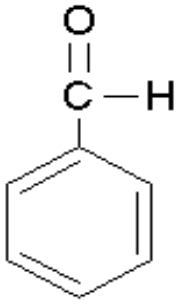
BENZALDEHYDE

SYNONYMS

Benzenecarbonal
Benzene carboxyaldehyde
Benzenemethylal
Benzoic aldehyde
Bitter almond oil
Benzenecarbonal
Phenylmethanal

CHEMICAL STRUCTURE



CHEMICAL FORMULA

C_7H_6O

IDENTIFIER DETAILS

CAS Number	:	100-52-7
CoE Number	:	101
FEMA	:	2127
EINECS Number	:	202-860-4
E Number	:	-

CLP CLASSIFICATION

Ingredient CLP Classification: Yes

Endpoint	Classification	Category
Acute Oral Toxicity	Acute Tox. 4 H302: Harmful if swallowed	4
Acute Dermal Toxicity	conclusive but not sufficient for classification	-
Acute Inhalation Toxicity	Acute Tox. 4 H332: Harmful if inhaled	4
Skin Corrosive/irritant	Skin Irrit. 2 H315: Causes skin irritation	2
Eye Damage/Irritation	Eye Irrit. 2 H319: Causes serious eye irritation.	2
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	STOT Single Exp. 3 H335: May cause respiratory irritation. Affected organs: Lungs Route of exposure: Inhalation	3
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: -26°C
Boiling point: 179°C

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
5	JECFA	1996	Group ADI

FDA Status:[CFR21]

Section Number	Comments
182.60	Synthetic flavouring substances and adjuvant

HUMAN EXPOSURE

Natural Occurrence: Benzaldehyde is present in cyanuric glucosides [amygdalin] in bitter almond, peach, apricot kernel, and other *Prunus* species; amygdalin is also present in various parts of the following plants: *Sambucus nigra, Chrysophyllum arten, Anacyclus officinarium, Anacyclus pedunculatus, Davillia brasiliensis, Lacuma deliciosa, Lacuna multiflora,* and others; free benzaldehyde has been reported found in several essential oils: hyacinth, citronella, orris, cinnamon, sassafras, labdanum and patchouli [Fenaroli, 2005]. Benzaldehyde has also been found in melon, grapes, tea and whiskey [Leffingwell, 1998].

Reported Uses: Benzaldehyde is reportedly used in baked goods at 233.4 ppm, frozen dairy at 166.8 ppm, fruit juice at 297.7 ppm, soft candy at 171.7 ppm, gelatin pudding at 138.5 ppm, non-alcoholic beverages at 57.55 ppm, alcoholic beverages at 48.63 ppm, hard candy at 335.4 ppm, and chewing gum at 1353.0 ppm [Fenaroli, 2005]. Benzaldehyde has a number of reported uses in the food, beverage, pharmaceutical, perfume, and soap and dyestuffs industries [Lawrence, 1998].

Sources other than foods: In its free state, benzaldehyde is reported to be present in several essential oils, notably hyacinth, citronella, cinnamon and patchouli oils [Furia, 1971; Leffingwell, 1998].

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 benzaldehyde at levels up to 12 ppm, “did not increase the overall toxicity of cigarette smoke” [Carmines, 2002].

***In Vivo* Toxicity Status**

Species	Test Type	Route	Reported Dosage
Rat	LD_{50}	Oral	1300-2850mg/kg
Mouse	LD_{50}	Oral	2020mg/kg
Guinea Pig	LD_{50}	Oral	1000mg/kg
Mouse	LD_{50}	Intraperitoneal	9.5mg/kg
Mouse	LD_{50}	Intraperitoneal	3265mg/kg
Mouse	LD_{50}	Intraperitoneal	1020mg/kg
Rabbit	LD_{50}	Dermal	>1250mg/kg
Mouse	LD_{50}	Intraperitoneal	1150mg/kg
Mouse	LD_{50}	Subcutaneous	5000mg/kg
Rat	LD_{50}	Oral	1300mg/kg
Guinea Pig	LD_{50}	Oral	100mg/kg

[JECFA, 1996; JECFA, 2002]

Groups of mice were given benzaldehyde by stomach tube. No signs of toxicity were reported for the group receiving the lowest dose of 300 mg/kg bw/day for 5 days a week for 13 weeks. Of the mice receiving 600 mg/kg bw/day, one out of the ten male mice showed signs of kidney damage. The male mice receiving 1200 mg/kg bw/day mostly died within the first week and all had kidney damage. One out of the ten female mice in the same dose grouping died. Detailed microscopic examination of a variety of tissues from the dose groups, including kidney, spleen, stomach & liver, failed to identify any abnormalities. All mice receiving a dose of either 1600 or 3200 mg/kg bw/day died within 2-3 days [BIBRA, 1989].

