VANILLIN

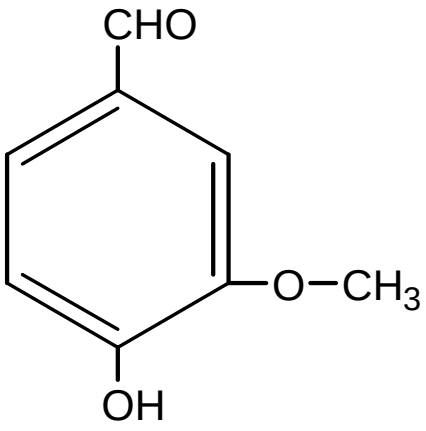
SYNONYMS

2-Methoxy-4-formylphenol
3-Methoxy-4-hydroxybenzaldehyde
4-Formyl-2-methoxyphenol
4-Hydroxy-3-methoxy-
4-Hydroxy-3-methoxybenzaldehyde
4-Hydroxy-5-methoxybenzaldehyde
4-Hydroxy-*m*-anisaldehyde
Benzaldehyde, 4-formyl-2-methoxyphenol
Benzaldehyde, 4-hydroxy-3-methoxy-
EINECS 204-465-2
Lioxin
Methylprotocatechuic aldehyde
m-Anisaldehyde, 4-hydroxy-
Protocatechualdehyde 3-methylether
Protocatechualdehyde, methyl-
p-Hydroxy-*m*-methoxybenzaldehyde
p-Vanillin
Vanilla
Vanillaldehyde
Vanillic aldehyde
Vanilline
Zimco

CHEMICAL FORMULA

$C_8H_8O_3$

CHEMICAL STRUCTURE



IDENTIFIER DETAILS

CAS Number	:	121-33-5
CoE Number	:	107
FEMA	:	3107
EINECS Number	:	204-465-2
E Number	:	-

SPECIFICATIONS

Melting Point: 81°C

Boiling point: 285°C

Smiles Code: c1(cc(ccc1O)C=O)OC

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	-	-

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/.

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

0-10	JECFA	1967	Previously established ADI was retained [JECFA, 2002]

FDA Status:(CFR21)

Section Number	Comments
182.60	Synthetic flavouring substances and adjuvants – GRAS
172.515	Synthetic flavouring substances and adjuvants
169.180	Vanilla – vanillin extract
169.181	Vanilla – vanillin flavouring
169.182	Vanilla – vanillin powder

HUMAN EXPOSURE

Natural Occurrence: Vanillin widely occurs in nature; it has been reported in the essential oil of Java citronella (*Cymbopogon nardus* Rendl.), in benzoin, Peru balsam, clove bud oil, and chiefly vanilla pods (*Vanilla planifolia, V. tahitensis, V. pompona*); more than 40 vanilla varieties are cultivated; vanillin is also present in the plants as glucose and vanillin. Reported found in guava, feyoa fruit, many berries, asparagus, chive, cinnamon, ginger, Scotch spearmint oil, nutmeg, crisp and rye bread, butter, milk, lean and fatty fish, cured pork, beer, cognac, whiskies, sherry, grape wines, cocoa, coffee, tea, roast barley, popcorn, oatmeal, cloudberry, passion fruit, beans, tamarind, dill herb and seed, sake, corn oil, malt, wort, elderberry, loquat, Bourbon and Tahiti vanilla and chicory root [Fenaroli, 2005].

Reported Uses: Vanillin is reported to be used (maximum levels) in baked goods at 186.1 ppm, breakfast cereals at 353 ppm, fats & oils at 100 ppm, milk products at 314.4 ppm, frozen dairy at 55.18 ppm, meat products at 2.72 ppm, soft candy at 407.9 ppm, confectionery & frosting at 768.2 ppm, sweet sauce at 363 ppm, gelatins & puddings at 116.8 ppm, snack foods at 200 ppm, non-alcoholic beverages at 97.42 ppm, alcoholic beverages at 47.09 ppm, hard candy at 192.8 ppm, and chewing gum at 444.7 ppm [Fenaroli, 2005].

TOXICITY DATA

As vanillin is the predominant component of vanilla also refer to vanilla extract (beans and pods).

