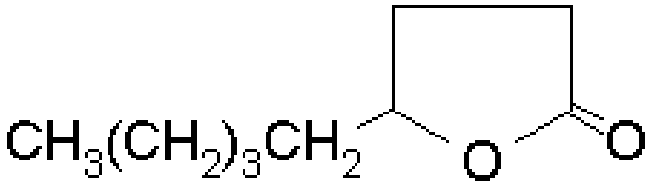
GAMMA-NONALACTONE

SYNONYMS

1,4-Nonanolactone
4-hydroxy Nonanoic acid lactone
Abricolin
Aldehyde C-18
Coconut aldehyde
gamma-n-amyl Butyrolactone
Nonan-4-olide
Prunolide

CHEMICAL STRUCTURE



CHEMICAL FORMULA

$C_9H_{16}O_2$

IDENTIFIER DETAILS

CAS Number	:	104-61-0
CoE Number	:	178
FEMA	:	2781
EINECS Number	:	203-219-1
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: 243°C

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
0 - 1.25	JECFA	1967 (maintained in 1997)	No safety concern at current levels of intake when used as a flavouring agent.

FDA Status:[CFR21]

Section Number	Comments
172.515	Synthetic flavouring substances and adjuvants permitted for direct addition to food for human consumption.

HUMAN EXPOSURE

Natural Occurrence: Gamma-Nonalactone (γ-Nonalactone) is reportedly found in nature – peaches and apricots, roasted barley, rum and tomato, being reported to be found in mangoes at concentrations of 0.3 - 04 mg/kg;

beer 0.3; other ale up to 0.1; beef [heated] 0.07; pork [heated] 0.1 - 0.3 and green tea at 0.1 - 0.3 mg/kg [Fenaroli, 2005; CoE, 2000].

Reported Uses: γ-Nonalactone is reportedly used in baked goods at 50.41 ppm, frozen dairy at 22.68 ppm, soft candy at 40.52 ppm, sweet sauce at 0.31 ppm, gelatin pudding at 26.62 ppm, non-alcoholic beverages at 13.45 ppm, alcoholic beverages at 0.36 ppm, hard candy at 17.59 ppm, and chewing gum at 2.90 ppm [Fenaroli, 2005].

Sources other than foods: Also found in mosquito larvacides, insecticides, repellents and mammal repellents.

γ-Nonalactone is one of the five gamma lactones produced naturally by the yeast *Sporidiobolus salmonicolor* [Dufosse *et al.,* 1997].

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 γ-Nonalactone at levels up to 6 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 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 gamma, nonalactone at 1.3 ppm, 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 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

Acute toxicity:

Test Type	Route	Species	Reported Dosage
LD_{50}	oral	rat	9780 mg/kg
LD_{50}	oral	g. pig	3440 mg/kg [Jenner *et al.,* 1964]
LD_{50}	oral	rat	6600 mg/kg [Moreno 1972]
LD_{50}	dermal	rabbit	>5000 mg/kg
Mild	dermal	rabbit	500 mg/24 hrs
Severe	dermal	rabbit	100 mg/24 hrs [RTECS 29/08/02]

In some limited feeding studies, no adverse effects were observed in 15 male and 15 female rats [FDRL strain] exposed to either 0 or 67.5 mg/kg for 90 days. Blood counts and, liver and kidney weights were reported to be not significantly different from control rat. Gross and microscopic examination revealed a non-treatment related incidence mild thyroid hyperplasia and pulmonary changes which were reported to be prevalent in this specific colony [Oser *et al.,* 1965].

In a chronic feeding study groups of 10 male and 10 female rats were exposed to or 0.1 - 0.5 % [0.5 % being equivalent to 5000 ppm, which approximates to 250 mg/kg] of γ-nonalactone in the diet for 2 years [Bär *et al.,* 1967]. It should, however, be noted that neither of these studies were carried out to currently acceptable standards, however, the results of the study did not give rise to any adverse affects [JECFA, 1998].