Another repeated dose study, where rats received a dose of 500 mg/kg bw/day of benzaldehyde in the diet for 14 days, noted a slight decrease in body weight and increase in liver weight [BIBRA, 1989].

A study cited by JECFA states that at doses close to the LD_{50}, tremors, sedation, respiratory distress, and weight loss were seen in male CD-1 mice. [JECFA, 1996].

Signs of toxicity associated with acute lethal oral doses are central nervous system effects in both mice [also following intraperitoneal injections] and rats, intestinal irritation and haemorrhage in guinea pigs, and a slight reduction in respiratory rate in dogs [also following intravenous exposure] (BIBRA, 1989).

Histopathological and gross examination of five male and five female rats receiving 0 or 0.1 % of benzaldehyde in their diet for 27-28 weeks or 1 % for 16 weeks, failed to reveal any signs of tissue damage [JECFA, 1996].

No overt signs of toxicity occurred when an unspecified number of healthy volunteers and ulcer patients were administered 0.2% benzaldehyde solution as part of test meal on “several consecutive” occasions. In a brief report

concerning inhalation of benzaldehyde to workers exposed to concentrations of 5 mg/ m^3 [duration of exposure unspecified] an increase in incidence of respiratory illness was noted. However, the authors concluded that they failed to show any deviation in the health status of workers directly related to benzaldehyde [BIBRA, 1989].

A 13-week repeated dose study on groups of rats, each group receiving a different oral dose of benzaldehyde 5 days/wk by gastric gavage resulted in a range of toxic effects associated with the central nervous system. A dose of 800 mg/kg bw/day produced hyperactivity, trembling and occasional inactivity. In addition, several deaths occurred. It was demonstrated that male rats had slightly reduced growth & those that survived showed a marked weight reduction of the testes & thymus. In female rats it was noted that the liver, kidneys, thymus & heart weights were increased. Damage to the brain and kidneys were also seen following comprehensive examination of a range of tissues. At the lower dose level of 400 mg/kg bw/day [as well as 800 mg/kg bw/day], mild injury to the forestomach was produced, with lesions occurring at doses of 200 mg/kg bw/day. All rats receiving a greatly increased dose of 1600 mg/kg bw/day died by day 2. These results demonstrate the dose-response nature of the test chemical [BIBRA, 1989].

A similar study carried out by the NTP involving the same dose levels, nature of exposure and exposure period, demonstrated similar results. The changes to the brain were also noted in the 400 mg/kg bw/day. These included necrosis in the cerebellum & hippocampus. Other details include hyperplasia and/or hyperkeratosis in the forestomach, and degeneration or necrosis of the liver and of the tubular epithelium in the kidney. The NTP continued an identical study for two years and concluded that there was no significant difference in bodyweights between any of the groups [rats & mice] compared to controls. The only significant changes noted were in the mouse forestomach and are discussed below [NTP, 2002].

Carcinogenicity and Mutagenicity

The NTP have conducted 2 year carcinogenicity study on benzaldehyde in both male & female rats [F344/N], as well as male & female mice [$B6C3F_1$]. The rats and male mice received a dose of 0, 200 or 300 mg/kg bw/day by gastric lavage, with 50 animals in each group. The groups of female mice received doses of 0, 300 or 600 mg/kg bw/day. It was concluded that there was no evidence of carcinogenicity in the male or female rats. There was some evidence of carcinogenicity in the groups of mice, with the identification of squamas cell papillomas in the forestomach, as well as some non-neoplastic lesions in the same area [hyperplasia] [NTP, 2002].

A recent mouse skin painting study [Gaworski *et al.*, 1999], investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including Benzaldehyde at 0.9 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].

Benzaldehyde was also non-genotoxic in *Drosphila melanogaster* dosed orally [150 ppm] and by injection [2500 ppm]. [JECFA, 1996]

Dermal Toxicity

A 24-hour covered contact test on rabbit skin using neat benzaldehyde produced 'moderate' irritation. The lowest concentration to cause redness in at least 25 % of guinea-pigs was 10 % benzaldehyde in either acetone, ethanol or diethyl phthalate when applied once directly to the skin, and 3 % when applied daily for 21 days [BIBRA, 1989].

A brief report by Dickel *et al*., (2001) failed to demonstrate any statistical significance between skin-sensitivity of white and black racial groups to benzyl alcohol [5 % in petrolatum], although they did demonstrate differences to other test chemicals [Dickel *et al.,* 2001].