Carmines *et al*., (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 conclude that the addition of the combined ingredients, including vanillin at levels up to 829 ppm, “did not increase the overall toxicity of cigarette smoke” [Carmines *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 vanillin at 65 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.

Vanillin was evaluated by JECFA at their 57th meeting in 2002 and the previously established ADI of 10mg/kg bw was retained. This ADI is based upon a NOEL of 1000mg/kg bw per day in a 2 year feeding study in rats. This NOEL provides a margin of safety that is 400 times the *per capita* intake of vanillin from its current use as a flavouring agent in Europe (0.9mg/kg bw per day) or in the USA (2.5mg/kg bw per day) [JECFA, 2002].

In Vivo **Toxicity Status**

Species	**Test Type**	**Route**	**Reported Dosage**
Rat	LD_{50}	Unspecified	1.58g/kg
Rat	LD_{50}	Unspecified	2.0g/kg
Rat	LD_{50}	Unspecified	2.8g/kg
Rabbit	LD_{50}	Unspecified	1.58g/kg
			[NTP, 13th August 2001]
Rat	LD_{50}	Intraperitoneal	1.16g/kg
Rat	LD_{50}	Subcutaneous	1.50g/kg
Mouse	LD_{50}	Intraperitoneal	0.48g/kg
Dog	LD_{50}	Intravenous	1.32g/kg
Guinea Pig	LD_{50}	Intraperitoneal	1.19g/kg
			[NTP, 13th August 2001]

An adult person received 100 mg of vanillin dissolved in water, and urine was collected for 24 h. The concentration of vanillic acid in the urine increased from a background value of 0.3 mg per 24h to 96mg per 24h, accounting for

about 94% of the dose [JECFA, 2002].

JECFA, (2002) summarised the acute and chronic oral data available for vanillin: 500 mg/kg bw of vanillin was fed to rats in their diet on a daily basis for 16 weeks. No adverse effects were observed. 1000 or 2500 mg/kg bw of vanillin dissolved in corn oil, was added to the diet of male rats on a daily basis for 1 year. No adverse effects were observed. 20 mg/kg bw or 64 mg/kg bw of vanillin was fed daily to rats for 126 or 70 days respectively. In the 70-day study half the rats were killed and the other half put on a recovery diet for 8 more weeks. Observations of appearance, behaviour and body-weight gain showed a reduced growth rate and myocardial, renal, hepatic, lung, spleen and stomach injuries at the dose of 64 mg/kg bw. 240 mg/kg bw of vanillin per day was given to rats for either 56 days or 126 days. No adverse effects were seen. 250, 500 or 1000 mg/kg bw of vanillin dissolved in propylene glycol, was added to the diet of 12 male and 12 female rats on a daily basis for 2 years. No adverse effects were seen. 500mg/kg bw of vanillin was administered to male BDF1 mice and micronucleus formation was investigated. Vanillin did not induce micronuclei *in vivo* [JECFA, 2002].

Vanillin was administered orally and intra-peritoneally to male Sprague-Dawley rats at doses of 150 and 300 mg/kg for 14 weeks. Analytically, whole blood cell counts, liver and kidney function, and brain gene expression were measured. The authors found that whilst at the 300 mg/kg injected dose rat would fall unconscious after 2 minutes but would recover after 10 minutes, there was ‘no toxic effect on blood cells, kidney or liver’. With regards to the gene expression analysis of brain homogenates the authors suggested that vanillin may have a neuroprotective effect as changes associated with xenobiotic metabolism, cell cycle arrest and apoptosis caused by administration of the ethanol vehicle, were reversed when vanillin was present. However the authors noted that vanillin maintained ‘normal’ levels of expression for gene associated with xenobiotic metabolism, cell progression, tumour suppressor, DNA damage and inflammation [Ho *et al.,* 2011].

Carcinogenicity and Mutagenicity

Feron *et al*., (1991), evaluated 12 aldehydes, including vanillin, found or used in food products for carcinogenic potential. The authors, citing Hagan *et al*., (1967), state that rats (12 of each gender) fed a diet containing 50,000 ppm for one year or 10,000 ppm for two years ‘showed no deleterious effect’, pointing to vanillin as a ‘rather harmless food component’ [Feron *et al*., 1991].

