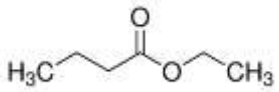
Ethyl butyrate

Ingredient synonym names

Butanoic acid, ethyl ester Butyric acid, ethyl ester

IDENTIFIER DETAILS

Ingredient chemical structure



CAS Number	FEMA Number	Additive Number	Ingredient EC Number
105-54-4	2427	-	203-306-4

CAS Additional Number	FL Number	CoE Number
-	09.039	264

Chemical formula C6H12O2

Ingredient CLP Classification

Ingredient REACH Registration Number

-

Acute Oral Toxicity	Eye Damage/Irritation	Carcinogenity
0	0	0
Acute Dermal Toxicity	**Respiratory Sensitisation**	**Reproductive Toxicity**
0	0	0
Acute Inhalation Toxicity	**Skin Sensitisation**	**Aspiration Toxicity**
0	0	0
Skin Corrosive/Irritant	**Mutagenicity/ Genotoxicity**	**Specific Target Organ Toxicity**
0	0	0

SPECIFICATIONS

Melting Point -93°C Boiling Point 120°C

STATUS IN FOOD AND DRUG LAWS

Acceptable Daily Intake (ADI, mg/kg) 0-15 mg/kg (JECFA1996)

Ethyl butyrate

Acceptable Daily Intake (ADI) comments	No safety concerns at current levels of intake when used as a flavouring agent
FDA Status	CFR21 182.60 Synthetic flavoring substances and adjuvants

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

HUMAN EXPOSURE

Ingredient Natural Occurence (if applicable)

Ethyl butyrate is reportedly found in strawberry juice; has also been identified by gas chromatography in olive oil and other vegetable oils [Fenaroli, 1995]. Reportedly found in apple, banana, citrus peel oils and juices, cranberry, blueberry, black currents, guava, grapes, papaya, strawberry, onion, leek, cheeses, chicken, beef, beer, cognac, rum, whiskies, cider, sherry, grape wines, coffee, honey, soybeans, olives, passion fruit, plums, mushroom, mango, fruit brandies, kiwifruit, mussels and paw paw [Fenaroli 2005]

References - Ingredient Natural Occurence

Fenaroli (1995) Fenaroli's Handbook of Flavor Ingredients, Volume II, 3rd Edition. CRC Press London.

Ingredient Reported Uses

Ethyl butyrate is reportedly used in baked goods at 136.6 ppm, fats and oils at 25.01 ppm, frozen dairy at 66.63 ppm, meat products at 18.60 ppm, soft candy at 104.1 ppm, gelatin pudding at 82.15 ppm, non-alcoholic beverages at 37.88 ppm, alcoholic beverages at 23.97 ppm, hard candy at 168.0 ppm, and chewing gum at 1393 ppm [Fenaroli, 1995].

References - Ingredient Reported Uses

Fenaroli (2005) Fenaroli's Handbook of Flavor Ingredients, 5th Edition. CRC Press London.

TOXICITY DATA

In Vivo Data

Acute Toxicity Data

13 g/kg, Rat, Oral [Jenner et al., 1964]
5 g/kg, Rabbit, Oral [RTECS, 2002]
>2 g/kg, Rabbit, Dermal [RTECS, 2002]

In Vivo Carcinogenicity/Mutagenicity

No data identified

References - In Vivo Carcinogenicity/Mutagenicity

No data identified

Ethyl butyrate

Dermal Toxicity

Ethyl butyrate is classified as a primary irritant based on the following data: Rabbit skin was exposed to 500 mg of ethyl butyrate for a 24 hours resulting in a moderate skin irritation effect [RTECS, 2002].

In a monograph on ethyl butyrate by Opdyke (1974), a study is described in which ethyl butyrate was applied at full strength to intact or abraded rabbit skin for 24h under occlusion and was found to be moderately irritating. However, when ethyl butyrate was tested at 5% in petrolatum it produced no irritation after a 48h closed-patch test in 25 human subjects. The same monograph describes a sensitisation study in which ethyl butyrate was tested at a concentration of 5% in petrolatum in 25 volunteers and produced no sensitisation reaction [Opdyke, 1974].