It was concluded that the level of γ-nonalactone that caused no observed effect level [NOEL] was 250 mg/kg/day in the rat, the authors concluded that the long term studies carried out make up for the lack of information on the metabolic fate of the compound at the time. However, as the studies were conducted only in one species then, metabolic studies and studies in another species were recommended [JECFA, 1967].

Dermal Toxicity

γ-Nonalactone was not a human skin irritant when tested at 10 % in petrolatum in human volunteers after a 48 hour closed patch test. It was also not found to be a skin sensitiser when tested in 25 human volunteers [Opdyke, 1975].

Application of γ-nonalactone full strength to abraded rabbit skin for 24 hours under occlusion was reported to be slightly irritating [Opdyke 1979].

Reproductive and developmental Toxicity

A mixture of lactones and carboxylic acids were added to the diet of a low number of rats [three females and four males]. The average daily consumption was calculated to be 100 mg of γ-butylactone per kg, and 32 mg/kg of δ-octalalctone, γ-nonalactone, γ-decalactone, γ-undecalactone and γ-do decalactone for between 4 - 6 months. Five successive generations were maintained on the same diet. There was reported to be no treatment-related effects of treatment [the low numbers of rats used limit the validity of the study, but are, however, consistent with the results of feeding studies for each individual lactone] [Adams *et al.,* 1998].

Inhalation Toxicity

In an inhalation study conducted in the rat by Rod products [1996] reported an LC_{50} value >5 mg//l for a particle size of 297 microns or less. The acute toxicity to birds using the species Northern bobwhite was reported to be above 2000 mg/kg, using a commercial repellent. However, the percentage content of furanone in the repellent was small and calculated to give an oral toxicity of >3.65 mg/kg [Rod Products 1996].

When tested at 0.7 ppm in cigarettes, in a 13-week inhalation study, the presence of γ-nonalactone "…had no discernible effect on the character or 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].

The addition of γ-nonalactone at 140 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 γ-nonalactone 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].

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 gamma-Nonalactone at 6 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].

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 gamma-nonalactone at levels up to 39 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other Relevant Studies

The metabolic fate of lactones has been extensively studied in both rats and humans. The administration of ^{14}C labelled γ butyrolactone to rats by intravenous administration was recovered as $^{14}CO_2$ after 2.5 hours [Adams *et al.,* 1998].

Behavioural Data

No data identified.

In vitro* Toxicity *Status

Carcinogenicity and Mutagenicity

γ-Nonalactone has been shown to be negative in the Ames test, both in presence and absence of a S9 fraction in the *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 at 37500 µg/plate [Heck *et al.,* 1989]. Similarly, γ-nonalactone failed to induce unscheduled DNA synthesis in rat hepatocytes exposed to 500 µg/ml [Heck *et al.,* 1989].

γ-Nonalactone was found to be positive in a mouse lymphoma assay [using the L5178y $TK^{(+/-)}$ strain] in the presence of S9 fraction at 400 µg/ml. Without the S9 metabolic fraction they found the assay to be negative at 1000 µg/ml. However, they suggest that the positive result could have been due to the assay conditions, *i.e.* low pH or high osmolarity [Heck *et al.,* 1989].

A gene mutation assay using human lymphocytes was found to be negative when human lymphocytes were dosed with γ-nonalactone at 0.7 mM [Withers 1966].

γ-Nonalactone was negative in the *E.Coli*. WP2 uvrA assay at 0.2 - 1.6 mg/plate. γ-Nonalactone was weekly positive in a rec assay with *Bacillus subtilis* strains M45 (rec-) and M17 (rec+) at a concentration of 20 µl/disk [Yoo, 1986].

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 gamma-Nonalactone at levels up to 6 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 gamma-nonalactone at 140 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].

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”.

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 *Nonalactone, gamma-* at levels up to 673 ppm.

In vitro toxicity testing of tobacco ingredients in burnt form (Internal document R-21).

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 gamma-nonalactone at levels up to 39 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 nonalactone, gamma 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.

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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