

ANISE STAR OIL

SYNONYMS

Anise Etoile
 Badiane
 Chinese star anise
Illicium verum
 Oils, star anise
 Star anise oil

CHEMICAL STRUCTURE

Ill defined (mixture of components)

CHEMICAL FORMULA

The principal constituent of star anise oil is anethole. Both *cis*- and *trans*-anethole forms have been identified [Opdyke, 1975]. The important constituents of star anise oil have been identified as follows:

Constituent	Level
Trans-anethole	71 - 88%
Cis-anethole	0.2 - 0.4%
α -pinene	0.02 - 0.57%
camphene	0.03 - 0.05%
β -pinene	0.03 - 0.05%
Limonene	1.18 - 2.38%
Safrole	Trace - 0.14%
Estragol	0.34 - 5.04%

[Natural Sources of Flavourings – Report No.1, 2000].

Active constituents identified as estragole and safrole.

The Council of Europe have classified star anise oil in Category 3 with limits on estragole and safrole.

Definition of Category 3:

“Plants animals and other organisms, and parts of these and products thereof, normally consumed as food items, herbs or spices in Europe which contain defined ‘active principles’ or ‘other chemical components’ requiring limits on use levels”. “These source materials and preparations are not considered to constitute a risk to health in quantities used provided that the limits set for the ‘active principles’ or ‘other chemical components’ are not exceeded.”

Limits set on the active principles by the Council of Europe: Both Estragole and Safrole are currently under evaluation by the committee and as yet the

limits have to be agreed. However, it is noted that the tolerable daily intake (TDI) has not been attributed for either of these substances as they are both considered to be potential genotoxic carcinogens [Natural Sources of Flavours – Report No.1, 2000].

IDENTIFIER DETAILS

CAS Number : 68952-43-2, 84650-59-9
CoE Number : 238
FEMA : 2096
EINECS Number : 283-518-1
E Number : -

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	-	-

SPECIFICATIONS

Melting Point: 19°C

Boiling point: Undefined

PURPOSE

Flavouring compound.

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
-	-	-	-

FDA Status:[CFR21]

Section Number	Comments
182.10	Spices and other natural seasonings and flavorings

HUMAN EXPOSURE

Natural Occurrence : Anise star oil is a herbaceous annual plant indigenous to Greece and Asia Minor, now extensively cultivated in Europe, Russia, and, to a lesser extent, in India. It has a perpendicular root, and erect cylindrical stalk, alternative leaves, yellowish white flowers, five-edged carpels and lens shaped seeds. The main commercial source of anise oil is Chinese star anise. [Fenaroli, 2005].

Reported Uses: Anise star oil is reportedly used in baked goods at 38.05 ppm, frozen dairy at 4.46 ppm, meat products at 0.32 ppm, soft candy at 260.90 ppm, gelatins and puddings at 3.08 ppm, non-alcoholic beverages at 15.18 ppm and alcoholic beverages at 167.40 ppm [Fenaroli, 2010].

Sources other than foods: *I. verum* has been in use as a basic spice and also used in the treatment of stomach disease, pain etc. in traditional Chinese and Japanese medicinal systems [Okuyama, 1993].

TOXICITY DATA

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 anise star oil at 65 ppm, were compared to a typical commercial tobacco blend without flavouring ingredients. The Ames assay (TA 98, 100,102, 1535 and 1537 $\pm$ 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 13 wk 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**

Species	Test Type	Route	Reported Dosage
Rat	LD ₅₀	Oral	2.57 ± 0.453g/kg
Rabbit	LD ₅₀	Dermal	> 5g/kg [Opdyke, 1975].

The menthol extract of star anise (*Illicium verum* Hook) had a hypothermic effect in mice when given orally at 3 g/kg. The ethyl acetate extract from the same plant when given orally at 100 mg/kg and 500 mg/kg resulted in convulsive effects and lethal toxicity respectively [Okuyama *et. al.*, 1993].

Dermal Toxicity

Star anise oil was applied undiluted to the backs of hairless mice or to intact or abraded rabbit skin for 24 h under occlusion and was found to be not irritating. When tested at 4% in petrolatum, it produced no irritation after a 48 h closed-patch test on human subjects [Opdyke, 1975].

Undiluted star anise oil was without effect when applied to the backs of hairless mice and swine [Opdyke, 1975]. Undiluted star anise oil applied to intact or abraded rabbit skin [24 hour occluded patch] was not irritating and was also reported to be without effect at a concentration of 4 % in petrolatum [48 hour closed patch] in human subjects [Opdyke, 1975]. Similarly a maximisation test carried out at a concentration of 4 % in petrolatum on 25 human volunteers was negative for a sensitisation effect, when challenged 10 - 14 days after the maximisation procedure [Opdyke, 1975]. However, sensitisation to anise oil has been reported, with the substance causing irritation being identified as anethole. The dermatitis that arises is reported to consist of erythema, scaling and vesiculation [Opdyke, 1973].

Its major component (anethole) has, however, been reported to cause dermatitis (erythema and scaling of the skin) in some people [Leung *et al.*, 1996].

A maximization test was carried out on 25 human volunteers. Star anise oil was tested at a concentration of 4 % in petrolatum and produced no sensitisation reactions [Opdyke, 1975].

