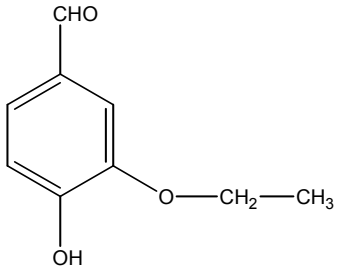
ETHYL VANILLIN

SYNONYMS

3-Ethoxy-4-hydroxybenzaldehyde;
Benzaldehyde, 3-ethoxy-4-hydroxy-
3-Ethoxyprotocatechualdehyde
Ethavan

CHEMICAL STRUCTURE



CHEMICAL FORMULA

$C_9H_{10}O_3$

IDENTIFIER DETAILS

CAS Number	:	121-32-4
CoE Number	:	108
FEMA	:	2464
EINECS Number	:	204-464-7
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	-	-

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/.

SPECIFICATIONS

Melting Point: 77-78°C

Boiling point: 285°C

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
0-3	JECFA	2002	No safety concern at current intake levels as a flavouring agent

FDA Status: [CFR 21]

Section Number	Comments
182.60	Synthetic flavoring substances and adjuvants

HUMAN EXPOSURE

Natural Occurrence: Ethyl vanillin is not reportedly found in nature; it can be distinguished from vanillin because of the yellow colour developed in the presence of concentrated H_2SO_4 [Fenaroli, 2005].

Reported Uses: Ethyl vanillin is reported used in baked goods at 92.97 ppm, breakfast cereals at 330.0 ppm, fats and oils at 0.15 ppm, milk products at 1403.0 ppm, frozen dairy at 26.61 ppm, meat products at 3.90 ppm, condiment relish at 13.0 ppm, soft candy at 89.64 ppm, confection frosting at 270.4 ppm, sweet sauce at 172.5 ppm, gelatin pudding at 39.93 ppm, non-alcoholic beverages at 29.72 ppm, alcoholic beverages at 10.04 ppm, hard candy at 30.26 ppm, and chewing gum at 37.46 ppm [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 conclude that the addition of the combined ingredients, including ethyl vanillin at levels up to 166 ppm, "did not increase the overall toxicity of cigarette smoke" [Carmines, 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 ethyl vanillin at 6.5 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 [Renne *et al.,* 2006].

In Vivo Toxicity Status

Species	Test Type	Route	Reported Dosage
Mouse	LD_{50}	I.P.	750
Rat	LD_{50}	Oral	1590
Rat	LD_{50}	Oral	>2000
Rat	LD_{50}	S.C	1800
Guinea-pig	LD_{50}	I.P	1140
Dog	LD_{50}	I.V	760

[JECFA, 1995]

The minimum lethal dose in rats has been reported to be 1800 mg/kg bw [JECFA, 1995].

The following is a summary of short-term toxicity studies carried out using ethyl vanillin at various dose levels:

- 300 mg/kg bw, fed to rats, twice a wk, 14 wks - no adverse effects.

- 20 mg/kg bw, fed to rats each day for 18 wks - no adverse effects.
- 64 mg/kg bw, fed to rats each day, for 10 wks - reduced growth rate, caused myocardial, renal, hepatic, lung, spleen and stomach injuries [the exact nature of the injuries was not specified].
- 30 mg, fed to rats weekly for 7 weeks - no adverse effects.
- 0, 2, or 5%, fed to rats for one year - no adverse effects.
- 0, 500, 1000 or 2000 mg/kg bw, fed to rats each day, 13 wks. A complete histological examination was performed on all rats in the control and top-dose groups and only on the rats in the low and intermediate dosage groups where treatment-related effects were suspected. No clinical signs or treatment-related deaths of toxicological significance were observed in treated animals during the study. At autopsy enlarged cervical lymph nodes were seen in the mammals that received 1000 mg/kg/day, with both sexes being affected at 2000 mg/kg/day. Both sexes at 2000 mg/kg/day had reduced body adipose. At both the intermediate and high dose groups their was an increase in the relative liver weights and an increased incidence of hepatic peribiliary changes, minor bile duct hyperplasia was seen in a few males at both of these doses. The authors concluded that no-treatment related changes were observed at 500 mg/kg bw/day which was considered to be the NOEL in this study. [It is worth noting that this study was designed in accordance with toxicological principles for the safety assessment of food additives established by the US FDA].
- 15, 32, 41 or 49 mg/kg in 10% aqueous glycerine, fed daily to single rabbits, for 13, 15, 26 and 43 days. Highest dose level, anaemia, diarrhoea and a lack of weight gain were observed. No toxicity signs were reported at any of the lower doses.
- 148-154 mg/kg bw was subcutaneously injected into rabbits each day for 6 days. No adverse effects were observed

[JECFA, 2002]

75 mg/kg bw was established as the maximum tolerated dose in strain A mice when administered i.p. 3 times/week for 8 weeks [Stoner *et al.,* 1973].

