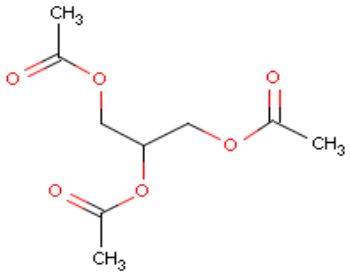
GLYCERYL TRIACETATE

SYNONYMS

1,2,3-Propanetriol-tri-acetate
Triacetin
Triacetylglycerol
Enzactin
Vanay
1,2,3-Propanetriol, triacetate

CHEMICAL STRUCTURE



CHEMICAL FORMULA

$C_9H_{14}O_6$

IDENTIFIER DETAILS

CAS Number	:	102-76-1
CoE Number	:	-
FEMA	:	2007
EINECS Number	:	203-051-9
E Number	:	E1518

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	-	-

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: 3°C

Boiling point: 258°C

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
Not specified	JECFA	1975	-

FDA Status: [CFR21]

Section Number	Comments
184.1901 181.27	Triacetin Substances classified as plasticisers

HUMAN EXPOSURE

Natural Occurrence: Glyceryl triacetate is reported found in several natural products [unspecified] [Fenaroli, 1995].

Reported Uses: Glyceryl triacetate is reportedly used in baked goods at 0.59 ppm, frozen dairy at 0.64 ppm, soft candy at 0.76 ppm, confectionery frosting at 0.02 ppm, gelatin pudding at 0.29 ppm, non-alcoholic beverages at 0.28 ppm, alcoholic beverages at 0.72 ppm, hard candy at 0.05 ppm, and chewing gum at 0.01 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 triacetin at levels up to 54 ppm, “did not increase the overall toxicity of cigarette smoke” [Carmines, 2002].

A battery of tests was used to compare toxicity of mainstream smoke from experimental cigarettes containing gliceryl triacetate (GTA) that was added individually to experimental cigarettes at three different levels 10,000, 50,000 and 100,000 ppm. Smoke from experimental and control cigarettes were evaluated using analytical chemistry, *in vitro* cytotoxicity (neutral red uptake), and mutagenicity (five bacterial strains) studies. Two 90-day inhalation studies were also performed, using different inclusion levels into either tobacco or cigarette filter (maximum inclusion level in filter 100,000 ppm). Several smoke constituent concentrations were reduced with the highest inclusion level of GTA in tobacco. Incorporation of GTA into the filter, and the other compounds into tobacco, produced effectively no changes. Cytotoxicity was reduced by the highest inclusion of GTA into tobacco for both gas–vapor and particulate phases of smoke; incorporation of GTA into the filter, and the other compounds into tobacco, showed no changes. Mutagenicity was reduced by the middle and high inclusion levels of GTA into tobacco (TA1537 strain with S9); incorporation of GTA into the filter, and the other compounds into tobacco, showed no changes. It was concluded Inclusion of GTA in tobacco at 100,000 ppm reduced the biological effects of the smoke in the various test systems reported in this study, although inclusion into the filter did not appear to have any major effect on the endpoints studied [Coggins *et al*., 2011].

In Vivo Toxicity Status

Test Type	Species	Route	Reported Dosage

LD_{50}	Rat	oral	3 g / kg bw
LD_{50}	Rat	I.P	2100 mg / kg bw
LD_{50}	Rat	Subcutaneous	2800 mg / kg bw
LD_{50}	Mouse	Oral	1100 mg / kg bw
LD_{50}	Mouse	I.P	1400 mg / kg bw
LD_{50}	Mouse	Subcutaneous	2300 mg / kg bw
LD_{50}	Mouse	Intravenous	1600 mg / kg bw
LD_{50}	Dog	Intravenous	1500 mg / kg bw
LD_{50}	Rabbit	Intravenous	750 mg / kg bw
LD_{50}	Guinea pig	Intramuscular	1740 mg / kg bw
LD_{50}	Frog	Oral	150 mg / kg bw

[RTECS, 1997]

Dermal toxicity

Human exposure to triacetin is reported to occur through dermal contact and inhalation as a topical antifungal agent or as a perfume fixative. However, triacetin reportedly appears innocuous when swallowed, inhaled or in contact with the skin, but may cause slight irritations to the eye in some sensitive individuals [HSDB, 2002].

Inhalation studies

When tested at 30 ppm in cigarettes, in a 13-week inhalation study, the presence of triacetin "…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)].

Similarly, a recent mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including triacetin at 1 ppm. The authors concluded that the study "did not indicate any substantive effect of these ingredients on the tumourogenicity 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)].

A 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 triacetin at 54 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 triacetin at 1400 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 triacetin 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].

The exposure of rats [number and strain unspecified] to 250 ppm heated triacetin vapour 6 hr / day, 5 days / week for 13 weeks reportedly caused no overt symptoms or changes in blood and urine analyses or weight or tissue structure of a 'limited' range of organs [unspecified] [BIBRA, 1997].

