TOLU BALSAM EXTRACT

SYNONYMS

Balsam tolu
Opobalsam
Resin tolu
Thomas balsam
Tolu balsam gum
Tolu resin

CHEMICAL STRUCTURE

Undefined (mixture of components)

CHEMICAL FORMULA

Main constituents of the balsam (in addition to 75 to 80% resinous substances include benzyl benzoate, benzyl cinnamate, a small amount of essent ial oil and traces of vanillin [Fenaroli, 2005].

IDENTIFIER DETAILS

CAS Number	:	9000-64-0, 8011-89-0, and 977136-92-7
CoE Number	:	297n
FEMA	:	3069
EINECS Number	:	232-550-4
E Number	:	-

SPECIFICATIONS

Melting Point: Undefined (mixture of components)
Boiling point: Undefined (mixture of components)

PURPOSE

Flavouring substance

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
172.510	Natural flavouring substances and natural substances used in conjunction with flavours

HUMAN EXPOSURE

Natural Occurrence: Balsam tolu occurs in the tree of *Myroxylon balsamum*. It is prepared by tapping the tree. The balsamic solid is then collected.

Reported Uses: Tolu balsam (gum; CAS: 9000-64-0) is reportedly used (maximum levels) in alcoholic beverages at 4.43 ppm, baked goods at 20.17 ppm, frozen dairy at 8.43 ppm, gelatins & puddings at 6.70 ppm, hard candy at 0.72 ppm, non-alcoholic beverages at 3.27 ppm, and soft candy at 18.85 ppm (Fenaroli, 2005). Tolu balsam (extr act; CAS: 977075-28-7) is reportedly used (maximum levels) in alcoholic beverages at 28.85 ppm, baked goods at 235.70 ppm, chewing gum at 35.50 ppm, frozen dairy at 142.70 ppm, gelatins & puddings at 142.20 ppm, hard candy at 0.25 ppm, non-alcoholic beverages at 28.85, and soft candy at 189.90 ppm [Fenaroli, 2005].

TOXICITY DATA

In Vivo Toxicity Status

Species	Test Type	Route	Reported Dosage
Rat	LD_{50}	Oral	>5g/kg
Rabbit	LD_{50}	Dermal	>10g/kg

[Opdyke, 1976].

Main constituents of the balsam (in addition to 75 to 80% resinous substances) include benzyl benzoate, benzyl cinnamate, a small amount of essential oil and traces of vanillin. The oleoresin contains 50 to 65% volatile oil (mainly benzyl benzoate and benzyl cinnamate and cinnamyl cinnamate). The crude balsam contains approximately 50 to 64% oil (cinnamein) and 20 to 28% resin [Fenaroli, 1995].

In the metabolism of balsam tolu, the main components of cinnamic and benzoic acids are excreted in the urine predominantly as hippuric acid [Opdyke 1976].

Carcinogenicity and Mutagenicity

A recent mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including tolu balsam at 56 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 compone nt (*i.e.* glycerine 20,000 ppm, propylene glycol at 24,000 ppm, and brown invert sugar at 24,000 ppm)] [Gaworski *et al.*, 1999].

Dermal toxicity

The frequency of allergic contact dermatitis (ACD) was determined for genital allergens and the top al lergens were identified. Data was analyzed for 1,238 patients tested between January 1990 and December 2006. Fifteen allergens caused reactions at rates greater than 1 %. Thirteen anatomic regions were assessed. Statistical analyses were by chi-square test and Fisher exact test. Adjusted level of significance due to multiple testing was $\alpha = 0.002$. Out of individuals with reported genital dermatitis (n = 37; aged 24 - 77 years, 48.6 % female), 41 % (15 of 37) had at least one positive patch-test result altho ugh only 30 % (11 of 37) had a final diagnosis of relevant ACD. Mean age was 46 years for males and 41 years for females. The top five allergens were balsam of Peru (10.8 %), fragrance mix I (8.1 %), tolu balsam (8.1 %), phenylmercuric acetate (8.1 %), and neomycin (5.4 %). Females were more often allergic (50 %) compared to males (37 %); 59.5 % of patients had no positive reactions [Bhate *et al*., 2010].