Ethyl vanillin was administered to NMRI mice [1000mg/kg bw] and male BDF1 mice [dose not reported] in two separate studies and micronucleus formation was investigated. In both studies ethyl vanillin did not induce micronuclei formation *in vivo* [JECFA, 2002].

Ethyl vanillin was amongst a group of known polyploidy inducers that was injected intraperitoneally (in the concentration range 23.2-28.5g) to BDF1 mice. 6, 24 and 48h. After treatment, formation of micronucleated polychromatic erythrocytes (MNPCEs) and tubulin polymerization was examined. Ethyl vanillin did not increase the frequency of MNPCEs and did not specifically inhibit tubulin polymerization to microtubules. The authors conclude that *in vivo* induced polyploidy cannot be mediated through the microtubular system [Furukawa *et al.,* 1989]. [It should be noted that this is only a published abstract and not a full paper. Furthermore in the last sentence the author refers to '*in vitro*'; it is assumed that the author means '*in vivo*'].

Ethyl vanillin was evaluated by JECFA at their 44th meeting in 1995 and an ADI was set at 0-3 mg/kg. This was based upon information showing daily intakes in the range of 0.06-7 mg/person/day, a NOEL of 500 mg/kg bw/day and a safety factor of 200 [JECFA, 1995]. More recently, it was again evaluated by JECFA at their 57th meeting in 2001 and the ADI was retained at 0-3 mg/kg bw [JECFA 2002].The estimated per capita intake was 2.2175 mg/kg/day [Fenaroli, 2005].

Carcinogenicity and Mutagenicity

Doses of 0, 0.5, 1 or 2 % and 2% or 5% (500, 1000 and 2000 mg/kg respectively) dissolved in propylene glycol was fed to rats for 2 years. 20 control rats were fed 3% propylene glycol in the diet as the control. No adverse effects on growth, haematology, organ weights or histology of major tissues were reported. The NOELl for ethyl vanillin was 1000 mg/kg/day [Hagan *et al.,* 1967].

Dermal Toxicity

Ethyl vanillin when tested at 2% in petrolatum in a 48hr-closed patch test in 25 human volunteers elicited only a mild irritation effect. Furthermore when tested at the same concentration in a maximisation test in human volunteers, no sensitisation reactions were produced [Opdyke, 1975].

Similarly, when it was tested at 5% in petrolatum in a 48h closed patch test in 100 human volunteers it was not found to be an irritant [Frosch, *et al.,* 1995].

A much more recent study based upon detailed consideration of mechanistic chemistry grouped 17 aldehydes including ethyl vanillin, into groups classified according to reactivity of the carbonyl group and the presence of alternative functionality within the compound. Ethyl vanillin was determined to contain a deactivated aldehyde group and thus was classified as non-sensitizing [Patlewicz *et al.,* 2001].

Ethyl vanillin was among a group of aldehydes assayed for skin sensitisation potential in the murine local lymph node assay (LLNA). This assay has been endorsed by both the Interagency Co-ordinating Committee on the Validation of Alternative Methods (ICCVAM) in the USA and by the European Centre for the validation of Alternative Methods (ECVAM) in Europe. Mice were exposed to various concentrations of ethyl vanillin [exact doses used not specified] daily for 3 consecutive days. Five days after the initial exposure, tritiated methyl thymidine was injected intravenously via the tail and 5 hours later the mice were sacrificed and the lymph nodes excised and pooled into a cell suspension and the incorporation of tritiated methyl thymidine was measured. Based on the results of this assay ethyl vanillin was considered to be a non-sensitiser [Basketter *et al.,* 2001].

A mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination,

including ethyl vanillin at 848 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)] [Gaworski *et al.,* 1999].

Reproductive and Developmental Toxicity

Four groups of 10 virgin Crl CD rats were given veratraldehyde, vanillin, ethyl vanillin [dose administered: 200, 1000 or 2000mg/kg bw per day] 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].

Four groups of 10 rats were orally administered ethyl vanillin at 0,200, 1000 and 2000 mg/kg/day 7 days prior to cohabitation, through cohabitation (max 7 days), gestation, delivery and 4 days postpartuition. Those rats that did not deliver a litter were necropsied on Day 25, with delivered pups being sacrificed on day 4 postpartum. The NOAEL for maternal toxicity was <200 mg/kg/day and offspring effects was 2000 mg/kg/day [Vollmuth *et al.,* 1990].

