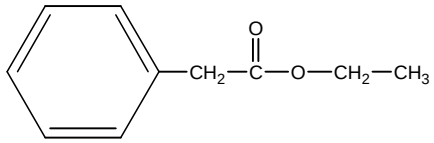
ETHYL PHENYLACETATE

SYNONYMS

Benzeneacetic acid, ethyl ester
Ethyl *alpha*-toluate
Phenylacetic acid, ethyl ester
alpha-Toluic acid

CHEMICAL STRUCTURE



CHEMICAL FORMULA

$C_{10}H_{12}O_2$

IDENTIFIER DETAILS

CAS Number	:	101-97-3
CoE Number	:	2156
FEMA	:	2452
EINECS Number	:	202-993-8
E Number	:	

CLP CLASSIFICATION

Ingredient CLP Classification: No

Endpoint	Classification	Category
Acute Oral Toxicity	Data lacking	-
Acute Dermal Toxicity	Data lacking	-
Acute Inhalation Toxicity	Data lacking	-
Skin Corrosive/irritant	Skin Irrit. 2 H315: Causes skin irritation	2
Eye Damage/Irritation	Eye Irrit. 2 H319: Causes serious eye irritation	2
Respiratory Sensitisation	Data lacking	-
Skin Sensitisation	Data lacking	-
Mutagenicity/Genotoxicity	Data lacking	-
Carcinogenicity	Data lacking	-
Reproductive Toxicity	Data lacking	-
Specific Target Organ Toxicity	Data lacking	-
Aspiration Toxicity	Data lacking	-

REACH Statement

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:
Boiling point: 226°C

PURPOSE

Flavouring substance.

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
-	50	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
Not specified	JECFA	2002	No safety concern

FDA Status:[CFR21]

Section Number	Comments
C172.515	Synthetic flavoring substances and adjuvants

HUMAN EXPOSURE

Natural Occurrence: Ethyl phenyl acetate is reported to be found in cocoa, honey, sake, tea, and wheat bread, guava, cognac, grape wines, figs apple juice, pineapple and papaya [Fenaroli, 2005].

Reported Uses: Ethyl phenyl acetate is reportedly used in baked goods at 18.57 ppm, frozen dairy at 9.84 ppm, soft candy at 13.52 ppm, sweet sauce at 10 ppm, gelatin pudding at 12.62 ppm, non-alcoholic beverages at 5.2 ppm, alcoholic beverages at 5 ppm, and hard candy at 20.32 ppm [Fenaroli, 2005].

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 phenylacetate at levels up to 269 ppm, "did not increase the overall toxicity of cigarette smoke" [Carmines, 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 phenyl acetate at 35 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].

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 phenylacetate at 6.5 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 [Renne *et al.,* 2006].

In Vivo Toxicity Status

Test Type	Species	Route	Reported Dosage
LD_{50}	Rat	Oral	3300 mg / kg bw [RTECS, 1997]

The estimated per capita intake is 0.000065 mg/kg [Fenaroli, 2005]

Dermal toxicity

Ethyl phenylacetate applied full strength to intact of abraded skin for 24 hours was non irritating. When applied to neat to the forearms of human volunteers it was non irritating. When tested at 8% in petrolatum in 25 human subjects, there was no irritation following 48 hours with a closed patch test [Opdyke 1973].

A maximisation test conducted on 25 human volunteers with ethyl phenylacetate at a concentration of 8% produced no sensitisation reactions [Opdyke 1973].

A mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including ethyl phenylacetate at <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 humectant and sugar component (*i.e.* glycerine ≈ 20,000 ppm, propylene glycol at ≈ 24,000 ppm, and brown invert sugar at ≈ 24,000 ppm) [Gaworski *et al.,* 1999].

Inhalation study

When tested at <0.1 ppm in cigarettes, in a 13-week inhalation study, the presence of ethyl phenylacetate "…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) [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 phenylacetate at 269 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 phenyl acetate at 35 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 phenyl 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]

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 phenylacetate at levels up to 6 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Behavioural data:

No data identified

***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".

Ethyl phenylacetate was negative in the Ames test, at concentrations up to 5 mg / plate [*S. typhimurium* strains TA98, 100 1535, 1537], and failed to induce chromosomal aberrations, at concentrations up to 1 mg / ml [Ishidate *et al.,* 1988].

Tests of the ability of ethyl phenylacetate to induce mutation in *Bacillus subtilis* H17 and M45 were inconclusive. In a study in which ethyl phenylacetate was incubated with *B. subtilis* H17 and M45 at 21 µg per disc, the difference in the zone of inhibition (0.8 mm) between the two strains indicated that it was not active (Oda *et al*., 1979). In a study with a lower concentration, ethyl phenylacetate was incubated at a concentration of 20 µl per disc with *B. subtilis* H17 and M45 in the same assay. The difference in the zone of inhibition (> 8 mm) between the two strains was considered to provide evidence of mutagenicity (Yoo, 1986).

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 phenylacetate at levels up to 269 ppm [a multiple of its typical use in a US cigarette] [Roemer *et al.,* 2002].

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 phenyl acetate* at levels up to 418 ppm.

In vitro toxicity testing of tobacco ingredients in burnt form (Internal document R-55).

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 phenylacetate at levels up to 6 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 phenyl 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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