Tolu balsam at a concentration of 5 % in petrolatum produced no irritation in 40 volunteers and 216 con tact dermatitis patients at 48, 72 and 96 hours after dosing with an occluded patch [Legacy 2003]. Tolu balsam applied neat to the backs of abraded or intact rabbit skin for 24 hours under an occluded patch was reported to be mildly irritating. A sensiti sation test in humans using a maximisation procedure with balsam tolu at a concentration of 2 % in petrolatum produced no sensitisation reactions in 25 humans [Opdyke, 1976].

Allergy to balsam of pine and or spruce was found in 5.5 % of 1247 patients examined with routine patch tests . The majority of the patients were found to be sensitive to both balsams. Many of the patients were reported to also be allergic to a mixture that contained balsam peru, tolu storax, benzoin each at a concentration of 5 % in acetone [Fregert *et al.,* 1963].

Inhalation toxicity

Steam inhalation of Friars balsam, a long used drug mixture that contains tolu balsam, was reported to have no effect upon specific mucous consistency or respiratory tract fluid volume , in rabbits, until doses were 1000 times the therapeutic dose. These large doses corresponded with toxicity, produced acute inflammation of the of the tracheal mucosa, an increase in the out put of the respiratory tract fluid apparently due to the large inhaled quantities o f alcohol inhaled, and an increase in the specific gravity due to the large amounts of benzoin storax, tolu balsam and aloe [Opdyke, 1976].

When tested at 56 ppm in cigarettes, in a 13-week inhalation study, the presence of tolu balsam, "…had no discerni ble 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].

Behavioural data

No data identified.

Other relevant studies

Transient receptor potential vanilloid subtype 1 (TRPV1) is a non-selective cation channel activated by capsaicin. TRPV1 is expressed not only on human sensory neurons but also on human epidermal and hair follicle keratinocytes. Therefore, TRPV1 could have the potential to be a therapeutic target for skin disorders. To search for novel TRPV1 agonists, 31 essential oils were screened using human TRPV1-expressing HEK293 cells. TRPV1 was activated by 4 es sential oils: rose, thyme geraniol, palmarosa, and tolu balsam. The dose-response curves for TRPV1 activation by the essential oils revealed a rank order potency [the half-maximal effective concentration (EC(50))] of rose>palmarosa>thyme geraniol>tolu bals am, and rank order efficiency (% activity in response to 1 μM capsaicin) of tolu balsam>rose>palmarosa>thyme geraniol. Moreover, the dose-response curves for TRPV1 activation by citronellol (main constituent of rose oil) and geraniol (main constituent of thyme geraniol and palmarosa oils) were consistent with the potency and efficiency of each essential oil. In contrast, benzyl cinnamate and benzyl benzoate (main constituent of tolu balsam oil) and geranyl acetate (main constituent of thyme geraniol oil) did not show TRPV1 activity. In this first-of-its-kind study, we successfully investigated the role of some essential oils in promoting human TRPV1 activation, and also identified two monoterpenes, citronellol and geraniol, as new human TRPV1 agonists [Ohkawara et al., 2010].

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 tha t the *in vitro* mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients, which included Tolu balsam extract at levels up to 127 ppm [In vitro toxicity testing of tobacco ingredients in burnt form (Internal d ocument R-53)].

Tolu balsam was negative in the Ames assay with *Salmonella* strains TA1535, TA1537, TA1538, TA 98 and TA100 both with and without metabolic activation at concentrations up to 10000 g/plate [Heck 1989].
At a concentration of 100 g/plate there was reported to be no unscheduled DNA synthesis in rat hepatocytes [Heck 1989].

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".

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