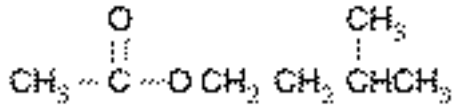
ISOAMYL ACETATE

SYNONYMS

3-Methylbutyl acetate
Acetic acid,3-methylbutyl ester
Amyl acetate
Banana oil
Isoamyl ethanoate
Isopentyl acetate
Isopentyl alcohol acetate
Isopentyl acetate
Pear oil

CHEMICAL STRUCTURE



CHEMICAL FORMULA

$C_7H_{14}O_2$

IDENTIFIER DETAILS

CAS Number	:	123-92-2
CoE Number	:	214
FEMA	:	2055
EINECS Number	:	204-662-3
E Number	:	-

SPECIFICATIONS

Melting Point: -78.5°C

Boiling point: 145°C

PURPOSE

Flavouring substance

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
60	500	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
0 - 3mg/kg	JECFA	1979	No safety concern at current levels of intake when used as a flavouring agent. The 1979 ADI was maintained at the forty-sixth meeting (1996).

FDA Status:

Section Number	Comments
172.515	Synthetic flavouring substances and adjuvant.

HUMAN EXPOSURE

Natural Occurrence: Isoamyl acetate is reportedly found in the volatile portion of banana fruit and cocoa bean. It is also reportedly found in banana at concentrations between 0.2 - 25 mg/kg; other fruit up to 0.5; beer at 0.1 - 7; other alcohol at up to 30; black tea at 1.3 and cocoa at 0.2 mg/kg [CoE 1992]. It is also naturally found in apple, apricot, currents, pineapple, peach, pear, butter, milk, cheese and wine [Fenaroli, 2005].

Reported Uses: Isoamyl acetate is r eportedly used in baked goods at 167.0 ppm, frozen dairy at 86.67 ppm, soft candy at 167.7 ppm, confection frosting at 100.0ppm, sweet sauce at 150.0 ppm, gelatin pudding at 109.7 ppm, non-alcoholic beverages at 72.98 ppm, alcoholic beverages at 38.83 ppm , hard candy at 234.9 ppm, and chewin g gum at 3027.0 ppm. Reported individual consumption is 0.1652 mg/kg/day [Fenaroli, 2005].

Sources other than foods: Amyl acetate is used in cosmetics primarily as a solvent in either nail polish removers or as a solve nt for nitrocellulose in nail polishes, enamel and lacquers. It is also reported to be used as a solvent for paints, lacquers, is used in cement, photographic film, adhesives, thinners and in the printing of and finishing of fabrics. Amyl acetate is also apparently used as a partition solvent, as an anti inflammatory agent, a weevil attractant and as an odorant [CIR, 1988].

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 Isoamyl acetate at levels up to 35 ppm, "did not increase the overall toxicity of cigarette smoke " [Carmines (2002), Rustemeier *et al* . (2002), Roemer *et al* . (2002) and Vanscheeuwijck *et al.* (2002)].

Renne *et* al., (2006) evaluated the effects of tobacco flavouri ng 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 isoamyl acetate at 1. 3ppm, 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 pe riodically. 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 o f 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	16600 mg/kg
LD_{50}	Oral	Rabbit	7422 mg/kg
LD_{50}	Dermal	Rabbit	>5000 mg/kg
LD_{lo}	Sub cut.	G.pig	>5000 mg/kg
TC_{Lo}	Inhalation	Human	200 ppm
LC_{Lo}	Inhalation	Cat	35000 mg/m^3

[Ganey, 1992]. [RTECS, 2002]

In ra bbits with subacute poisoning, changes in liver were present as well as congestion and hypertrophy of the spleen and congestion of the kidney, with degenerative and reparative changes in tubular epithelium [Browning, 1965].

Guinea pigs and one rabbit, aft er 237 and 340 days exposure, respectively, to doses which were reported to be as high as 7000 ppm did eventually produce central nervous system depression, showed loss of weight and appetite; at 1900 ppm there was reported to be no marked cerebral disturb ance. Thirty-six three hour exposures to 500 and 1000 ppm were followed by albuminuria which was interpreted as being resultant to kidney damage. With maximal doses [actual maximal concentration not established] after 5 minutes animals showed spasmodic mov ements, slow respiration and salivation. In cats and rabbits at 900 ppm there was some irritation of eyes and nose, at 5000 ppm, lethargy but no cerebral disturbance was reported [Browning, 1965].

Intraperitoneal injection of either 750 mg/kg or 1500 mg /kg of amyl acetate to two groups of four guinea pigs, lead to the deaths of 3/4 animals dosed at 1500 mg/kg. After 24 hours after injection the livers were removed and

analysed histologically, there was found to be no evidence of necrosis or fat deposition in those animals dosed at 750 mg/kg with moderate deposition of fat in the livers of animals dosed at 1500 mg/kg. The authors concluded that amyl acetate had a relatively low order of hepatotoxicity [Divincenzo *et al.,* 1974].