Vanillin did not cause tumours in strain A mice injected intraperitoneally with doses of vanillin of 3.6-18.0 g/kg over a period of 24 weeks. Neither the Environmental Protection Agency (EPA) nor the International Agency for Research on Cancer (IARC) have classified vanillin as to its carcinogenicity [Forsyth, 1997].

A mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including vanillin at 864 ppm. The authors concluded that the study “did not

indicate any substantive effect of these ingredients on the tumorigenicity 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)).

Vanillin has recently been reported to show anti-mutagenic activity and to inhibit chemical carcinogenesis. Lirdprapamongkol *et al.,* (2005) examined the effect of vanillin on the growth and metastasis of 4T1 mammary adenocarcinoma cells in BALB/c mice. Mice that were orally administered vanillin showed significantly reduced numbers of lung metastasized colonies compared to controls. In vitro studies revealed that vanillin, at concentrations which were not cytotoxic, inhibited invasion and migration of cancer cells and inhibited enzymatic activity of MMP-9 secreted by the cancer cells. Vanillin was also reported to show a growth inhibitory effect towards cancer cells in vitro. However, vanillic acid, a major metabolic product of vanillin in human and rat, had no activiy in these in vitro activity assays. It was suggested that vanillin has an anti-metastatic potential by decreasing the invasiveness of cancer cells. Lirdprapamongkol *et al.,* (2005) have suggested that since vanillin is generally regarded as safe, it may be of value in the development of anti-metastatic drugs for cancer treatment [Lirdprapamongkol *et al.,* 2005]

Dermal toxicity

In closed-patch tests vanillin caused no primary skin irritation when tested at concentrations of 20 and 2% in normal subject, and at 0.4% on subjects with dermatoses. Concentrations of 2 and 5% in petrolatum produced no sensitisation reactions. However, vanillin can cause dermal reactions in people previously sensitised to other compounds [Forsyth 1997].

Inhalation toxicity

Results of sub-acute and acute experiments in which rats and mice were subjected to inhalation of saturated vanillin vapour concentrations (41.7 mg/m^3) for 30 days revealed that vanillin was of low toxicity. Vanillin had weak cumulative properties and induced habituation. Vanillin was capable of causing noxious effects on the body with long-term inhalation. Further studies on its toxicity in chronic experiments were recommended [Makaruk 1980].

Rats exposed to a 'saturated' (concentration unspecified) vapour of vanillin for 4 hours / day, 5 days / week for 30 days showed weight loss and a reduction in movement. Microscopic examination revealed damage (unspecified) due to hyperaemia of the liver, kidneys and lungs and a reduction in the number of both red and white blood cells [BIBRA, 1990].

When tested at 864 ppm in cigarettes, in a 13-week inhalation study, the presence of vanillin "…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)).

A 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 vanillin at up to 829 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 vanillin at 2550 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 vanillin 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 subchronic inhalation study (150 mg/m^3 TPM 6 h a day for 90 consecutive days) in Sprague-Dawley rats investigated the effects of vanillin added to experimental cigarettes. Vanillin concentrations in the cigarettes were 0, 67, 1233, and 3109 ppm and at these concentrations vanillin "did not influence a broad range of toxicological endpoints". Most of the observed changes were resolved 42 days post-inhalation. Additional studies included the mutagenicity of mainstream smoke in Salmonella bacterial strains (with and without metabolic activation), cytotoxicity of particulate and vapour phase (neutral red uptake assay), and analytical chemistry. Similar responses were seen across all four experimental cigarettes with regards to mutagenicity, cytotoxicity, and analytical chemistry, [Lemus *et al*, 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 Vanillin at levels up to 261 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Reproductive and Developmental Toxicity

Using single injections, at doses up to 10mg / egg, vanillin was shown not to be teratogenic to developing chick embryos after 96 hours [Verrett *et al.,* 1980].

