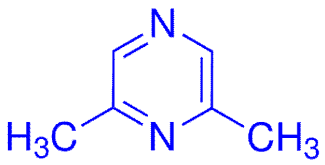
2,6-Dimethylpyrazine

Ingredient synonym names

Pyrazine, 2,6-dimethyl- ;3,5-Dimethylpyrazine; 2,6-Dimethyl-1,4-diazine; Pyrazine

IDENTIFIER DETAILS

Ingredient chemical structure



CAS Number	FEMA Number	Additive Number
108-50-9	3273	-
Ingredient EC Number	**FL Number**	**CoE Number**
203-589-4	14.021	2211

Chemical formula C6H8N2

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
37.00 to 48.00 °C. @ 760.00 mm Hg	154.00 to 155.00 °C. @ 760.00 mm Hg

STATUS IN FOOD AND DRUG LAWS

2,6-Dimethylpyrazine

Acceptable Daily Intake (ADI, mg/kg)	Acceptable (JECFA 2001)
Acceptable Daily Intake (ADI) comments	No safety concerns at current levels of intake when used a flavouring agent.
FDA Status	-

CoE limits - Beverages (mg/kg)	1	CoE limits - Food (mg/kg)	30	CoE limits - Exceptions (mg/kg)	-

HUMAN EXPOSURE

Ingredient Natural Occurence (if applicable)

Reported found in bread, tea, peppermint oil, cheeses, chicken, beef, pork, beer, sherry, whiskies, cocoa, coffee, tea, oatmeal, rice bran, buckwheat and malt (Fenaroli, 2005).

References - Ingredient Natural Occurence

Fenaroli (2005). Fenaroli's Handbook of Flavour Ingredients. 5th Edition. CRC Press London.

Ingredient Reported Uses

2,6 Dimethylpyrazine has been reported in baked good at a level of 10 ppm, in confection and frosting at a level of 3ppm, gelatins and puddings at 3 ppm, in gravies at a level of 5 ppm, in instant coffee at amaximum level of 0.15 ppm, in nut products at a level of 5 ppm , in snack foos at a level of 10 ppm and in soups at 5 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

880 mg/kg bw, Rat, Oral
Drug and Chemical Toxicology. Vol. 3, Pg. 249, 1980.

1080 mg/kg bw, Mouse, Intraperitoneal
Toxicology and Applied Pharmacology. Vol. 17, Pg. 244, 1970.

In Vivo Carcinogenicity/Mutagenicity

Moran et al. (1980) investigated the acute oral lethal doses in rats or mice for 63 flavoring substances, including 2-ethyl-3,(5 or 6)-dimethylpyrazine. The LD50 values of the pyrazines ranged from 456–1910 mg/kg body weight, an approximately four-fold range. The LD50 of 2-ethyl-3,(5 or 6)-dimethylpyrazine in rats was reported as 456 mg/kg with a 95% confidence interval of 394–527 mg/kg. In another unpublished report submitted to the World Health Organization by FEMA, the LD50 of 2-ethyl-3,(5 or 6)- dimethylpyrazine is reported as 460 mg/kg (Oser, 1969a). The LD50 data suggests that 2-ethyl-3,(5 or 6)-dimethylpyrazine is moderately toxic by the oral route

(Derelanko and Hollinger, 1995). In a material safety data sheet (MSDS) from Sigma–Aldrich Chemical Co., it has been reported that 2-ethyl-3,(5 or 6)-dimethylpyrazine is irritating to skin, eye and upper respiratory tract (Sigma-Aldrich, 2003). No information on data sources was given in the MSDS. (Moran, et al., 1980).

The Council of Europe cites a confidential 90-day oral feeding study in rats where the no-effect level of 2-ethyl-3,(5 or 6)-dimethylpyrazine was reported as greater than 12.5 mg/kg/day in a single dose level study (CoE, 2000). The details of the study were not available for independent evaluation. The study does not meet the core standards of FDA (FDA, 1982). JECFA, in its evaluation of a group of 44 pyrazines, including 2-ethyl-3,(5 or 6)-dimethylpyrazine, cited an unpublished subchronic toxicity study in rats (Oser,1969b; Mattia et al., 2001). To paraphrase the JECFA summary of this unpublished report:

A control and a test group of albino weanling rats (Food and Drug Research Laboratories strain) (15/sex/group) were maintained either on control diet or diet containing 2-ethyl-3,(5 or 6)-dimethylpyrazine, adjusted every two weeks to deliver approximately 15 mg/kg body weight/ day for 90 days. Clinical observations were recorded daily, and food consumption and body weights were performed weekly. During weeks 6 and 12 of the study, hematological, clinical chemistry and urinary analyses were performed on 10 animals of each sex. At the end of study, all animals were killed and subjected to detailed necropsy and histological examinations of ‘‘major organs and tissues.” In female rats, daily dietary intake of 2-ethyl- 3(5 or 6)-dimethylpyrazine resulted in slight to moderate (statistically non-significant) decreases in body-weight gain (7–10%) and statistically significant decreases in food use efficiency. No pathological lesions were found. In male rats fed 2-ethyl-3,(5 or 6)-dimethylpyrazine, no changes in body-weight gain or food use efficiency were noted. No other differences were found between test and control groups in either sex (Oser, 1969b).
Based on these unpublished reports, the JECFA determined the no-observed effect level to be 17 and 18 mg/kg/ day for male and female rats, respectively. The details of either study were not available for independent review, nor does either study meet the core FDA standards (FDA, 1982).

In a series of studies, Gaworski et al. (1999) investigated the tumor promoting potentials of cigarette smoke condensate prepared from cigarettes containing 150 flavor ingredients including 2-ethyl-3,(5 or 6)-dimethylpyrazine in female SENCAR mice,. The target concentration of 2-ethyl-3,(5 or 6)-dimethylpyrazine applied to tobacco used for preparation of cigarette smoke condensate was 0.7 ppm. Tumors were initiated in groups of 35–50 female mice by applying a single dose of 50 lg of 7,12-dimethylbenz(a)anthracene (DMBA) in 0.1 ml acetone to shaved 2 3 cm dorsal skin. One week after DMBA initiation, mice were treated thrice weekly for 26 consecutive weeks with either 10 or 20 mg cigarette smoke condensate from test cigarettes in 0.1 ml acetone. At the end of 27 weeks, after DMBA initiation, mice were examined for tumor incidence. No substantial changes were noted in tumor promotion capacity from cigarette smoke condensate prepared from cigarettes with flavor ingredients, including 2-ethyl-3,(5 or 6)-dimethylpyrazine, compared with cigarette smoke condensate prepared from cigarettes without flavor ingredients.

No genotoxicity studies of 2-ethyl-3,(5 or 6)-dimethylpyrazine were found in the published literature. However, it has been reported that pyrazine and four alkyl derivatives (2-methylpyrazine, 2-ethyl pyrazine, 2,5-dimethylpyrazine and 2,6-dimethylpyrazine) were not mutagenic in Ames-Salmonella assays in strains TA98, TA100 and TA1535 with and without S-9 metabolic activation, at concentrations up to 100 lg/plate. In in vitro studies with Chinese ovary cells, pyrazine and all of the alkylated derivatives above, at doses ranging from 5–40 mg/ml induced increases in chromosomal aberrations (Stich et al., 1981). In assays with Saccharomyces cerevisiae, no recombinant genetic changes were found, but the surviving cells had increased mutant colony frequencies. JECFA, in its evaluation of the safety of pyrazine derivatives, including 2-ethyl-3,(5 or 6)-dimethylpyrazine, stated that ‘‘the relevance of the positive results for pyrazine and certain alkylpyrazines in assays with S. cerevisiae and Chinese hamster ovary cells in vitro reported by Stich et al. (1980) is unclear. The studies were performed at high, nearly toxic concentrations of the weakly basic pyrazines, which may have altered cellular homeostasis.”

Pyrazines, as a class, are weak bases (pKb = 13.4) and are readily absorbed in the gastrointestinal tract. As an

alkyl substituted pyrazine, the metabolism of 2-ethyl-3,(5 or 6)-dimethylpyrazine is expected to occur primarily by oxidation of the alkyl side-chains with oxidation of one or both methyl groups yielding, in this case, the corresponding pyrazine-2-carboxylic acids. These carboxylic acids may be excreted unchanged or as the glycine, glucuronic acid or sulfate conjugates. A limited amount of pyrazine may be metabolized by hydroxylation of the pyrazine ring. The percentage of pyrazine excreted in the urine may be as high as 97% of a gavaged dose and for 2-ethyl-3,(5 or 6)-dimethylpyrazine, most of these excretory products should be carboxylic acids and glycine conjugates (Schranker, 1957).

References - In Vivo Carcinogenicity/Mutagenicity

Moran EJ, Easterday DD and Oser BL, 1980. Acute oral toxicity of selected flavor chemicals. Drug Chem.Toxicol. 3(3), 249-258.

FDA, 1982. Toxicological principles for the safety assessment of direct food additives and color additives used in food (‘Redbook’). US Food and Drug Administration, Washington, DC.