Dermal toxicity

Undiluted amyl acetate was reported to be produce only the least visible compillary injection when tested on the clipped skin of five albino rabbits. Amyl acetate was also reported to be a marginal skin sensitizer when applied following a maximisation procedure [concentration not specified] [CIR, 1988].

Several drops of liquid amyl acetate squirted on to the eyes of rabbits and washed off with water 2 minutes later caused temporary corneal epithelial injury, but recovery was complete in 1-2 days. By standardised testing on rabbit eyes, amyl acetate has been graded only 2 [slightly injurious] on scale of 1 to 10 [Grant, 1986]. At a concentration of 300ppm in air, the amyl acetate vapour is noticeably irritating to the eyes. At higher concentrations it reportedly caused a burning sensation in eyes and hyper aemia of conjunctiva, but no corneal damage was observed [Grant, 1986].

The application of a sun cream containing 0.2 % amyl acetate was evaluated for eye irritation potential in six New Zealand white rabbits. T he application of 0.1 ml of sun cream to the conjunctival sac leads to no ocular reactions [CIR, 1988].

Isoamyl acetate applied neat to either the intact or abraded rabbit skin under an occluded patch for 24 hours was reported to be non-irritating. When tested at a concentration of 8 % in petrolatum under an occluded patch for 48 hours, it was found to be non-irritating to human volunteers [Opdyke, 1975].

When a maximisation procedure was conducted on 25 human volunteers using isoamyl acetate at a conce ntration of 8 % in petrolatum, a subsequent challenge lead to no sensitisation reactions [Opdyke, 1975].

No evidence of delayed contact hypersensitivity, phototoxicity, or photoallergy due to isoamyl acetate was observed in repeated insult patch test stud ies. It was concluded that isoamyl acetate is safe as currently used in cosmetic products [CIR, 1988].

Inhalation Toxicity

Isoamyl acetate administered via the inhalation route to dogs at a concentration of 5000 ppm [27 mg/l] for 1 hour caused nasal irri tation and drowsiness. Light CNS depression and delayed death were observed in cats exposed to 7200 ppm [38 mg/l] for 24 hr. For cats exposed to inhala tion of isoamyl acetate at 4000 ppm [21 mg/l] 20 minutes duration leading to irritation of the eyes and nose [Clayton *et al.,* 1994].

Mice have been reported to tolerate inhalation of isoamyl acetate at 1000 ppm for 2 - 3 hours without ill effects. In another study mice recovered in a day following exposure to 10500 ppm, with no deaths being observed in animals after exposure to 5000 ppm. CNS depression was produced in mice by exposure for 4 - 6 hours to 3800 ppm. Inhalation at 4000 ppm produced complete loss of reflexes in rabbits within an hour [Browning, 1965].

The toxicological effects of isoamyl acetate are probably similar to those of amyl acetate: chiefly irritation of the conjunctiva and upper respiratory tract followed by gradual onset of CNS depression, with slow recovery after exposure ceases. Men exposed to 950 ppm for 30 min reported only irritation of the nose and throat, headache, and weakness [Mackinson, 1981].

In persons with impaired pulmonary function, especially those with obstructive airway diseases, the breathing of isoamyl acetate might cause exacerbation of symptoms, due to its irritant properties. Persons with existing skin disorders may be more susceptible to the effects of isoamyl acetate. Although isoamyl acetate is not known as a kidney toxin in humans, the importance of this organ in the elimination of toxic substances justifies special consideration in those with possible impairment of renal function. Although isoamyl acetate is not known as a liver toxin in humans, the importance of this organ in the biotransformation and detoxification of foreign substances should be considered before exposing persons with impaired liver function [Makinson *et al.,* 1981].

Cats exposed to 1900 ppm isoamyl acetate for 8 hours a day for six-days was reported to show signs of irritation, weakness and weight loss [HSDB, 2003].

From the available data it is clear that the prevention of irritation to the upper respiratory tract and eyes form the basis of the proposed limit value. Irritation of the upper respiratory tract of man was reported to occur at concentrations between 185 – 300 ppm [1000 - 1620 mg/m^3] for a short period of exposure. The systemic effects on experimental animals in the literature which have been reported were reported by the authors to have been provoked with very high doses and one may expect general intoxication to occur at these dosages. The recommended health based occupational exposure limit for humans was 100 ppm [540 mg/m^3] for a time-weighted average of 15 minutes [Dutch Expert Committee for Occupational Standards 1990].

However, later ACGIH documentation has revealed a TLV-TWA of 50 ppm (266 mg/m3) and a TLV-STEL of 100 ppm (532 mg/m ^{3}). These new levels were anticipated to reduce the potential for irritation of eyes mucus membranes and narcosis hepatotoxicity and developmental effects (at higher concentrations) [ACGIH, 2001].

