4, 8 and 13 after which tissues were microscopically examined for neuropathology. The results from these studies indicate a LOEL of 350 ppm for systemic toxicity based on the decreased body weight gain in male rats and a LOEL of 1500 ppm for neurotoxicity based on the transient reduction in motor activity in female rats. The authors concluded that there was no evidence that sub chronic exposure up to 1500 ppm ethyl acetate produced any enduring neurotoxic effect in rats [Christoph *et al.*, 2003].

A study by Kleinbeck *et al.*, (2008) was designed to investigate ethyl acetate on three different dimensions: behavioural, physiological and psychological indicators of adverse chemosensory effects were investigated during acute exposures to different concentrations of ethyl acetate. Twenty-four subjects were challenged with ethyl acetate in three exposure patterns (2 ppm, 400 ppm, 400 ppm including peaks of 800 ppm). While the odour intensity is rated "strong", trigeminal perceptions were rated less than "moderate". The absence of substantial trigeminal ratings was supported by physiological data. There was neither an effect of concentration on blinking frequency nor on nasal resistance which both are indicators of irritation. Furthermore, there are no effects of ethyl acetate concentration on behavioural measures indicating no olfactory or trigeminally mediated disturbance of cognitive processing. In conclusion, the results of this multilevel approach revealed no adverse chemosensory effects at ethyl acetate concentrations as recommended by the German MAK-value. [Kleinbeck *et al.*, (2008)].

***In Vitro* Toxicity Status**

Carcinogenicity and mutagenicity

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".

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 acetate at levels up to 515 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 acetate at 643 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].

Ethyl acetate was found to be negative in the Ames test when tested in *Salmonella* strains, TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, and TA1538 both in the absence and presence of S9 when tested up to maximum concentrations of 10mg/plate [Zeiger, 1992].

Ethyl acetate was found to be negative when tested in the Ames test in *Salmonella* bacterium strains [TA92, TA1535, TA100, TA1537, TA94 and TA98; maximum dose being 5 mg/plate] both in the absence and presence of S9. In the same study ethyl acetate was also evaluated for chromosome damage in the Chinese hamster fibroblast cell line [maximum dose tested 9 mg/ml; in the absence of S9] and the authors concluded that the result was positive based on the criteria that the total incidence of cells with aberrations [including gaps] was 10% or more [Ishidate, 1984].

Ethyl acetate gave a negative result for mutagenicity in the rec assay in *Bacillus subtilis*, which is an indirect measure of DNA damage [BIBRA Toxicity Profile, 1992].

Myocardial contractibility was depressed in isolated guinea pig myocardial strips exposed to ethyl acetate. The depressant activity of ethyl alcohol was reported to be 10 times greater than that of ethanol [Nakano *et al.*, 1973].

Ethyl acetate induced aneuploidy in the yeast *Saccharomyces cerevisiae* but did not induce other chromosome aberrations [BIBRA Toxicity Profile, 1992].

It has also been reported as causing chromosome damage in the bean *Vicia fabia* [BIBRA Toxicity Profile, 1992].

Ethyl acetate did not cause DNA damage in human cells or in hamster cells in either the presence or absence of S9. However, in a further study sister chromatid exchange [a form of DNA damage] was detected in the presence of S9 only [BIBRA Toxicity Profile, 1992].

A number of esters and ketones including ethyl acetate were used to study the induction of mitotic chromosomal malsegregation, mitotic recombination and point mutation in a diploid yeast strain D61.M. Ethyl ester was found to strongly induce aneuploidy but not recombination or point mutation at a concentration of 2.44%. The authors proposed that the mutagenic compounds act directly on the tubulin during growth [Zimmermann, 1985].

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 acetate* at levels up to 127 ppm.

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 added ingredients, which included Ethyl acetate at levels up to 10,000 ppm 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 Acetate at levels up to 1065 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 ethyl acetate 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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