Inhalation Toxicity

When tested at 848 ppm in cigarettes, in a 13-week inhalation study, the presence of ethyl 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)] [Gaworski *et al.,* 1998].

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 ethyl vanillin at 166 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 ethyl vanillin at 2920 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 ethyl 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 total of 31 ingredients were tested in 90-day nose-only rat inhalation studies using mainstream cigarette smoke. Studies were designed following conventional toxicity testing methods employed for food additives and other consumer products. The authors concluded that these added ingredients, which included Ethyl vanillin at levels up to 8,060 ppm 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 Ethyl Vanillin at levels up to 84 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Behavioural data

No data identified

Other Relevant Studies

The authors aimed to assess novel pharmacological properties of ethyl vanillin (EVA). EVA exhibited an inhibitory activity in the chorioallantoic membrane angiogenesis. Anti-inflammatory activity of EVA was convinced using the two in vivo models, such as vascular permeability and air pouch models in mice. Antinociceptive activity of EVA was assessed using acetic acid-induced writhing model in mice. EVA suppressed production of nitric oxide and induction of inducible nitric oxide synthase in the lipopolysaccharide (LPS)-activated RAW264.7 macrophage cells. However, EVA could not suppress induction of cyclooxygenase-2 in the LPS-activated macrophages. EVA diminished reactive oxygen species level in the LPS-activated macrophages. EVA also suppressed enhanced matrix metalloproteinase-9 gelatinolytic activity in the LPSactivated RAW264.7 macrophage cells. EVA at the used concentrations couldn't diminish viability of the macrophage cells. Taken together, the anti-angiogenic, anti-inflammatory and anti-nociceptive properties of EVA are based on its suppressive effect on the production of nitric oxide possibly via decreasing the reactive oxygen species level [Jung *et al*., 2010].

Tai *et al*., systematically evaluated the antioxidant activity of ethyl vanillin, a vanillin analog, as compared with the activities of vanillin and other vanillin analogs using multiple assay systems. Ethyl vanillin and vanillin exerted stronger antioxidant effects than did vanillyl alcohol or vanillic acid in the oxygen radical absorbance capacity (ORAC) assay, although the antioxidant activities of vanillyl alcohol and vanillic acid were clearly superior to those of ethyl vanillin and vanillin in the three model radical assays. The antioxidant activity of ethyl vanillin was much stronger than that of vanillin in the oxidative hemolysis inhibition assay, but was the same as that of vanillin in the ORAC assay. Oral administration of ethyl vanillin to mice increased the concentration of ethyl vanillic acid, and effectively raised antioxidant activity in the plasma as compared to the effect of vanillin [Tai et al., 2011].

The impact of ethyl vanillin was evaluated on the activities of CYP2C9, CYP2E1, CYP3A4, CYP2B6 and CYP1A2 in human liver microsomes (HLM) in vitro, and impact on the activities of CYP1A2, CYP2C, CYP3A and CYP2E1 in rat liver microsomes (RLM) in vivo. The in vitro results demonstrated that ethyl vanillin had no significant effect on the activity of five human CYP450 enzymes with concentration ranged from 8 to 128μM. However, after rats were orally administered ethyl vanillin once a day for seven consecutive days, CYP2E1 activity was increased and CYP1A2 activity was decreased in RLM. The in vivo results revealed that drug interaction between ethyl vanillin and the CYP2E1/CYP1A2-metabolizing drugs might be possible, and also suggested that the application of the above additives in foods and drugs should not be unlimited so as to avoid the adverse interaction [Chen *et al*., 2012].

In Vitro Toxicity Status

Carcinogenicity and Mutagenicity

The following is a summary of genotoxicity assays conducted on ethyl vanillin:

0-10 mg/plate was tested in the Ames test using *S. typhimurium* strains TA92, 94, 98, 100, 1535 and 1537 (+ / - S9 metabolic activation). The result was negative [Ishidate *et al.,* 1984].

0-10 mg/plate was tested in the Ames test using *S. typhimurium* strains TA98, 100, 1535 and 1537 (+ / - S9). The result was negative [JECFA, 1995].

0-3.6 mg/plate was tested in the Ames test using *S.typhimurium* strains TA98, 100, 1535 and 1537 and 1538 (+ / - S9). The result was negative [Wild *et al.,* 1983].

0-10 mg/plate was tested in the Ames test using *S. typhimurium* strains TA98, 100, 1535 and 1537 and 1538 (+ / - S9). The result was negative [Heck *et al.,*1989].