An Open Epicutaneous Test (OET) of Ethyl butyrate (CAS No: 105-54-4)) was conducted on guinea pigs to assess the skin sensitization potential caused by test chemical. On day 1 during induction, 0.1 ml of the Ethyl butyrate was applied at concentrations of 100%, 30%, 10%, 3%, 1%, or 0.3% in vehicle to an area measuring 8 cm2 on the clipped flank skin of the guinea pigs. The applications are repeated daily for 3 weeks or done 5 times weekly during 4 weeks, usually on the same skin sites. The application sites were left uncovered and the reactions, if continuous daily applications were performed, can be read 24 h after each application, or at the end of each week. To determine whether or not contact sensitization was induced, all groups of guinea pigs previously treated for 21 days, as well as 10 untreated, or only pretreated with the vehicle, controls are tested on days 21 and 35 on the contralateral flank with the test material. This test was performed by applying with a pipette 0.025 ml of each concentration to skin areas measuring 2 cm2. The reactions were read after 24,48 and/or 72h. None of the treated guinea pigs showed any signs of skin sensitization at challenge concentration of 5%.Thus the chemical Ethyl butyrate (CAS No: 105-54-4)) was considered to be not sensitizing on skin of guinea pigs in an Open Epicutaneous Test (OET) [G. Klecak., 1985]

References - Dermal Toxicity

RTECS (Registry of Toxic Effects of Chemical Substances). Search carried out on 24th January 2002. RTECS no. ET1660000.

Opdyke (1974). Monographs on Fragrance Raw Materials: Ethyl butyrate and ethyl cinnamate. Fd. Cosmet. Toxicol., 12, 719.

G. Klecak (1985) The Freund's Complete Adjuvant Test and the Open Epicutaneous Test. Curr. Probl. Derm., vol.14, pp.152-171 (Karger, Basel 1985)

Reproductive/ Developmental Toxicity

No data identified

References - Reproductive/ Developmental Toxicity

No data identified

Inhalation Toxicity

In a repeated inhalative toxicity study, male and female Sprague-Dawley Crl:CD rats were exposed to Ethyl butyrate 1 hours/day, 5 days/week for 13 weeks of exposure and 13 weeks of recovery. Exposure to smoke from reference or test cigarettes in both studies induced increases in blood carboxyhemoglobin ((COHb)) and plasma nicotine, decreases in minute volume, differences in body or organ weights compared to air controls, and a concentration-related hyperplasia, squamous metaplasia, and inflammation in the respiratory tract. All these effects were greatly decreased or absent following the recovery period. Comparison of rats exposed to similar concentrations of test and reference cigarette smoke indicated no difference at any concentration. Thus, the results did not indicate any consistent differences in toxicological effects between smoke from cigarettes containing the

flavoring or casing ingredients and reference cigarettes. Therefore, NOAEL was considered to be 0.06 mg/L when male and female Sprague-Dawley Crl:CD rats were exposed to Ethyl butyrate by inhalation of cigarette smoke for 13 weeks [Roger et al., 2006].

References - Inhalation Toxicity

Roger A. Renne, Hiroyuki Yoshimura, Kei Yoshino, George Lulham, Susumu Minamisawa, Albrecht Tribukait Dennis D. Dietz, Kyeonghee Monica Lee, and R. Bruce Westerberg (2006) Effects of Flavoring and Casing Ingredients on the Toxicity of Mainstream Cigarette Smoke in Rats. Inhalation Toxicology, 18:685–706.

Cardiac Toxicity

No data identified

References - Cardiac Toxicity

No data identified

Addictive Data

No data identified

References - Addictive Data

No data identified

Behavioral data

To screen for inositol-depleting valproate-like compounds as potential mood stabilizing drugs, yeast Saccharomyces cerevisiae, was employed as a model in which inositol de novo synthesis has been extensively characterized, to test the effects of ethyl butyrate (EB), 2-ethyl-butyric acid, sodium butyrate, and n-propyl hexanoate on inositol biosynthesis. Cell growth was followed by measuring the optical density of the cultures (spectrophotometrically), RNA abundance was determined by Northern blot analysis, intracellular inositol was measured by a fluorometric assay, and 1-d-myo-inositol-3-phosphate synthase activity was examined using a chromatographic method. Of the tested compounds, only EB exhibited an inositol-depleting effect. The inositol-depleting effect of EB was achieved without significant adverse effect on cell growth, pointing to lesser toxicity compared to valproate. The authors report the results indicate that EB is a potential candidate for mood-stabilizing therapy [Azab et al., 2009].

