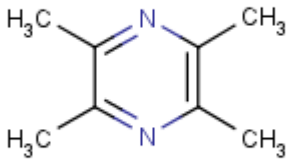
2,3,5,6-TETRAMETHYL PYRAZINE

SYNONYMS

2,3,5,6-Tetramethylpyrazine (natural)
Bs factor
NSC 36080
NSC 46451
Pyrazine, tetramethyl-
Tetramethylpyrazine
Tetrapyrazine
UNII-V80F4IA5XG
Ligustrazine

CHEMICAL STRUCTURE



CHEMICAL FORMULA

C_8-H_{12}-N_2

IDENTIFIER DETAILS

CAS Number	:	1124-11-4
CoE Number	:	734
FEMA	:	3237
EINECS Number	:	214-391-2
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: 84-86^{o}C

Boiling point: 190^{o}C

PURPOSE

Flavour

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
3	5	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
-	-	-	-

FDA Status:

Section Number	Comments
-	-

HUMAN EXPOSURE

Natural Occurrence: Tetramethylpyrazine is an amide alkaloid found in the stem of *Jatropha podagrica Hook* and is the active principle in *Ligusticum wallichii* [Feng, 2009].

Reported found in French fried potato, bell pepper, wheaten bread, Swiss cheese, Camembert cheese, Gruyere cheese, boiled and cooked beef, grilled and roasted beef, fried beef, lamb and mutton liver, grilled and roasted uncured pork , beer, black tea, green tea. Also reported present in cocoa products, coffee, dairy products, oatmeal, galbanum oil, peanuts, soybean, beans, mushroom, trassi, coriander seed, rice bran, sukiyaki, malt, liquorice, dried bonito, wild rice, shrimp, crab, clam, scallop, filberts, rum, whiskey and soy products [Fenaroli, 2005].

Reported Uses: 2,3,5,6-Tetramethyl pyrazine is reportedly used for Alcoholic beverages at 1.03ppm, Baked goods at 4.53ppm, Frozen dairy at 3.25ppm, Pudding gelatins at 2.76ppm, Meat products at 4ppm, non-alcoholic beverages at 2.76ppm, and Soft candy at 3.53ppm [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 2,3,5,6-tetramethylpyrazine at levels up to 24 ppm, “did not increase the overall toxicity of cigarette smoke” [Carmines, 2002].

In Vivo Toxicity Status

Species	Test Type	Route	Reported Dosage
Mouse	LD_{50}	intraperitoneal	0.8mg/kg
Mouse	LD_{50}	intravenous	239mg/kg
Rat	LD_{50}	oral	1910mg/kg

ChemIDplus (2010)

The toxicity of 2,3,5,6-tetramethylpyrazine was tested in a 90-day feeding study in rats. A control and a test group, each consisting of 15 male and 15 female albino weanling rats (Food and Drug Research Laboratories strain), were maintained individually in housing with controlled temperature and humidity and access to water and food *ad libitum*. The concentration of the test material in the diet was adjusted every 2 weeks to maintain a constant dietary intake of approximately 44 mg/kg bw per day of 2,3,5,6-tetramethylpyrazine. Clinical observations were recorded daily, and food consumption and body weights were determined weekly. During weeks 6 and 12 of the study, haematological, clinical chemical, and urinary parameters were measured on 10 animals of each sex. After 90 days, all animals were killed and subjected to detailed necropsy. Tissues and organs from each

animal were preserved, and histopathological examinations were performed on major organs and tissues. In female rats, daily dietary intake of 55 mg/kg bw per day of 2,3,5,6-tetramethyl pyrazine resulted in slight to moderate decreases in body-weight gain and decreases in food use efficiency. No pathological lesions were found. No effects on body-weight gain or food use efficiency were reported in males fed this flavouring agent at similar doses of 50 mg/kg bw per day of 2,3,5,6-tetramethylpyrazine. No other differences were found between test and control groups in either sex [IPCS INCHEM, 2009].

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 2,3,5,6-tetramethylpyrazine at <0.1 ppm. The authors concluded that the study "did not indicate any substantive effect of these ingredients on the tumorigenicity of cigarette smoke condensate" [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].

Dermal Toxicity

No data identified.

Reproductive and Developmental Toxicity

Four groups of 10 virgin Crl:CD BR VAF Plus rats were given tetramethylpyrazine by gavage at a dose of 0, 25, 125, or 250 mg/kg bw per day 7 days before cohabitation and throughout cohabitation (maximum of 7 days), gestation, parturition, and a 4-day post-parturition period. The maternal indices monitored included daily measurement of body weight and food consumption, duration of gestation, and fertility parameters (mating, fertility, and gestation indices, number of offspring per litter). The indices in offspring included daily observations for clinical signs, examination for gross external malformations, and measurement of body weight. The only effects reported were reduced body-weight gain in dams at the two higher doses, accompanied by a statistically significant reduction in food consumption in those at the highest dose. No effects were observed in dams at the lowest dose or in offspring at any dose. It was concluded that tetramethylpyrazine had no reproductive or developmental effects [IPCS INCHEM, 2009].