0-0.25 mg/ml was tested *in vitro* for chromosomal aberrations using Chinese hamster ovary cells. The result was negative. However, ethyl vanillin did induce a significant increase in polyploidy cells. The significance of this finding is not discussed [Ishidate *et al.,* 1984].

2 x 0-1000 mg/kg bw was administered i.p. to NMRI mice. The mice were then killed and bonemarrow smears were prepared 30h after treatment. The micronucleus test was performed on these smears. The result was negative. [Wild *et al.,* 1983].

0-2 M was tested in human lymphocytes *in vitro* for sister chromatid exchange (SCE). The result was positive. The chemical name for ethyl vanillin is 3-ethoxy-4-hydroxybenzaldehyde. In this study 14 benzaldehydes were tested and all but one was found to induce SCE [Jansson *et al.,* 1988].

0-100 μM was tested in Chinese hamster ovary cells *in vitro* for SCE. The result was negative. However it should be noted that when ethyl vanillin [at doses 10-100 μM] was applied to cells following treatment with Mitomycin C [a known mutagen], an obvious dose-dependent increase in the frequencies of SCEs was observed [Sasaki *et al.,* 1987].

199 μg/ml was tested *in vitro* in the rat hepatocyte unscheduled DNA synthesis (UDS) assay. The result was negative [Heck *et al.,* 1989].

125-500 μg/ml was tested in the L5178Y mouse lymphoma cell TK+/- mutagenesis (MLY) assay (+/- S9). The result was a weak or borderline positive. The authors state in their conclusions, that the large number of positive results seen in the MLY assay should be viewed with caution. They speculate that since each of the positive materials were acidic in nature, the pH may have effected the osmolarity of the assay resulting in an some false positive results [Heck *et al.,* 1989].

50 mM was tested for heritable mutations in *Drosophila melanogaster*. The result was negative [Wild *et al.,* 1983].

500 μg/ml showed marked antimutagenic activity against mutagenicity induced by 4-nitroquinoline 1-oxide, furylfuramide, captan or methylglyoxal in *Escherichia coli* WP2 but was ineffective against mutations induced by Trp-P-2 in *S. typhimurium* strain TA98. The authors propose that the antimutagenic activity may be due to enhancement of an error-free recombinant repair system [Ohta *et al.,* 1986].

Ethyl vanillin at concentrations up to 5 μg/disk was found to be negative in the rec assay in *B. subtilis* [The majority of the paper is in Japanese and these results are derived from a table; Oda *et al.,* 1978].

Ethyl vanillin was assayed for estrogenic activity in an *in vitro* yeast bioassay [concentration tested not clear] and was found not to elicit estrogenic activity in this assay [Miller *et al.,* 2001].

Ethyl vanillin was among 90 chemicals screened for potential chemopreventive action in a variety or rodent cells and human cells using a number of biochemical markers of carcinogenesis. It was found to be an effective inhibitor of tyrosine kinase activity and DNA-binding ability of a known carcinogen [Sharma *et al.,* 1994].

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 ethyl vanillin at levels up to 194 ppm.

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, 100, 102, 1535 and 1537 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 ethyl vanillin at levels up to 166ppm [a multiple of its typical use in a US cigarette; Roemer *et al.,* 2002].

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 ethyl vanillin at 2920 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].

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 added ingredients, which included Ethyl vanillin at levels up to 4.490 ppm 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 Ethyl Vanillin at levels up to 84 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other Relevant Studies

Metabolism studies indicate that ethyl vanillin was rapidly absorbed, metabolized and excreted in the rat. Within 24 hours radioactive doses of ethyl vanillin administered to rats had been excreted in the urine. Peak plasma radioactivity occurred within 2h after dosing at all levels (50, 100 or 200 mg/kg bw; single oral doses). The principal metabolite at all dose levels was ethyl vanillic acid [JECFA, 2002].

In humans ethyl vanillic acid has also been shown to be the major metabolite. Patients known to have consumed foodstuffs flavoured with ethyl vanillin or with vanilla excreted ethyl vanillic acid in their urine. Unchanged ethyl vanillin was not detected [JECFA, 1995]. An adult received a100 mg of vanillin (a related substance), dissolved in water. The concentration of vanillic acid in the urine increased from the back ground level of 0.3 mg per 24 hours of urine collection to 96 mg per 24 hours (accounting for 94% of the dose) [JECFA 2002].

35 commonly used flavouring ingredients including ethyl vanillin were evaluated for humoral and cell-mediated immune responses. Ethyl vanillin up to a maximum dose of 2500 mg/kg/day was administered intragastrically on a daily basis for 5 days to female CD1 mice and was found not to modulate the cell-mediated or humoral immune responses [Gaworski *et al.,* 1994].

PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of ethyl 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.

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