Four groups of 10 virgin Crl CD rats were given veratraldehyde, vanillin (125, 250 or 500 mg/kg bw per day), ethyl vanillin or piperonal by gavage once a day for 7 days before cohabitation and throughout cohabitation, gestation, parturition and a 4-day post-parturition period. Maternal and offspring indices measured included twice-daily observations, body weight, food consumption and fertility parameters. In view of the lack of adverse effects on offspring at all doses and on dams at the low dose of each substance, the authors concluded that the compounds had no reproductive or developmental effects [JECFA, 2002].

Other relevant studies

In an immunotoxicity assay vanillin failed to modulate the cell mediated or humoral immune response of female CD_1 mice when administered intragastrically for five days at doses up to 1000 mg / kg / day [Gaworski *et al.*, 1994].

Vanillin, covalently binds with sickle haemoglobin (Hb S), and inhibits cell sickling. shifting the oxygen equilibrium curve towards the left. These effects would potentially benefit patients with sickle cell disease (SCD). However, vanillin has no therapeutic effect if given orally because orally administered vanillin is rapidly decomposed in the upper digestive tract. A vanillin prodrug, MX-1520, which is biotransformed to vanillin in vivo, was synthesized by Zhang *et al.* (2004) to over come this problem . Zhang et al., (2004) conducted studies using transgenic sickle mice, which nearly exclusively develop pulmonary sequestration upon exposure to hypoxia, and demonstrated that oral administration of MX-1520 prior to hypoxia exposure significantly reduced the percentage of sickled cells in the blood. The survival time under severe hypoxic conditions was prolonged from 6.6 +/- 0.8 min in untreated animals to 28.8 +/- 12 min ($P < 0.05$) and 31 +/- 7.5 min ($P < 0.05$) for doses of 137.5 and 275 mg/kg respectively. Intraperitoneal injection of MX-1520 to bypass possible degradation in the digestive tract showed that doses as low as 7 mg/kg prolonged the survival time and reduced the percentage of sickled cells during hypoxia exposure. Zhang *et al.,* (2004) stated that these results demonstrated the potential for MX-1520 to be a new and safe anti-sickling agent for patients with SCD [Zhang *et al.,* 2004].

Vanillin has been shown to be rapidly absorbed in the gastrointestinal tract, metabolised in the liver to yield benzoic acid derivatives and excreted primarily in the urine either unchanged or conjugated [JECFA, 2002].

Beaudry *et al.* (2010) investigated the pharmacokinetics of vanillin in rats (n = 6). The rats were administered 100 mg/kg of vanillin p.o. Pharmacokinetic results following p.o. administrations were C(max) 290.24 ng/mL, T(max) 4 h, relative clearance 62.17 L/h/kg, T(1/2) 10.3 h and a bioavailability of 7.6% [Beaudry *et al.,* 2010].

Behavioural data

A study using wistar albino rats was designed to investigate the supposed anxiolytic effects of vanillin. The rats were divided into five groups (n = 6), and vanillin was administered orally at doses of 10, 100, 200 mg/kg/day. The behaviour of the rats was compared to that of the control group, and those administered 1 mg/kg/day of diazepam (oral). The experiment was conducted over 10 days. Two pharmacologically validated models, elevated plus maze and bright and dark arena were used to evaluate the rats behaviour. The data was analysed using Kruskal Wallis followed by Mann-Whitney Test. $P < 0.05$ was considered as statistically significant. The results indicated that vanillin significantly reduced the time spent in closed arm, increased the entries into open arm both in chronic and acute model of elevated plus maze ($P < 0.05$) in all three doses (10, 100, 200 mg/kg) used. The authors concluded that the study demonstrates the anxiolytic activity of vanillin in wistar albino rats [Bhagwat *et al*. 2013].

Using an "odor-induced crawling" testing procedure, it was observed that neonates exposed prenatally to vanilla or alcohol crawl for a longer distance towards the experienced odor than to other odors or than control pups. Blocking mu, but not kappa opioid receptors, reduced the attraction of vanilla odor to neonates exposed to vanilla in utero, while the response to alcohol in pups exposed prenatally to this drug was affected by both antagonists. Results confirm that exposure to a non-alcohol odor enhances postnatal responses to it, observable soon after birth, while also suggesting that the mu opioid receptor system plays an important role in generating this effect. The results also imply that with alcohol exposure, the prenatal opioid system is wholly involved, which could explain the longer retention of the enhanced attraction to alcohol following prenatal experience with the drug [Gaztanaga, Fernandez & Chotro, 2015].