Inhalation Toxicity

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 2,3,5,6-tetramethylpyrazine at 1.95 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 1h/day, 5 days/wk for 13 weeks to smoke at concentrations of 0.06, 0.2 or 0.8mg/L from the test or reference cigarettes, or to air only. Plasma nicotine, COHb and respiratory parameters were measured periodically. Rats were necropsied after 13wk 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.

When tested at <0.1 ppm in cigarettes, in a 13-week inhalation study, the presence of 2,3,5,6-tetramethylpyrazine 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 2,3,5,6-tetramethylpyrazine at 23 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 2,3,5,6-tetramethylpyrazine 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 2,3,5,6-tetramethyl pyrazine at levels up to 3 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other Relevant Studies

Tetramethylpyrazine was found to have inhibitory effects on platelet function. Antithrombotic activity of tetramethylpyrazine is at least partly due to its potent antiplatelet effect. Tetramethylpyrazine inhibits platelet aggregation induced by ADP, collagen, thrombin, and A23187 but not by diacyl-glycerol [Liu and Sylvester, 1990].

Qi *et al.* (2002) measured the plasma and brain levels of 2,3,5,6-tetramethylpyrazine following a single transdermal application of the compound in Sprague-Dawley rats. The elimination half-life for Tetramethylpyrazine in plasma and brain was 26.5 and 31.2 minutes, respectively [Qi *et al*., 2002].

Feng *et al.* (2009) studied the brain pharmacokinetic behaviours of tetramethyl pyrazine following its intranasal or intravenous administration in rats. A dose of 10mg/kg of tetramethyl pyrazine was given to intranasally and intravenously exposed groups. The results indicated that the mean corrected tetramethyl pyrazine concentration of 1.49 ug/ml was obtained at 5 min following i.n. dosing while no tetramethyl pyrazine in the dialysate sampled 5 min after i.v. injection was detected, in the range of our measurement limit. Compared with i.v. application, intranasal administration of tetramethyl pyrazine could obtain significantly fast absorption from nasal to ipsilateral striatum and equal bioavailability [Feng, 2009].

The haemodynamic action of intravenous administration of tetramethylpyrazine phosphate was studied in anesthetized dogs by Huang *et al.* (1996). When given alone, tetramethylpyrazine increased left ventricular systolic pressure, peak positive first derivative of left ventricular pressure, coronary blood flow and heart rate while decreasing mean aortic pressure [Huang *et al.,* 1996].

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”.

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 2,3,5,6-tetramethylpyrazine at 23 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 2,3,5,6-tetramethylpyrazine at levels up to 13 ppm.

The authors state that Cyclooxygenase (COX)-2 plays an important role in tumorigenesis and has been implicated to be a critical factor for invasion and metastasis of lung cancer. Tetramethylpyrazine (TMP), an effective component of the traditional Chinese medicine Chuanxiong, has been traditionally used in treating neurovascular and cardiovascular diseases. Recently TMP has been reported to have beneficial effect in cancer patients. However, the function and the mechanism of TMP in lung cancer have not been elucidated to date. In this study, we investigated the in vitro and in vivo effect of TMP in tumorigenesis and whether COX-2 is a molecular target of TMP. We showed that TMP exhibited a dose- and time-dependent inhibition on A549 cell proliferation by suppressing cell cycle progression. In vitro treatment of A549 cells with TMP resulted in a significant inhibition of invasion, associated with reduced activities of COX-2 and MMP-2/TIMP-2. Furthermore, in vivo experiments showed that TMP significantly suppressed metastatic growth of A549 cells and COX-2 expression in metastatic nude mouse model. This preclinical study provides the first evidence for the novel anti-tumor effects of TMP as a COX-2 pathway inhibitor in human adenocarcinoma cell line A549. It was suggested by the authors that these studies suggest that TMP may serve as an effective agent for the treatment and chemoprevention of non-small cell lung cancer. (Zheng CY, 2012)

A total of 95 ingredients were tested individually through addition at different concentrations to the tobacco of experimental cigarettes. Mainstream cigarette smoke chemistry analysis, bacterial mutagenicity testing, and cytotoxicity testing were conducted. The authors concluded that these ingredients, which included 2,3,5,6-tetramethylpyrazine applied at levels up to 10,000 ppm on cigarettes produced minimal changes in the overall toxicity profile of mainstream cigarette smoke, and in some cases, the addition of high levels of an ingredient caused a small reduction in toxicity findings, probably due to displacement of burning tobacco [Gaworski *et al*., 2011].

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 2,3,5,6-

tetramethyl pyrazine at levels up to 3 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other Relevant Studies

Cellular and biochemical analyses demonstrated that 50microM tetramethylpyrazine significantly preserved neuronal morphology and survival in retinal cell cultures following 4-week *in vitro* cultivation as well as lethal exposures to hydrogen peroxide (10microM or 50microM for 24h) [Yang *et al.,* 2007].

PYROLYSIS AND TRANSFER STUDIES

No data identified.

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