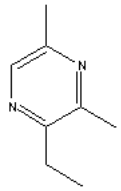
Ethyl-3,(5 or 6)-dimethylpyrazine, 2-

Ingredient synonym names

2-Ethyl-3,-dimethylpyrazine Pyrazine, 2-ethyl-3,?-dimethyl-
3,5-Dimethyl-2-ethylpyrazine
2,5-Dimethyl-3-ethylpyrazine
2-Ethyl-3,(5)-dimethylpyrazine
2-Ethyl-3,5(6)-dimethylpyrazine
2-ethyl-3,5-dimethylpyrazine; 3-ethyl-2,5-dimethylpyrazine

IDENTIFIER DETAILS

Ingredient chemical structure



CAS Number	FEMA Number	Additive Number
27043-05-6, 13925-07-0, 55031-15-7	3149/3150	-

Ingredient EC Number	FL Number	CoE Number
248-182-2	14.016	727

Chemical formula C8H12N2

Ingredient CLP Classification

Ingredient REACH Registration Number

-

Acute Oral Toxicity	Eye Damage/Irritation	Carcinogenity
4	0	0
Acute Dermal Toxicity	**Respiratory Sensitisation**	**Reproductive Toxicity**
0	0	0
Acute Inhalation Toxicity	**Skin Sensitisation**	**Aspiration Toxicity**
0	0	0
Skin Corrosive/Irritant	**Mutagenicity/ Genotoxicity**	**Specific Target Organ Toxicity**
0	0	0

SPECIFICATIONS

Melting Point - Boiling Point -

Ethyl-3,(5 or 6)-dimethylpyrazine, 2-

STATUS IN FOOD AND DRUG LAWS

Acceptable Daily Intake (ADI, mg/kg)	ACCEPTABLE (JECFA, 2001)
Acceptable Daily Intake (ADI) comments	Acceptable at current levels of intake when used as a flavouring agent
FDA Status	-

CoE limits - Beverages (mg/kg)	5	CoE limits - Food (mg/kg)	5	CoE limits - Exceptions (mg/kg)	-

HUMAN EXPOSURE

Ingredient Natural Occurence (if applicable)

2-Ethyl-3,5-dimethylpyrazine is reported to be present in the following foods: roasted barley, cocoa products, coffee, meat, peanuts, filberts, pecans, popcorn, rum and whiskey, beer, cooked potatoes, fried pork, soy products; corn tortillas; malt and cooked shrimps. [Fenaroli 2005].

References - Ingredient Natural Occurence

Fenaroli (2005) Fenaroli’s handbook of flavour ingredients, 5th Edition CRC press. London

Ingredient Reported Uses

2-Ethyl-3,5-dimethylpyrazine is reportedly used in baked goods at 6.43 ppm, frozen dairy at 5.31 ppm, meat products at 2.10 ppm, soft candy at 6.15 ppm, gelatin pudding at 6.15 ppm, non-alcoholic beverages at 2.06 ppm, and alcoholic beverages at 1 ppm [Fenaroli 2005].

References - Ingredient Reported Uses

Fenaroli (2005) Fenaroli’s handbook of flavour ingredients, 5th Edition CRC press. London

TOXICITY DATA

In Vivo Data

Acute Toxicity Data

LD50 Rat Unspecified 460 mg/kg
[JECFA 2002].

In Vivo Carcinogenicity/Mutagenicity

No data identified

References - In Vivo Carcinogenicity/Mutagenicity

No data identified

Dermal Toxicity

No data identified

References - Dermal Toxicity

No data identified

Reproductive/ Developmental Toxicity

No data identified

References - Reproductive/ Developmental Toxicity

No data identified

Inhalation Toxicity

No data identified

References - Inhalation Toxicity

No data identified

Cardiac Toxicity

No data identified

References - Cardiac Toxicity

No data identified

Addictive Data

No data identified

References - Addictive Data

No data identified

Behavioral data

No data identified

References - Behavioral data

No data identified

In Vivo - Other Relevant Studies

The biotransformation of alkyl-, alicyclic-, and alkylaryl-substituted pyrazine derivatives is expected to occur by oxidation of the alkyl side-chains. Methyl-substituted pyrazines are oxidized to yield the corresponding pyrazine-2-carboxylic acids. Products of oxidative metabolism can be excreted unchanged or conjugated with glycine, glucuronic acid, or sulphate before excretion (JECFA 2002).

The LD50 of 2-ethyl-3,(5 or 6)-dimethylpyrazine in rats was reported as 460 mg/kg and is irritating to the skin,

eyes and the upper respiratory tract. Burdock et al., report that, according to the data available (two 90 day studies: no effect level of 12.5 mg/kg/day and NOAEL 17 mg/kg/day), the current level used in food flavouring is safe (Burdock and Carabin, 2008).

In a 90 day toxicity study groups of 15 male and 15 female rats received 2-ethyl-3,5 (or 6)-dimethylpyrazine in the diet at a single dose and a control group received un treated diet. The NOEL for 2-ethyl-3,5 (or 6)-dimethylpyrazine was reported to be 17 mg/kg/day for males and 18 mg/kg/day for females (the only dose tested), there was a slight decrease in bodyweight gain for females but this was not accompanied by any macroscopic or microscopic lesions [JECFA 2002].