References - Behavioral data

Azab et al., (2009). Ethylbutyrate, a valproate-like compound, exhibits inositol-depleting effects- a potential mood-stabilising drug.

In Vivo - Other Relevant Studies

The US National Library of Medicine HSDB database contains a record on ethyl butyrate that was last updated on the 8th September 2001 and contains the following information:

- The oral administration of ethyl butyrate to dogs at 3g in 60 ml of water caused no toxic effects
- In rabbits administration of 2.14 ml/kg caused an increase in respiratory volume.
- An intravenous injection of 177-222 mg/kg to dogs had no effect

[HSDB, 2002].

In a feeding experiment on 15 males and 15 females for 12 weeks no adverse effect was noted at 14.4 mg/kg body-

weight/day [Oser, 1967].

Ethyl butyrate was metabolised by non-Michaelis-Menten kinetics in the horse and pig liver via carboxylesterases [Stoops et al., 1975].

References - In Vivo - Other Relevant Studies

HSDB (Hazardous Substances Data Bank). http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB. Search carried out on 24th January 2002. HSDB no. 406.

Oser (1967) Unpublished report [as cited in JECFA 1967].

Stoops et al., (1975) Carboxylesterases (EC3.1.1). A comparison of some kinetic properties of horse, sheep, chicken, pig, and ox-liver carboxylesterases. Can. J. Biochem. 53(5), 565-573.

In Vitro Data

In Vitro Carcinogenicity/Mutagenicity

The US National Library of Medicine database CCRIS lists information on the mutagenicity of ethyl butyrate. Ethyl butyrate was found to be negative in two strains of Salmonella typhimurium [TA97 and TA102] with and without metabolic activation [CCRIS, 2002].

A study carried out by Ishidate et al., (1984) found that ethyl butyrate was negative in the Ames test using the following S. typhimurium strains, TA92, TA1535, TA100, TA1537, TA94 and TA98 both in the presence and absence of a metabolic activation system. The same study then investigated ethyl butyrate in a chromosomal aberration test using Chinese Hamster fibroblast cells [CHL]. Ethyl butyrate did not induce chromosomal aberrations even at the maximum dose tested [2 mg/ml] after a 48-hour exposure, without metabolic activation [Ishidate et al., 1984].

A chromosomal aberration study was performed to determine the mutagenic nature of Ethyl butyrate. The cells were exposed to the test material at three different doses with 2 mg/mL being the maximum concentration for 24 and 48 hr. Colcemid (final concn 0.2 µg/ml) was added to the culture 2 hr before cell harvesting. The slides were stained with Giemsa solution for 12-15 min. A hundred well-spread metaphases were observed under the microscope. In the present studies, no metabolic activation systems were applied. The incidence of polyploid cells as well as of cells with structural chromosomal aberrations such as chromatid or chromosome gaps, breaks, exchanges, ring formations, fragmentations and others, was recorded on each culture plate. Untreated cells and solvent-treated cells served as negative controls, in which the incidence of aberrations was usually less than 3.0%. The results were considered to be negative if the incidence was less than 4.9%, equivocal if it was between 5.0 and 9.9%, and positive if it was more than 10.0%. Ethyl butyrate did not induce chromosomal aberration in the Chinese hamster fibroblast cell line CHL. Hence, Ethyl butyrate is not likely to classify as a gene mutant in vitro [Ishidate et al., 1984].

A gene mutation toxicity study was performed on Ethyl butyrate using Salmonella typhimurium strains TA102 and TA97 with and without rat liver Aroclor 1254-induced S9 metabolic activation system. Preincubation assay was performed at dose levels of 0.01 to 1 mg/plate using DMSO as the solvent. The plates were observed for a dose-dependent increase in the number of revertants/plate. As seen by the results, Ethyl butyrate did not induce gene mutation in S. typhimurium strains TA97 and TA102 in the presence or absence of S9 metabolic activation system. Hence, ethyl butyrate is not likely to classify as a gene mutant in vitro [US National Library of Medicine, 2017].

References - In Vitro Carcinogenicity/Mutagenicity

CCRIS (Chemical Carcinigenesis Research Information System). http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS. Search carried out on 24th January 2002. CCRIS Record no. 6554.