In Vitro Toxicity Status

Carcinogenicity and Mutagenicity

Vanillin was not mutagenic in the Ames test, in both the presence and absence of a metabolic activation system, using five strains (TA98, TA100, TA1535/7/8) of *Salmonella typhimurium* at up to 10,000 ug / plate (Heck *et al.,* 1989; Ishidate *et al.,* 1984). It did not induce unscheduled DNA synthesis in rat hepatocytes (at up to 500 ug / ml of perfusion agent) (Heck *et al.,* 1989). Similarly, vanillin was not mutagenic in either a mouse lymphoma assay (at up to 1500 ug / ml tester medium) (Heck *et al.,* 1989), or in Chinese hamster cells (at up to 1 mg / ml) (Ishidate *et al.,* 1984). It is reported that vanillin induced chromosomal damage in human white blood cells (test dose and procedure unspecified) [BIBRA, 1990].

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 conclude that the *in vitro* mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients which included vanillin at levels up to 829 ppm (a multiple of its typical use in a US cigarette) [Roemer *et al.,* 2002].

So far, 7 different studies looking at the effect of vanillin (highest dose tested 10,000 mg/plate) in the Ames test in various bacterial strains (TA1535, TA1537, TA1538, TA98, TA100, TA97, TA94, TA2637) have been reported in the literature. All studies were reported as negative [JECFA, 2001].

There have been 5 studies looking at the effect of vanillin (highest dose tested 1000 mg/ml) on chromosomal aberrations in a number of mammalian cell types (Chinese hamster fibroblasts, Chinese hamster B241 cells, Chinese hamster V79 lung cells, Human lymphocytes) have been reported in the literature. All 5 studies were reported as negative [JECFA, 2002].

In addition, 3 studies looking at the effect of vanillin (highest dose tested 300 mg/ml) on sister chromatid exchange in a number of mammalian cell types (Human lymphocytes, Chinese hamster ovary K1 cells) have been reported in the literature. Two out of the three studies were reported as positive; 150-300mg/ml vanillin in Human lymphocytes) [JECFA, 2002].

One study reporting the effect of vanillin on micronucleus formation in Human hepatoma (Hep-G2) cells found that 50mg/ml vanillin was negative but 500mg/ml was positive [JECFA, 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 vanillin at 2550 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].

Vanillin has recently been found to be an inhibitor of non-homologous DNA end-joining (NHEJ) which is a major repair pathway for double strand break in human cells. Direct inhibition of DNA-PK, a crucial NHEJ component in a selective manner was observed in the presence of vanillin (no further information provided). Sub-chronic concentrations of vanillin increased toxicity to cisplatin. The author concluded that inhibition of NHEJ is consistent with antimutagenic and other biological properties of vanillin, which may alter the balance between DSB repair by NHEJ and homologous repair [Durant & Karran, 2003].

Long-term (1-3 weeks) treatment of HCT116 cells (human colon cancer cells deficient in mismatch repair) with vanillin at minimally toxic concentrations

(0.5-2.5 mM) reduced the spontaneous HPRT mutant fraction (mutants/ 10(6) survivors) by 19-73%. Short-term (4 h) vanillin treatments reduced the spontaneous mutation fraction by 64%. The cells treated with vanillin demonstrated expression changes in 64 genes (Affymetrix microarrays) involved in DNA damage, stress responses, oxidative damage, apoptosis, and cell growth. Authors claim this is the first report of antimutagenic activity of vanillin against spontaneous mutations in human cells. It is suggested that vanillin induces DNA damage that in turn elicits recombinational DNA repair which reduces the spontaneous mutations, [King *et al*, 2007].