The estimated per capita intake is 0.000197 mg/kg [Fenaroli 2005].

References - In Vivo - Other Relevant Studies

Burdock GA and Carabin IG (2008) Safety assessment of 2-ethyl-3,(5 or 6) dimethylpyrazine as a food ingredientRegul Toxicol Pharmacol. 2008 50(3):303-12

Fenaroli (2005) Fenaroli's handbook of flavour ingredients, 5th Edition CRC press. London

JECFA (2002) Safety evaluation of certain food additives and contaminants. Prepared by the fifty-seventh meeting of the joint FAO/WHO Expert Committee on Food Additives (JECFA). ICPCS Geneva.

In Vitro Data

In Vitro Carcinogenicity/Mutagenicity

2-ethyl-3,5-dimethylpyrazine was found to be negative in the Ames assay when tested with Salmonella strains TA98, TA100, TA1535, TA1537 and TA1538 at concentrations up to 50 mg/plate both with and without metabolic activation. In addition, 2-ethyl-3,5-dimethylpyrazine failed to induce unscheduled DNA synthesis in rat hepatocytes at a dose level of 100 µg/ml [Heck et al., 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".

References - In Vitro Carcinogenicity/Mutagenicity

Heck et al., [1989] An evaluation of food flavouring ingredients in a genetic toxicity screening battery. The Toxicologist 9(1): 257

An Interim report on data originating from Imperial Tobacco Limited's Genotoxicity testing programme September 2003 – internal document

An updated report on data originating from Imperial Tobacco Limited's external Genotoxicity testing programme – Round 2 August 2007 – internal document

In Vitro - Other Relevant Studies

No data identified

References - In Vitro - Other Relevant Studies

No data identified

Emissions and Associated 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 2-ethyl-3,5-dimethylpyrazine 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)].

A recent mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including 2-ethyl-3,5-dimethylpyrazine at 0.7 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)] [Gaworski et al., 1999].

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 2-ethyl-3,5-dimethylpyrazine 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].

When tested at 0.7 ppm in cigarettes, in a 13-week inhalation study, the presence of 2-ethyl-3,5-dimethylpyrazine "…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)] [Gaworski et al., 1998].

The addition of 2-ethyl-3,5 (or 6)-dimethylpyrazine 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 2-ethyl-3,5-dimethylpyrazine 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 2-ethyl-3,(5 or 6)-dimethylpyrazine at levels up to 3 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

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 2-ethyl-3,5-dimethylpyrazine at levels less than 1 ppm [a multiple of its typical use in a US cigarette] [Roemer et al., 2000].

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 2-ethyl-3,5 (or 6)-dimethylpyrazine 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 2-ethyl-3,5 (or 6)-dimethylpyrazine at levels up to 127 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 2-ethyl-3,(5 or 6)-dimethylpyrazine at levels up to 3 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

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”.

Information relating to the pyrolysis and/or transfer of 2-ethyl-3,5-dimethylpyrazine 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.

References - Emissions and Associated Toxicity Data

Baker RR, et al., (2004) An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. Food Chem Toxicol. 42 Suppl: S53-83

Carmines (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 1: Cigarette design, testing approach, and review of results. Fd Chem Toxicol 40, 77-91

Gaworski et al., (1998). Toxicologic evaluation of flavour ingredients added to cigarette tobacco: 13-week inhalation exposure in rats. Inhalation Toxicol., 10, 357-381.

Gaworski et al., (1999). Toxicologic evaluation of flavour ingredients added to cigarette tobacco: skin painting bioassay of cigarette smoke condensate in SENCAR mice. Toxicology, 139, 1-17.

In vitro toxicity testing of tobacco ingredients in burnt form (Internal document R-53).

Roemer et al., (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 3: In vitro genotoxicity and cytotoxicity. Fd Chem Toxicol 40, 105-111

Roemer (2014) Toxicological assessment of kretek cigarettes: Part 1: background, assessment approach, and summary of findings. Regul Toxicol Pharmacol.; 70 Suppl 1: 2-14.

Roemer (2014) Toxicological assessment of kretek cigarettes Part 6: the impact of ingredients added to kretek cigarettes on smoke chemistry and in vitro toxicity. Regul Toxicol Pharmacol.; 70 Suppl 1: 66-80.

Rustemeier et al., (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke. Fd Chem Toxicol 40, 93-104

Schramke (2014) Toxicological assessment of kretek cigarettes. Part 7: the impact of ingredients added to kretek cigarettes on inhalation toxicity. Regul Toxicol Pharmacol; 70 Suppl 1: 81-9.

Vanscheeuwijck et al., (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 4: Subchronic inhalation toxicity. Fd Chem Toxicol 40, 113-131

An Interim report on data originating from Imperial Tobacco Limited's Genotoxicity testing programme September 2003 – internal document

An updated report on data originating from Imperial Tobacco Limited's external Genotoxicity testing programme – Round 2 August 2007 – internal document

Report on Thermochemical Properties of Ingredients (Internal document)