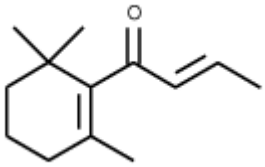
DAMASCONE, BETA-

SYNONYMS

1-(2,6,6-Trimethyl1-cyclohexen-1-yl)-2-buten-1-one
1-(2,6,6-Trimethylcyclohexenyl)-2-buten-1-one
4-(2,6,6-Trimethylcyclohex-1-enyl)-but-2-en-4-one
Damascone
trans-2,6,6-Trimethyl-1-crotonylcyclohex-1-ene
Rose dihydroketone

CHEMICAL STRUCTURE



CHEMICAL FORMULA

$C_{13}H_{20}O$

IDENTIFIER DETAILS

CAS Number	:	23726-91-2, 23726-92-3
CoE Number	:	2340
FEMA	:	3243
EINECS Number	:	245-842-1
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: -

Boiling point: 81-82ºC at 0.8 mm Hg

PURPOSE

Flavouring substance

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
1	1	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
ACCEPTABLE	JECFA	2002	No safety concern at current levels of intake when used as a flavouring agent; secondary components do not raise a safety concern.

FDA Status: [CFR21]

Section Number	Comments
-	-

HUMAN EXPOSURE

Natural Occurrence: beta-Damascone is reportedly found in tea [Fenaroli, 2005].

Reported Uses: beta-Damascone is reportedly used in soft candy at 0.5 ppm, gelatin, pudding at 0.5 ppm, non-alcoholic beverages at 0.5 ppm, alcoholic beverages at 0.5 ppm, confectionery frosting at 0.5 ppm, hard candy at 0.5 ppm and chewing gum at 0.5 ppm [Fenaroli, 2005].

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 beta damascone at levels less than 1 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)].

In Vivo Toxicity Status

Species	Test Type	Route	Reported Dosage
Rat	LD_{50}	Oral	2920mg/kg[1]
Rat	LD_{50}	Oral	>2000mg/kg[1]
Rabbit	LD_{50}	Dermal	>2000mg/kg[2]

1[JECFA, 1999], [2][Lapczynski *et al., 2007*]

A combined *in vivo* micronucleus assay/liver Comet assay was performed after the oral administration of beta-damascone (purity 95.6 %). This study is an unpublished study conducted by Covance Laboratores Ltd, which was submitted by EFSA to the FLAVIS secretariat in 2013, under the author: Beevers C. Male Han Wistar rats were orally administered with 3 doses of 125, 250 and 500 mg/kg bw (in groups of 6) at 0, 24 and 45 hours. Rats were sacrificed and sampled 48 hours after the initial dose. Hepatocyte vacuolation was present in animals given 500 mg/kg/day and was characterised by scattered, occasionally shrunken hepatocytes with perinuclear cytoplasmic eosinophilia and peripheral cytoplasmic vacuolation. Single cell necrosis was present in one animal given 500 mg/kg/day. There was a dose-related reduction in the level of glycogen vacuolation in animals given 250 or

500 mg/kg/day. In addition, increased mitosis was present in all animals in all groups given beta-damascone. Collectively, these findings indicate that the test animals were systemically exposed to beta-damascone. With regards to the genotoxic effects of beta-damascone, there was no statistically significant increase in micronucleus induction in rat bone-marrow cells following oral gavage. The Comet assay performed on the in the liver tissue and the duodenum tissue of the dosed rats did not show a statistically significant increase in tail intensity and tail moment. The results from the combined *in vivo* micronucleus induction study and Comet assay show that orally administered beta-damascone did not induce micronucleated erythrocytes in rat bone-marrow cells and there were no genotoxic events in the liver and duodenum of the dosed rats. EFSA conclude that the combined evidence from previous *in vitro* data and the *in vivo* genotoxicity data described here, does not indicate a genotoxic potential for beta-damascone [EFSA, 2014].

Trans-Beta-Damascone was tested in a 90 day oral toxicity study in 32 male and female CF/Gif rats (16/sex). Trans-Beta-Damascone was absorbed into cellulose and added to the diet such that the approximate daily dose was 2.26 mg/kg of bodyweight. A control group (16/sex) received the basic diet alone. Each diet was fed *ad libitum*. Weekly observations were made of growth, physical appearance, and behaviour. The efficiency of feed utilisation was calculated. During the 7th week haematological studies were conducted on 16 animals (8/sex) and on all animals at the termination of the study. Each animal was sacrificed after 90 days and a gross necropsy was conducted. There were no mortalities and physical appearance and behaviour were considered normal during the course of the study. Body weight gains were reduced by 4.8 % and 0.4 % for treated males and females respectively. Feed consumption showed an increase for both males and females. A moderate decrease of feed efficiency was observed in both sexes equal to -9.04 % in the males and -9.60 % in the females. A significant increase in absolute liver and kidney weights was observed in females and a significant increase in relative liver and kidney weights for both males and females was observed; however, as these changes did not correlate with any histological modifications it was concluded that these changes were due to adaptation and not to pathology. Clinical haematology parameters did not reveal any significant changes. A slight modification in relative distribution of leukocytes was observed in females in week 7. In view of the fact that no toxicologically significant modifications were observed either in the haematological or histological examinations, it was concluded that the changes observed were not of any biological significance [Lapczynski *et al., 2007*].

Dermal Toxicity

Summary of human sensitization studies

Test Method	**Test Concentration %**	**Results**
Human repeat Insult patch test (HRIPT)	0.5	No sensitization 0/104
HRIPT	1	No sensitization 0/54

Maximization	0.2	No sensitization 0/23 [Lapczynski *et al., 2007*]

A Local Lymph Node Assay (LLNA) was conducted in female CBA/J Hsd mice (6/dose). A 25 µl aliquot of trans-Beta-damascone was applied daily to the dorsum of each ear for three consecutive days at concentrations of 0.1 %, 0.25 %, 0.5 %, 1.0 %, 2.5 % and 5 % in acetone/olive oil (4:1). Isoeugenol at 0.5 % and 5 % was used as a positive control. Animals were allowed to rest for two days. On day 6, the mice were injected with 2 µC1 of ^{125}I-labelled iododeoxyuridine, 10^{-5}M FuDR in phosphate buffered saline. The mice were euthanized after 5 hours and auricular lymph nodes were excised. The nodes were dissociated, washed and the radioactivity was measured using a gamma counter. All animals appeared healthy and there were no signs of irritation at the dosing site. The EC3 value was calculated to be 2.4 % (600 µg/cm^2). Under the conditions of the test, trans-beta-damascone was considered to a sensitizer [Lapczynski *et al., 2007*].

Inhalation toxicity

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 beta damascone at less than 1 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 damascene, beta at 18 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 damascene, beta 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 beta-damascone at levels up to 15 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other relevant studies

JECFA [1999] in a review of structurally related compounds including beta damascone concluded that the two major pathways of metabolism lead to the formation of polar metabolites which are excreted in the urine either unchanged or conjugated with glucuronic acid [JECFA 1999].

The per capita intake was calculated to be 43 µg/day in Europe and 10 µg/day in USA [JECFA 1999].

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".

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 beta damascone at levels less than 1 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 damascone, beta at 18 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 *beta-damascone* at levels up to 127 ppm [In vitro toxicity testing of tobacco ingredients in burnt form (Internal document R-53)].

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".

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 beta-damascone at levels up to 15 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of Damascone, beta 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.

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