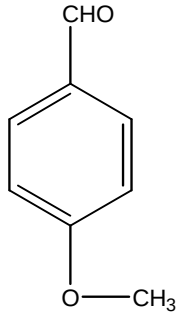
PARA-METHOXYBENZALDEHYDE

SYNONYMS

Aldehyde anisic
Anisaldehyde
Anisic aldehyde
Aubepine
Para-anisaldehyde
4-methoxybenzaldehyde

CHEMICAL STRUCTURE



CHEMICAL FORMULA

$C_8H_80_2$

IDENTIFIER DETAILS

CAS Number	:	123-11-5
CoE Number	:	103
FEMA	:	2670
EINECS Number	:	204-602-6
E Number	:	-

CLP CLASSIFICATION

Ingredient CLP Classification: No

Endpoint	Classification	Category
Acute Oral Toxicity	conclusive but not sufficient for classification	-
Acute Dermal Toxicity	conclusive but not sufficient for classification	-
Acute Inhalation Toxicity	conclusive but not sufficient for classification	-
Skin Corrosive/irritant	conclusive but not sufficient for classification	-
Eye Damage/Irritation	conclusive but not sufficient for classification	-
Respiratory Sensitisation	conclusive but not sufficient for classification	-
Skin Sensitisation	conclusive but not sufficient for classification	-
Mutagenicity/Genotoxicity	conclusive but not sufficient for classification	-
Carcinogenicity	conclusive but not sufficient for classification	-
Reproductive Toxicity	conclusive but not sufficient for classification	-
Specific Target Organ Toxicity	conclusive but not sufficient for classification	-
Aspiration Toxicity	conclusive but not sufficient for classification	-

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: 2.5°C

Boiling point: 247°C

Smiles Code: c1(ccc(cc1)OC)C=O

PURPOSE

Flavouring substance

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (ppm)	Food (ppm)	Exceptions (ppm)

5	50	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
Acceptable	JECFA	2001	No safety concerns at current levels of intake when used as a flavouring agent.

FDA Status:

Section Number	Comments
172.515	Synthetic flavouring substances and adjuvants permitted for direct addition to food for human consumption.

HUMAN EXPOSURE

Natural Occurrence: *Para*-Methoxybenzaldehyde is reported found in essential oils and extracts: vanilla, *Acacia farmesuiana, Willd Magnolia salicifolia* Maxim., *Erica arborea*, *Pirus comunis*, *Boswellia serrata*, and others; also in anise, fennel, star anise [especially when aged due to oxidation of anethole] [Fenaroli, 2005]. It is also reportedly found in cranberries at 0.1 mg/kg [CoE 2000].

Reported Uses: Para-methoxybenzaldehyde is used in baked goods at 88.01 ppm, frozen dairy at 45.09 ppm, soft candy at 66.95 ppm, confection frosting at 1.0 ppm, gelatin pudding at 47.94 ppm, non-alcoholic beverages at 23.14 ppm, alcoholic beverages at 20.0 ppm, hard candy at 9.57 ppm, and chewing gum at 30.54 ppm. Individual consumption has been reported to be 0.004703 mg/kg/day [Fenaroli, 2005].

Sources other than foods: Metabolic product of odoriferous fungus [*Lentinus lepidus*]; of wood-rotting fungus *Polyporus benzoinus*; of *Daldalea juniperina* as cited in The Merck index (1976).

TOXICITY DATA

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/.

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 p-methoxybenzaldehyde at levels up to 84 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 methoxybenzaldehyde, para at 0.65 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 1 h/day, 5 days/wk for 13 weeks to smoke at concentrations of 0.06, 0.2 or 0.8 mg/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.

In Vivo Toxicity Status

Test Type	Species	Route	Reported Dosage
LD_{50}	rat	oral	1510 mg/kg/ day
LD_{50}	guinea pig	oral	1260 mg/kg/day [Jenner *et al.,* 1964]
LD_{50}	mouse	oral	1859 mg/kg/day
LD_{50}	rabbit	skin	>5000 mg/kg
Moderate	rabbit	skin	500mg/24 hr [RTECS 19/08/02]

Following chronic administration of anisaldehyde at 10,000 ppm to groups of 5 male and 5 female rats for 15 weeks, no treatment-related effects were reported [Hagan *et al.,* 1967]. Administration of anisaldehyde at 1000 ppm to groups of 5 male and 5 female rats for 28 weeks, there was also reported to be no treatment-related effects [Hagan *et al.,* 1967].

Carcinogenicity and mutagenicity

In a mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including p-methoxybenzaldehyde 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” [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].

Dermal toxicity

Anisic aldehyde applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was moderately irritating [Opdyke 1979]. When tested on humans at a 10% concentration in petrolatum, there was no irritation after a 48 hr closed-patch test on human subjects [Opdyke 1979].

Reproductive and Developmental toxicity

A combined repeat dose and reproductive/developmental toxicity screening test was performed via oral gavage of 4-methoxybenzaldehyde at doses of 0, 20, 100, 500 mg/kg to groups of 13 male and female Crj/CD rats. Males were dosed for 42 days and females from 14 days prior to mating until day 4 of lactation. No animals died in any group. Temporary salivation after dosing was observed, increased in bodyweight gain associated with increased food consumption. There was a decrease in platelet count, increase in the A/G ratio, GOT activity and inorganic phosphorus concentration, increase in liver weight and decrease in epididymidal weights (males) were observed in males and females dosed at 100 and/or 500 mg/kg groups. In the 100 and/or 500 mg/kg groups, hyperplasia of squamous epithelium in the forestomach and centrilobular hypertrophys of hepatocyte were detected [Hatano Research Institute 2008].