Mattia, A., Renwick, A.G., Sipes, I.G., 2001. Safety evaluation of certain food additives and contaminants. Pyrazine derivatives. WHO Food Additives Series: 48.

Oser, B.L., 1969b. The acute oral toxicity to rats of nine pyrazine derivatives. Submitted to WHO by the Flavor and Extract Manufacturers’ Association (cited in Adams et al. 2002).

Schranker, L.S., Shore, P.A., Brodie, B.B., Hogben, C.A.M., 1957. Absorption of drugs from the stomach I. The rat. The Journal of Pharmacology and Experimental Therapeutics 120, 528–539.

Dermal Toxicity

No data identified

References - Dermal Toxicity

No data identified

Reproductive/ Developmental Toxicity

Although no teratogenicity or reproduction toxicity studies of 2-ethyl-3,(5 or 6)-dimethylpyrazine were found, one study was available on the related alkylpyrazine, tetramethylpyrazine, at up to levels of 250 mg/kg/day, which reported no reproductive or developmental effects (Vollmuth et al., 1990). Also available were several reports of studies in mice by Yamada and coworkers (Yamada et al., 1992, 1994, 2002, 2004) involving another related pyrazine, 2,5-dimethylpyrazine at levels up to 100 mg/kg/ day. This substance was reported to inhibit the growth of testes and sex-accessory glands in juvenile male mice. It had an inhibiting effect on the weight increase of the uterus and prostate or seminal vesicles in female and male rats, respectively, following intraperitoneal administration (Yamada et al., 1992, 1994, 2002, 2004). These studies may not be applicable to oral low level exposure of 2-ethyl-3,(5 or 6)-dimethylpyrazine and the concentration of 2,5-dimethylpyrazine used in these studies was high compared to current pyrazine consumption.

References - Reproductive/ Developmental Toxicity

Vollmuth, T.A., Bennett, M.B., Hoberman, A.M., Christian, M.S., 1990. An evaluation of food flavoring ingredients using an in vivo reproductive and developmental toxicity screening test. Teratology 41, 597 (cited in Adams et al. 2002).

Yamada, K., Sano, M., Aoyagi, Y., 2002. Inhibitory effect of 2,5- dimethylpyrazine on oxytocic agent-induced

uterine hypercontraction of pregnant female rats. Japanese Journal of Pharmacology 88, 257.

Yamada, K., Shimizu, A., Komatsu, H., Sakata, R., Ohta, A., 1994. Effects of 2,5-dimethylpyrazine on plasmatestosterone and polyamines- and acid phosphatase-levels in the rat prostate. Biological & Pharmaceutical Bulletin 17, 730–731 (cited in Adams et al. 2002).

Yamada, K., Takahashi, H., Ohta, A., 1992. Effects of 2,5-dimethylpyrazine on reproductive and accessory reproductive-organs in female rats. Research Communications in Chemical Pathology and Pharmacology 75, 99–107 (cited in Adams et al. 2002).

Yamada, K., Kobayashi, Y., Fujihara, H., Ohta, A., 2004. Inhibitory effect of 2,5-dimethylpyrazine on oxytocic agent-induced uterine hypercontraction of normal or pregnant female rats. Biological & Pharmaceutical Bulletin 21, 538–540.

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

2-Ethyl-3,(5 or 6)-dimethylpyrazine (CAS No. 27043-05-6), a heterocyclic, nitrogen-containing compound, is used in the food industry as a flavor ingredient for its characteristic roasted odor and flavor, reminiscent of roasted cocoa or nuts. Pyrazines, including 2-ethyl-3,(5 or 6)-dimethylpyrazine, are widely distributed in foods and because of their natural unavoidable occurrence in cooked food; therefore, pyrazine compounds, including 2-ethyl-3,(5 or 6)-dimethylpyrazine, are commonly consumed in the daily diet. 2-Ethyl-3,(5 or 6)-dimethylpyrazine is oxidized in rats almost exclusively via its aliphatic side-chain to carboxylic acid derivatives. The LD50 of 2-ethyl-3,(5 or 6)-dimethylpyrazine in rats was reported as 460 mg/kg and it is reported to be irritating to the skin, eyes

and the upper respiratory tract. Two 90-day rat feeding studies have been conducted on 2-ethyl-3,(5 or 6)-dimethylpyrazine, with the one reporting a no effect level of 12.5 mg/kg/day (both sexes) and a second study reporting a NOAEL of 2-ethyl-3,(5 or 6)-dimethylpyrazine 17 and 18 mg/kg/day for male and female rats, respectively. Although no genotoxicity studies were found on 2-ethyl-3,(5 or 6)-dimethylpyrazine, structurally similar pyrazine derivatives were reported as clastogenic in mammalian cells and non-mutagenic in bacterial assays. The relevance of the positive results in assays with Saccharomyces cerevisiae and Chinese hamster ovary cells in vitro is unclear. The data and information available, including a prolonged history of safe use, indicate that at the current level of intake, the food flavoring use of 2-ethyl-3,(5 or 6)-dimethylpyrazine is safe (Burdock and Carabin, 2008).