Sasaki *et al*., (1987) report on an evaluation of the effects of (what they describe as) antimutagenic flavourings (including vanillin) on the initiation of Sister Chromatid Exchanges (SCEs) induced by known chemical mutagens in cultured Chinese hamster ovary cells. The authors state that vanillin, at up to 100 M per plate, did not induce SCE by itself, but that vanillin in the presence of cells pre-treated with an alkylating agent (mutagen), such as mitomycin C, did induce SCEs and that this effect was dose-dependent (Sasaki *et al*., 1987). However, Jansson *et al*., (1988) report on phenolic and related constituents derived from cigarette smoke as 'potent inducer(s) of SCE'. They studied (induction of SCE) a number of weakly acidic semi-volatile substances derived from cigarette smoke, which are structurally related to benzaldehyde that are commonly encountered in food, beverages, and cosmetics (including vanillin). Using concentrations up to 1 mM the authors report that vanillin possesses SCE-inducing properties [Jansson *et al*., 1988].

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 *Vanillin* at levels up to 4855 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 Vanillin at

levels up to 261 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other relevant studies

The mechanism of vanilloid-induced apoptosis was discussed in a review. It was reported that vanilloids interaction with membranes/proteins causes pleiotrophic effects including cytotoxicity. Vanilloids also bind to cation channels on nociceptive sensory neurons to regulate Ca^{2+} uptake, consequently promoting neurotoxicity causing apoptosis and necrosis. The effects of vanilloids on the plasma membrane and mitochondria, i.e. targeting enzymatic reactions through functioning as a coenzyme Q antagonist caused the production of reactive oxygen species and consequently the disruption of redox homeostasis resulting in apoptosis was also reported [Hail, 2003].

Vanillin has been recently been shown to act as a peroxynitrile (PON) scavenger. PON, a reactive nitrogen species is implicated in several neurodegenerative diseases including Alzheimer's & Parkinson's disease as well as pathological conditions such as inflammation and septic shock. To assess nitration of tyrosine by PON, high performance liquid chromatography (HPLC) was conducted. Inhibition of nitration of tyrosine by PON and oxidation of dihydrorhodamine 123 to fluorescent rhodamine 123 was also inhibited in the presence of vanillin [Kumar *et al.,* 2004].

Ho *et al.* (2009) investigated the cytolytic and cytostatic properties of vanillin against HT-29, a human colorectal cancer cell line. Methods used included cell viability assay, acridine orange (AO)-ethidium bromide (EB) double staining cell morphological analysis, Cell cycle analysis, annexin V-propidium iodide apoptosis test and 5-bromo-2-deoxyuridine (BrdU)-labeling cell proliferation assay. Results showed that apoptosis was induced by vanillin and the IC_{50} for HT-29 and NIH/3T3 normal cell lines were 400 microg/ml and 1000 microg/ml, respectively. Different concentrations of vanillin arrest cell cycle at different checkpoints. 5-Bromo-2-deoxyuridine-labeling cell proliferation assay showed that G0/G1 arrest was achieved at lower concentration of vanillin (200 microg/ml) while cell cycle analysis by flow cytometer showed that G2/M arrest occurs at higher concentration of vanillin (1000 microg/ml). The authors concluded that the cytolytic and cytostatic effects shown by vanillin showed that it could be a useful colorectal cancer preventive agent [Ho *et al.* 2009].

Previous studies reported that vanillin is a good antimutagen and anticarcinogen. However, there are some contradictory findings showing that vanillin may be a comutagen and cocarcinogen. Ho *et al*. investigated whether vanillin is an anticarcinogen or a cocarcinogen in rats induced with azoxymethane (AOM). Rats induced with AOM will develop aberrant crypt foci (ACF). AOM-challenged rats were treated with vanillin orally and intraperitoneally at low and high concentrations and ACF density, multiplicity, and distribution were observed. The gene expression of 14 colorectal cancer-related genes was also studied. Results showed that vanillin consumed orally had no effect on ACF. However, high concentrations (300 mg/kg body weight)

of vanillin administered through intraperitoneal injection could increase ACF density and ACF multiplicity. The expression of colorectal cancer biomarkers, protooncogenes, recombinational repair, mismatch repair, and cell cycle arrest, and tumor suppressor gene expression were also affected by vanillin. Vanillin was not cocarcinogenic when consumed orally. However, it was cocarcinogenic when being administered intraperitoneally at high concentration.

PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of vanillin 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

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