Nine out of 94 patients suffering from dermatitis who had also previously been shown to be sensitised to balsam Peru were also sensitive to 5% benzaldehyde in petrolatum when exposed for 24/48 hour using a closed patch skin test. Five of the patients were also tested for an exposure time of 30 minutes [same concentration], only one developed an immediate urticarial rash [BIBRA, 1989].

In a separate study, a concentration of 4% benzaldehyde in petrolatum, applied for 48 hours [closed patch] to the skin and re-applied 5 times consecutively, followed by a challenge patch 10-14 days later [again applied for 48 hours] failed to produce any local reaction in 25 volunteers [BIBRA, 1989].

Benzaldehyde was applied to guinea pigs at the minimally irritant concentration, daily for 3 weeks. Subsequent challenges using the same concentration both immediately afterwards, and with a 2 week rest period, failed to produce any signs of sensitisation. Sensitisation has been demonstrated in guinea pigs using an intradermal route of exposure [BIBRA, 1989].

Patlewicz *et al.,* (2001) concluded that benzaldehyde was not a skin-sensitizer based on examination of *in vivo* data and detailed consideration of mechanistic chemistry involved in structure-activity relationships [Patlewicz *et al.*, 2001].

Reproductive and Developmental Toxicity

In reproductive studies and multigeneration studies using benzoic acid administered to rats via the diet there was reported to be no effects of treatment at doses up to 750 mg/kg/day [SCF 2002]. Gavage studies conducted on the mouse using benzyl alcohol at a lowest-observed-affect-

level [LOAEL] was reported to be 750 mg/kg/day, for effects on the pup weight and a no observed adverse effect level [NOAEL] of 550 mg/kg/day were reported [SCF, 2002].

In developmental toxicity studies foetotoxicity effects were reported to be found only in one study, using benzyl benzoate administered by oral gavage to rats at 1000 mg/kg/day with a NOAEL of 500 mg/kg/day [SCF, 2002].

A "limited" reproductive toxicity study reported that female rats given benzaldehyde at a dose equivalent to 5 mg/kg bw/day every other day for 32 weeks by stomach tube, had fewer pregnancies when they were mated with untreated males 75 and 108 days after the initial dose. No changes were observed on the number, weight or viability of pups born and the NOAEL level was about 5 mg/kg bw/day [BIBRA, 1989; JECFA, 1996].

In assessing the teratogenic potential of benzaldehyde, JECFA (2002) concluded, "…the data reviewed were sufficient to demonstrate a lack of teratogenic and reproductive potential" [JECFA, 2002].

Inhalation Toxicity

The addition of benzaldehyde at 351 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 benzaldehyde 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 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 benzaldehyde at 12 ppm; a level described as a multiple of its typical use in a US cigarette. This data, along with that from a number of other studies, indicates 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].

An inhalation study reported reduced respiration rate in mice following an exposure of 333-394 ppm benzaldehyde for 10 minutes. BIBRA give no details concerning the test conditions. Similarly for rats, an exposure concentration of 1423 ppm resulted in the same response. Another laboratory also reported upper respiratory tract irritation in laboratory animals [species not named] following exposure to atmospheric concentrations of 0.5 g/m^3. No information was given regarding the length of exposure [BIBRA, 1989].

A 120 day inhalation study, exposure to 6 mg/m^3 benzaldehyde for 5 hours/day had no effect on rats. However a concentration of 26 mg/m^3 for the same exposure period led to a reduction in body weight gain, and altered haematology. These changes were reported to be temporary, and values

returned to normal after an unspecified recovery period [BIBRA, 1989].

In a 14 day inhalation study, hypothermia and reduction of motor activity were observed in rats at 500, 750 and 1000 ppm benzaldehyde. At the highest level rats suffered abnormal gait and tremors indicative of severe CNS impairment. Histopathological examination of the tissues showed a goblet cell metaplasia, largely confined to the respiratory epithelium lining the nasal septum in male rats. Biochemical effects were also observed in both sexes [Laham *et al*., 1991].

When tested at 0.9 ppm in cigarettes, in a 13-week inhalation study, the presence of benzaldehyde "…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 Benzaldehyde at levels up to 279 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other relevant studies

One study conducted in rats shows that following inhalation, benzaldehyde was rapidly absorbed and cleared from the lungs. Excretion in the urine was also rapid, with hippuric acid being the main metabolite [BIBRA, 1989].