The fertility index and the number of pups were decreased in the 500 mg/kg group. From the results, the no observed effect dose level (NOEL) for systemic toxicity of 4-methoxybenzaldehyde is considered to be 20 mg/kg/day in males and females, and NOELs for reproductive and developmental toxicity are considered to be 100 mg/kg/day in males and females, and 100 mg/kg/day in pups [Hatano Research Institute 2008].

Inhalation toxicity

The addition of methoxybenzaldehyde para at 234 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 p-methoxybenzaldehyde 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].

Inhalation of vaporised anisaldehyde 3 - 9 mg/kg body weight was added to a vaporiser, and administered to anaesthetised rabbits. The administration lead to an increased the volume output and mucus content of respiratory tract fluid. The authors suggested that steam inhalation of small amount of anisaldehyde

or other volatile oils could produced an expectorant action of some value in the treatment of cough caused by desiccation of the mucosa of the respiratory tract [Boyd *et al.,* 1970].

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 [Vanscheeuwijck *et al.,* 2002]. These ingredients included p-methoxybenzaldehyde at 84 ppm, a level described as a multiple of its typical use in a US cigarette. The data from this study along with that from a number of other biological and chemical studies indicate that the addition of the combined ingredients "did not increase the inhalation toxicity of the smoke, even at the exaggerated levels used" [Vanscheeuwijck *et al.,* 2002].

When tested at <0.1 ppm in cigarettes, in a 13-week inhalation study, the presence of p-methoxybenzaldehyde "…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].

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 Methoxybenzaldehyde at levels up to 24 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other relevant studies

Anisic aldehyde undergoes a very slight degree of demethylation with oxidation of its aldehyde group to an acid group and the major metabolite identified being anisic acid.

The interaction of the aromatic aldehyde, p-anisaldehyde, with rat liver mitochondria was examined *in vitro*. Both pyruvate/malate- and succinate-mediated respiration was inhibited by the aromatic aldehyde [0.5 - 1.0 mM concentrations]. Cytochrome c oxidase was reportedly not inhibited. Several sites of inhibition, possibly both at the site of transport of substrates and the active sites of enzymes were reported to possibly exist [Wolf *et al.,* 1982].

Anisaldehyde has been reported to be unstable in tooth paste above pH 7, with a very high rate of degradation in the presence of sulphated castor oil [Guven *et al.,* 1984].

Behavioural data

No data identified.

***In Vitro* Toxicity Status**

Mutagenicity and carcinogenicity

Roemer *et al.,* (2002) reported on a study in which cigarettes containing various additives in three different combinations were produced. Smoke condensates prepared from these cigarettes were then tested in two different *in vitro* assays. The mutagenicity of the smoke condensate was assayed in the *Salmonella* plate incorporation [Ames] assay with tester strains TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of an S9 metabolic activation system. The cytotoxicity of the gas/vapour phase and the particulate phase was determined in the neutral red uptake assay with mouse embryo BALB/c 3T3 cells. The authors concluded that the *in vitro* mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients, which included p-methoxybenzaldehyde at levels up to 84ppm [a multiple of its typical use in a US cigarette] [Roemer *et al.,* 2000].

p-Anisaldehyde has been reported to be negative in the Ames test with the following *Salmonella typhimurium* strains TA97 and TA102 at concentrations between 0.01 - 1 mg/plate [Fujita *et al.,* 1987], and with strainTA102 at 0 - 5000 μg/plate [Mueller *et al.,* 1993], in all cases both with and without metabolic activation [Fujita *et al.,* 1987; Mueller *et al.,* 1993].

p-Anisaldehyde has been reported to be positive in the mouse lymphoma assay with L5178Y [TK+/-] both without metabolic activation at concentrations between 1.01-5.08 mmol/l^{-1}, and with metabolic activation at concentrations between 0.2-0.4 μg/ml [Wangenheim 1988; CCRIS, 2002].

In the chromosomal aberration assay p-anisaldehyde was negative with Chinese hamster lung (CHL/IU) cells, with no increases in chromosomal aberrations being seen in with or without metabolic activation in either short term (doses up to 1362 μg/ml) or continuous treatment (doses up to 681 μg/ml) [Bio safety Research Center Japan, 2008].

Anisaldehyde was reported to decrease the number of x-ray induced chromosome aberrations when administered at 250, 313 or 500 mg/kg after irradiation. The presence of micronuclei was reported to be decreased by 55 - 60% compared to the controls without toxicity to the bone marrow [Sasaki *et al.,* 1990].

Anisaldehyde has been reported to cause DNA strand breaks in PM2 strain in DNA in the presence of copper (II) chloride ($CuCl_2$). Neither compound caused DNA breaks on its own. The maximum number of strand breaks was found to be dependent upon the concentration of $CuCl_2$. The endogenous antioxidants catalase and neocuproine were found to nearly totally inhibit the formation of DNA strand breaks [Becker *et al.,* 1996].

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 methoxybenzaldehyde para at 234 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].

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 Methoxybenzaldehyde at levels up to 24 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other relevant studies

p-Anisaldehyde has been reported to have potent nematicidal activity against *C. elegans* being effective at 5 - 10 ppm [Stadler *et al.,* 1994].

p-Methoxybenzaldehyde was reported to cause no damage to diploid human lung fibroblasts incubated with p-methoxybenzaldehyde at a concentration of 25mM for thirty minutes [Thelestam *et al.,* 1980]. p-Methoxybenzaldehyde was also reported to have no toxicity against the beating of ciliary in embryo chick trachea when tested at 5mM [Pettersson *et al.,* 1982].

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 *para-methoxybenzaldehyde* at levels up to 127 ppm [In vitro toxicity testing of tobacco ingredients in burnt form (Internal document R-053)].

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 p-methoxybenzakdehyde 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].

PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of methoxybenzaldehyde, para 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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