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 [Vanscheeuwijck *et al.* , 2002]. These ingredients included isoamyl acetate at 35 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 toxic ity of the smoke, even at the exaggerated levels used" [Vanscheeuwijck *et al.*, 2002].

The ad dition of Isoamyl acetate at 70 ppm to ref erence 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. T his indicated that addition of i soamyl 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].

Behavioural data

No data identified.

Other relevant studies

The metabolism of amyl acetate is not known, however the metabolism of esters with a similar chemical structure suggest that it is like to undergo enzymatic hydrolysis to acetic acid and amyl alcohol [CIR, 1988].

Isoamyl acetate is reported to be 2.6 times more potent as a narcotic that ethyl acetate, with isoamyl acetate being considered more irritating than butyl acetate [HSDB, 2003].

Isoamyl acetate is reported to be rapidly hydrolysed in blood to isoamyl alcohol (which has been studied extensively) and acetic acid, [NTP, 2003] The percentage hydrolysis for a structurally related compound in pancreatin (after 2 hours) was reported to be 20 % and 100 % in whole homogenate of pig jejunum [JECFA, 1998].

***In Vitro* Toxicity Status**

Carcinogenicity and mutagenicity

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 isoamyl acetate at levels up to 42 ppm [In vitro toxicity testing of tobacco ingredients in burnt form (Internal document R-19)].

Baker *et al.,* [2004], examined the effects of the addition of 482 tobacco ingredients upon the biological activity an d chemistry of mainstream smoke.

The ingredients, essentially different groups of flavourings and casings, were added in different combinations to reference cigarettes. The addition of Isoamyl acetate at 70 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].

When isoamyl acetate was tested in *Drosophila melanogaster*, using the sex linked recessive lethal assay, it was found to be non mutagenic via the two routes of administration, *i.e.* feeding and injection [concentration not specified] [Foreman *et al.*, 1994].

Isoamyl acetate was not mutagenic to the yeast *Saccharomyces cerevisiae*, although toxicity was observed at low concentrations [Zimmermann *et al.,*1985], and it has been reported to be non mutagenic in a combined study using the Ames *, Eschericia coli* , *Bacillus subtilis* DNA repair and the mouse forward mutation assay [Sernau *et al.,* 1973].

Isoamyl acetate was negative in the Ames test in *Salmonella typhimurium* strains TA92, TA94, TA100, TA1535 and TA1537 at concentrations up to 5 mg/plate both with and without a S9 fraction [Ishidate *et al.,* 1984]. Isoamyl acetate was also found to be negative in the TA97, TA98, strains with and without metabolic activation [Zeiger *et al.,* 1992].

Isoamyl acetate was not mutagenic in the Ames test with *Salmonella typhimurium* strains TA97 , TA98, TA1535, TA100, and TA1537 with and without metabolic activation [NTP, 1986].

Amyl acetate was reported to be non mutagenic in the Ames assay with *Salmonella typhimurium* strains TA1538, TA98, TA1535, TA100, and TA1537 both with and without metabolic activation [CIR, 1988].

Isoamyl acetate was found to be negative in a Chinese hamster fibroblast assay *in vitro* at concentrations up to 2 mg/ml [Ishidate *et al.,* 1984].

Isoamyl acetate was negative, being non mutagenic in the recombinant assay test using *Bacillus subtilis* M45 [rec-] and H17 [rec+] at the concentrations tested [Yoo, 1986].

Isoamyl acetate was not mutagenic to the yeast *Saccharomyces cerevisiae*, although it was toxic to the yeast at a concentration of 0.24 % [Zimmermann *et al.,* 1985].

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 Iso amyl acetate at levels up to 35 ppm [a multiple of its typical use in a US cigarette] [Roemer *et al.* (2002)].

Other relevant studies

Amyl acetate was reported to be non- cytotoxic to Ehrlich-Landschutz diploid ascites tumour cells at the incubation me dia concentrations of 50 o r 100 ppm [CIR, 1988].

Amyl acetate has been reported to have an anti haemolytic a ctivity with rat erythrocytes. The addition of 350 ppm [2.37 mM] reduced haemolysis by 50 % in comparison to control cells. The application of 1000 ppm [6.77 mM] was reported to afford 100 % protection against haemolysis. Anti-haemolysis was reported to be associated with an increase in the critical cell volume indicative that the protective effect was related to a solvent increase in membrane stability [CIR, 1998].

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 Imper ial 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 che mistry analysis, bacterial mutagenicity testing, and cytotoxicity testing were conducted. The authors concluded that these ingredients, which included isoamyl acetate 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].

PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of isoamyl acetate is detailed in the Report on Thermochemical Properties of Ingredients document (FileNet reference: 003822859). In the aforementioned document, the term 'pyrolysis' means t he 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 quantitativ e terms following the smoking of a tobacco product to which it has been applied.

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/.

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