Star anise oil at a concentration of 1 and 2 % produced active sensitisation in 5 % of test subjects and positive patch tests in 36 and 34 % respectively of consecutive patients with dermatitis. Patients positive to this oil are frequently positive to anethole and to other constituents of this oil [Rudzki & Grzywa, 1976].

No photo toxic effects were reported for undiluted star anise oil on hairless mice and swine [Opdyke, 1975].

Star anise oil is used interchangeably with anise oil in the United States with both being recognised as anise oil by the U.S.P. and F.C.C. [Leung *et al.*, 1996].

Inhalation Toxicity

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 anise star oil at levels up to 39 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other relevant studies

Anise star oil is used as a calmative, stimulant, mild spasmolytic, weak bactericide and expectorant in cough mixtures and lozenges. It is also reported to be used to hide undesirable odour in cosmetic and pharmaceutical preparation. Anise star oil is also used as a fragrance component in toothpaste, soaps, creams, lotions perfumes and detergents with a maximum recommended use level of 0.4 % [Leung *et al.*, 1996].

Anise star oil has also been repeatedly used in traditional folk medicine as an oestrogenic agent to increase milk production, stimulate menstruation, increase libido and stimulate child birth [Leung *et al.*, 1996].

Ethanollic extracts of *Illicium verum* extracts were fed to C57B1/6 male and female mice for 10 and 14 days respectively. The extract was found to induce 7-ethoxy coumarin-*o*-deethylase and epoxide hydratase in both male and female liver samples [Hendrich *et al.*, 1986].

Star anise (*Illicium verum*), was assessed for its anti-carcinogenic potential in *N*-nitrosodiethylamine (NDEA) initiated and phenobarbital (PB) promoted hepato-carcinogenesis. Carcinogenesis was induced in rats by injecting with a single dose of NDEA (200 mg/kg body weight) intraperitoneally as initiator, followed by promotion with PB (0.05 %) in drinking water for 14 consecutive weeks. Treatment with Star anise throughout for 20 weeks reduced the nodule incidence and nodule multiplicity, lowered the lipid peroxidation (LPO) in liver and erythrocytes, restored the liver and erythrocyte super-oxide dismutase (SOD) activities, increased liver glutathione (GSH) level significantly and the erythrocyte GSH level to some extent. Liver and erythrocyte glutathione-S-transferase (GST) activity was increased in all the groups treated with NDEA and PB and treatment with Star anise decreased GST level significantly. The authors concluded that these results indicated that treatment with Star reduced the tumour burden, lowered oxidative stress and increased the level of phase II enzymes, which may have contributed to its anti-carcinogenic potential (Yadav and Bhahnager, 2007).

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”.

A study was conducted that looked at the genotoxicity of a number of compounds including anise oil. The Ames test was performed in *Salmonella typhimurium* tester strains TA100, TA1535, TA98, TA1537 and TA1538 and *E. Coli* WP2 uvrA. Anise oil was negative in all of the bacterial strains tested both in the absence and presence of S9 [the dose tested is not stated]. In the same study, anise oil was also tested in the DNA repair test in *B. Subtilis* (Rec assay) in the absence of S9 and was found to be positive. The authors state that they had difficulty in diffusing the oils in the aqueous agar layer in the Rec assay and consequently this may have influenced the result [Sekizawa and Shibamoto, 1982].

Anise oil at a concentration range of 0.005-5.0 μ l [il as quoted in legacy document. However, assumed to be μ l] per plate. Was not mutagenic in the absence and presence of rat liver S-9 in the *Salmonella* strains TA1535, TA1537, TA987 and TA100. It has also been reported that anise oil is not mutagenic in the Chinese Hamster Ovary chromosomal abnormality test. However, one study did report anise oil to be slightly mutagenic in *Salmonella typhimurium* strain TA100 in the presence of rat liver S-13 supernatant. The authors concluded in this study that ‘although the results of these experiments indicate biological activity in this mammalian system, they do not seem to be potent enough or persistent enough to be of serious practical concern at this time’ [Legacy, 2002].

Zeiger and Margolin, (2000) identified the flavour anise oil as a compound not found to be mutagenic in the Ames test using *Salmonella typhimurium* strains TA98, TA100, TA 97 and TA 1535, [in the absence and presence of rats and hamster S-9], [no concentrations given], [Zeiger and Margolin, 2000]. Similarly anise oil was observed to be negative for mutagenicity in *Salmonella typhimurium* tester strains TA 97, TA98, TA100, TA102, TA104, TA1535, TA1537 and TA1538 [no further details given] [NTP, 1994].

A study was conducted in which anise oil was assessed for its ability to produce DNA damage in an unscheduled DNA synthesis assay [UDS] in rat primary hepatocytes at a concentration range of 0.0003 - 0.03 μ l/ml. Anise was reported not to cause significant increase in UDS, which was measured

by an increase in net nuclear autoradiographic grain counts in comparison to solvent treated control hepatocytes [Legacy, 2002].

No significant number of revertant colonies were observed in *Salmonella* tester strains or with *Escherichia coli* WP2 uvrA in the absence of S-9 however, anethole [a constituent of anise oil], gave a clear dose-dependent increase of induced mutation frequencies in TA 100 [Legacy, 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 *anise star oil* at levels up to 10 ppm.

In vitro toxicity testing of tobacco ingredients in burnt form (Internal document R-44).

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 anise star oil at levels up to 39 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

PYROLYSIS AND TRANSFER STUDIES

No data identified

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