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 glyceryl triacetate at levels up to 225 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other relevant studies

Bailey *et al.,* (1991) described a study where triacetin was infused at isocaloric or hypercaloric rates in mongrel dogs for 3 hr. Ketone body and glucose production rates were quantified with [$^{13}C_2$] acetoacetate and [^{3}H] glucose, respectively. Four additional animals were infused with glycerol to serve as controls for the hypercaloric triacetin infusion. Energy expenditure was determined in the isocaloric experiments. The author reported no evidence of acute toxicity being observed during triacetin infusion at either rate. Plasma acetate concentrations increased from basal levels to approximately 1 and approximately 13 mmol / litre in the isocaloric and hypercaloric experiments, respectively. Plasma lactate and pyruvate concentrations decreased dramatically after 30 min of both isocaloric and hypercaloric triacetin infusions. Glucose production rates did not increase in either group, but glucose clearance decreased significantly in both groups [$p < 0.05$] over the last hour of triacetin infusion. Plasma ketone body concentrations increased from 1.4 to 3.5 and 1.8 to 13.5 ∝mol / kg / min, respectively, during isocaloric and hypercaloric triacetin infusion. Resting energy expenditure increased from 3.0 +/- 0.3 to 4.0 +/- 0.5 kcal / kg / hr during isocaloric triacetin infusion ($p < 0.05$). These studies reportedly indicate that triacetin can be administered to dogs at high rates without overt toxicity. The decrease in glucose clearance may represent competition between carbohydrate [glucose] and lipid [acetate]. Triacetin infusion resulted in significant increases in ketone body production and concentration. These preliminary data indicate that triacetin may have a

role as a parenteral nutrient, and that further studies of its use are warranted [Bailey *et al.*, 1991].

Canavan disease (CD) is a rare autosomal recessive neurodegenerative disorder presenting in early infancy. The course of the disease is variable, but it is always fatal. CD is caused by mutations in the ASPA gene, which codes for the enzyme aspartoacylase (ASPA), which breaks down N-acetylaspartate (NAA) to acetate and aspartic acid. The lack of NAA-degrading enzyme activity leads to excess accumulation of NAA in the brain and deficiency of acetate, which is necessary for myelin lipid synthesis. Intragastric administration of GTA to tremor mice results in greatly increased brain acetate levels, and improved motor functions. GTA given to infants with CD at a low dose (up to 0.25 g/kg/d) resulted in no improvement in their clinical status, but also no detectable toxicity. The study presented for the first time the safety profile of high dose GTA (4.5 g/kg/d) in 2 patients with CD. Two infants with CD at ages 8 months and 1 year were treated with high dose GTA, for 4.5 and 6 months respectively. No significant side effects and no toxicity were observed. Although the treatment resulted in no motor improvement, it was well tolerated [Segel et al., 2011].

Behavioural data

No data identified

***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 triacetin at levels up to 54 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 triacetin at 1400 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 glyceryl triacetate at levels up to 2024 ppm.

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 glyceryl triacetate at levels up to 225 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other relevant studies

Opdyke, (1978) stated that triacetin was found to be metabolised [by hydrolysis] by pig intestinal lipase and pancreatic lipase to glycerol and acetic acid [Opdyke, 1978].

In an assessment of triacetin, the Joint FAO / WHO Expert Committee on Food Additives [JECFA] reportedly considered it unnecessary to assign an ADI, as triacetin was 'metabolised like other fats in foods'. JECFA, (1975) concluded that, based on the available data and anticipated daily intake, triacetin did not represent a hazard to health [JECFA, 1975].

The i.v. administration of triacetin to ten mongrel dogs (unknown concentration) is reported to undergo intravascular hydrolysis with a majority of the resulting acetate, oxidised. Therefore, the authors concluded that 'energy in the form of short-chain fatty acids may be delivered to a resting gut via intravenous infusion of a short chain triglyceride' [Bleiberg *et al*., 1993].

Triacetin is reported to undergo hydrolysis (*in vivo*) to glycerol (which is endogenous) and carboxylic acids. JECFA have not yet finalized their evaluation on triacetin and therefore have not made any conclusions based on its current levels of intake, [JECFA, 2002].

A report on the final safety assessment of triacetin reported that cosmetic formulations included triacetin at concentrations ranging from 0.8-4 %. Triacetin was reported to be non-toxic by the oral or dermal route and non-toxic in short-term inhalation or parenteral studies (as well as sub-chronic feeding and inhalation studies). Triacetin was reported to be slightly irritating

to guinea-pig skin, (although a single study reported erythema, slight edema, alopecia and desquamation, and was shown to cause some irritation in rabbit eyes). Triacetin was also reported to be non-sensitising to guinea pigs and not an irritant or sensitizer in a clinical maximization study, (a mild reaction was reported in the Duhring-chamber test using a 50 % dilution. Ocular irritation without injury has also been reported. Triacetin was also reported to be non-mutagenic however there was an absence of reproductive and developmental data. (the authors did however report that triacetin was rapidly metabolised to glycerol and acetic acid and these are not reported developmental toxins). Reports that 1,2-glyceryl diesters may be present in triacetin and could affect cell growth and proliferation raised the possibility of hyperplasia and/or tumour promotion. However, the Cosmetic Ingredient Review (CIR) expert panel concluded that the 'the effects of 1,2-glyceryl diesters on cell growth and proliferation require longer ester chains on the glycerine backbone than those present when acetic acid is esterified with glycerine, as in Triacetin. With this information the CIR expert Panel concluded that 'Triacetin is safe as used in cosmetic formulations, [Fiume *et al*., 2003].

PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of glyceryl triacetate 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.

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 glyceryl triacetate was found to transfer 98.5% intact, other breakdown product included diacetin (1.4%) and 1 unidentified compounds (0.1%).

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