Ishidate et al., (1984). Primary Mutagenicity Screening of Food Additives Currently Used in Japan. Fd. Chem. Toxic. 22 (8), 623-636.

M. Ishidate Jr, T. Sofuni, K. Yoshikawa, M. Hayashi, T. Nohmi, M. Sawada, A. Matsuoka (1984) Primary mutagenicity screening of food additives currently used in Japan. Food and Chemical Toxicology 22(8):623-636.

U. S. National Library of Medicine (2017) Gene mutation toxicity study for Ethyl Butyrate. Chemical Carcinogenisis Research Information System, U. S. National Library of medicine.

In Vitro - Other Relevant Studies

Concentrations of ethyl butyrate in excess of 5 mM were effective in inhibiting the proliferation and differentiation of erythroleukemia cells. Butyrate and its analogues had similar inhibitory effects on the histone deacetylase activity in the nuclei of both mouse Ds19 cells and human K562 cells [Lea et al., 1995].

References - In Vitro - Other Relevant Studies

Lea (1995) Discordant effects of butyrate analogues on erythroleukemia cell proliferation, differentiation and histone deacetylase.

Emissions and Associated 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 butyrate at levels up to 28 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 butyrate 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.

When tested at <0.1 ppm in cigarettes, in a 13-week inhalation study, the presence of ethyl butyrate "…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].

Ethyl butyrate

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 butyrate at 28 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 butyrate at 292 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 butyrate 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 butyrate at levels up to 8190 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

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 conclude that the in vitro mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients, which included ethyl butyrate at levels up to 28 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 butyrate at 292 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/was not increased by the addition of the ingredients, which included Ethyl butyrate at levels up to 127 ppm.

Information relating to the pyrolysis and/or transfer of ethyl butyrate 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.

Ethyl butyrate

A 2004 study by Baker and Bishop analysed the pyrolytic breakdown of 291 tobacco ingredients using combustion conditions that simulate cigarette combustion. Due to the combustion conditions the results likely predict the natural behaviour of these compounds during combustion on the cigarette, and allow estimation of the degree of intact transfer into the mainstream smoke. Under pyrolysis ethyl butyrate was found to transfer 100% intact.

References - Emissions and Associated Toxicity Data

Baker RR, et al., (2004) An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. Food Chem Toxicol. 42 Suppl: S53-83

Baker and Bishop, (2004). The pyrolysis of tobacco ingredients. J. Anal. Appl. Pyrolysis. 71, 223-311.

Carmines (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 1: Cigarette design, testing approach, and review of results. Food and Chemical Toxicology 40, 77-91.

Gaworski et al., (1998). Toxicologic evaluation of flavor ingredients added to cigarette tobacco: 13-week inhalation exposure in rats. Inhalation Toxicol., 10, 357-381.

Renne, R.A., Yoshimura, H., Yoshino, K., Lulham, G., Minamisawa, S., Tribukait. Dietz, D.D., Lee, K.M., Westerberg, R.B. (2006). Effects of flavouring and casing ingredients on the toxicity of mainstream cigarette smoke in rats. Inhalation Toxicology. 18, 685-706.

Roemer et al. (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 3: In vitro genotoxicity and cytotoxicity. Food and Chemical Toxicology 40, 105-11.

the mainstream smoke.

Roemer (2014) Toxicological assessment of kretek cigarettes: Part 1: background, assessment approach, and summary of findings. Regul Toxicol Pharmacol.; 70 Suppl 1: 2-14

Roemer (2014) Toxicological assessment of kretek cigarettes Part 6: the impact of ingredients added to kretek cigarettes on smoke chemistry and in vitro toxicity. Regul Toxicol Pharmacol.; 70 Suppl 1: 66-80

Rustemeier et al., (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke. Food and Chemical Toxicology 40, 93-104.

Schramke (2014) Toxicological assessment of kretek cigarettes. Part 7: the impact of ingredients added to kretek cigarettes on inhalation toxicity. Regul Toxicol Pharmacol; 70 Suppl 1: 81-9

Vanscheeuwijck et al., (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 4: Subchronic inhalation toxicity. Food and Chemical Toxicology 40, 113-131.

In vitro toxicity testing of tobacco ingredients in burnt form (Internal document R-53).