Pyrazines, as a class, are weak bases (pKb = 13.4) and are readily absorbed in the gastrointestinal tract. As an alkyl substituted pyrazine, the metabolism of 2-ethyl-3,(5 or 6)-dimethylpyrazine is expected to occur primarily by oxidation of the alkyl side-chains with oxidation of one or both methyl groups yielding, in this case, the corresponding pyrazine-2-carboxylic acids. These carboxylic acids may be excreted unchanged or as the glycine, glucuronic
acid or sulfate conjugates. A limited amount of pyrazine may be metabolized by hydroxylation of the pyrazine ring. The percentage of pyrazine excreted in the urine may be as high as 97% of a gavaged dose and for 2-ethyl-3,(5 or
6)-dimethylpyrazine, most of these excretory products should be carboxylic acids and glycine conjugates (Schranker, 1957; JECFA, 2012)

References - In Vivo - Other Relevant Studies

Burdock, G., Carabin, I., (2008), Safety Assessment of 2-ethyl-3,(5 or 6) dimethylpyrazine as a food ingredient, Regult. Tox. and Pharmacol. 50, 3, pp. 303-312

In Vitro Data

In Vitro Carcinogenicity/Mutagenicity

In a study on mutagenic activity of four coffee flavors, pyrazine was found to be negative in a Salmonella typhimurium (TA1535, TA1537, TA1538, TA98 and TA 100) plate incorporation assay and a mouse lymphoma L5178 TL+/ assay (Fung et al., 1988). These observations are in agreement with those previously reported in the Salmonella assay (Stich et al., 1980). Sims et al. (1982) reported that pyrazines lack an inhibitory effect on poly (ADP-ribose) polymerase activity in normal human lymphocytes, but did stimulate unscheduled DNA synthesis in UV-irradiated human lymphocytes. In a mutagenicity assay of products obtained from a maltol-ammonia browning model system, fractions containing 2-ethyl-3,5-dimethylpyrazine (4% of total) and 2-ethyl-3,6-dimethylpyrazine (6%) were positive in an Ames assay with TA98 in the presence of exogenous metabolic activation (S9), but were negative without S9. The fractions were reported to contain approximately 26 other compounds (Shibamoto et al., 1981). In an antimutagenicity study of Maillard reaction products, Jenq et al. (1994) found that the dichloromethane extract of eight sugar/ amino acid reaction systems, which contained mostly pyrazines, inhibited the metabolic activation and mutagenicity of 2-amino-3-methyl-imidazo(4,5-f)quinoline in Ames tester strain TA98.

References - In Vitro Carcinogenicity/Mutagenicity

Jenq, S., Tsai, S.H., Lee, H., 1994. Antimutagenicity of Maillard reaction products from amino acid/sugar model systems against 2-amino-3- methylimidazo-(4,5-f) quinoline: The role of pyrazines. Mutagenesis 9, 483–488.

Shibamoto, T., Nishimura, O., Mihara, S., 1981. Mutagenicity of products obtained from a maltol-ammonia browning model system. Journal of Agricultural and Food Chemistry 29, 643–646.

2,6-Dimethylpyrazine

In Vitro - Other Relevant Studies

Liu and Sylvester (1994) studied the inhibition of platelet aggregation of a series of alkyl pyrazines. When fresh human platelets were treated at 1.8 mM with various alkylpyrazines, followed by adenosine diphosphate to stimulate aggregation, 2,3-diethylpyrazine exhibited greater platelet aggregation inhibition (55%) than tetramethylpyrazine. The investigators concluded that enhanced antiplatelet activity was associated with increasing the number of alkyl groups on the pyrazine ring as well as increasing the length of unbranched alkyl side chains.

References - In Vitro - Other Relevant Studies

Liu, S.Y., Sylvester, D.M., 1994. Antiplatelet structure–activity relationship of tetramethylpyrazine. Life Science 55, 1317–1326.

Emissions and Associated Toxicity Data

No data identified

References - Emissions and Associated Toxicity Data

No data identified