Following a single dose of 700-1100 mg/kg bw by intraperitoneal injection. Benzaldehyde was detected in the plasma of CD-1 mice within 5 minutes. Prior inhibition of aldehyde dehydrogenase by administration of disulfiram, produced increased plasma levels of benzaldehyde [368%] [JECFA, 1996].

Pure benzaldehyde administered to groups of five male Sprague-Dawley rats at a dose of 400, 750 or 1000 mg/kg bw/day for 13 days, by gavage, demonstrated the metabolism of benzaldehyde to benzylmercapturic acid. This metabolite was found in the urine of all treated animals, but none of the controls [who received tap water]. This suggests that benzaldehyde can undergo reduction to form benzyl alcohol, as benzylmercapturic acid is formed by glutathione conjugation with the sulphate ester of benzyl alcohol [JECFA,

1996].

In rabbits, benzaldehyde is metabolised by hydrogenation to benzyl alcohol, which is then oxidised to benzoic acid and excreted as hippuric acid [the glycine conjugate of benzoic acid]. Small amounts of free benzoic acid and benzoylglucuronic acids have also been detected in rabbit urine following administration of benzaldehyde. Both hippuric acid and free benzoic acid have previously been detected in the urine of control rabbits indicating that they are endogenous [JECFA, 1996].

At a recent meeting [57th], the Joint FAO/WHO Expert Committee on Food Additives [JECFA] concluded that there was no safety concern regarding benzaldehyde based on the current levels of intake, which is reported as 3,300 µg/day for Europe & 36,000 µg/day for USA. Benzaldehyde is rapidly metabolised by oxidation to benzoic acid, which is endogenous in humans. JECFA decided to maintain the group ADI of 5 mg/kg bw/day, which was originally set at their 46th meeting [JECFA, 2001].

JECFA have previously set a group ADI for benzyl compounds of 5 mg/kg bw set in 1967, based on toxicity data available and fact that these compounds are metabolised along a common pathway [JECFA, 1996].

Behavioural Data

Benzaldehyde exerted a powerful sedative effect on mice exposed via inhalation for one hour, reducing motility by around 44 %. This effect was strong enough to counter the over agitation caused by previous caffeine exposure [Buchbauer *et al.,* 1993].

***In Vitro* Toxicity Status**

Carcinogenicity and mutagenicity

Benzaldehyde was found to be negative in the Ames test when tested against *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 & TA100, both in the absence and presence of an S9 metabolic activation system. It also failed to induce unscheduled DNA synthesis in rat hepatocytes. However, benzaldehyde did produce a positive result in the mouse lymphoma forward mutation assay in the presence of an S9 fraction using the L5178y mouse lymphoma cell line. The authors suggest that this could have been due to high osmolarity or low pH resulting in non-physiological culture conditions and they warn that this result should be viewed with caution. These conditions have previously been shown to produce positive results in this and other assays in the absence of known genotoxic chemicals. The authors conclude that overall; the 63 GRAS flavouring ingredients tested did not exhibit *in vitro* genetic activity [Heck *et al.,* 1989].

The BIBRA toxicity profile for benzaldehyde states that several other studies demonstrate similar results supporting these findings. BIBRA note Florin *et al*., (1980); Haworth *et al*., (1983); Kasamaki *et al*., (1982); Nohmi *et al*. 1985;

Sasaki & Endo, (1978) as having found benzaldehyde to be non-genotoxic in *Salmonella typhimurium* in the Ames test [BIBRA 1989].

The National Toxicology Program [NTP] reiterates that benzaldehyde is not mutagenic in 6 strains of *S. typhimurium* and does not induce chromosomal aberrations in CHO cells, with or without a metabolic activation system [NTP, 2002].

Benzaldehyde did not influence the cell cycle rate or the number of spontaneous sister chromatid exchanges in CHO-K1 cells *in vitro*. Similarly, when treated with mitomycin C, benzaldehyde did not increase the rate of mitomycin C induced sister chromatid exchanges [Sasaki *et al.,* 1989].

Conversely, the BIBRA toxicity profile for benzaldehyde comments that effects on chromosomes [increased sister chromatid exchange] occurred *in vitro* [cultured mammalian cells, including lymphocytes]. It is also stated that activity was seen in the absence and presence of a liver metabolic activation system. Both positive and negative results have been reported in the literature regarding chromosomal damage induced by benzaldehyde in CHO cells [BIBRA, 1989].

Benzaldehyde has been examined in a novel mouse lymphoma assay. It was positive without a metabolic activation system. However, concentrations that induced significant increases in the mutant fraction were very close to the toxic dose. The authors question the relevance of the results to man, in that the mechanism needs to be elucidated before biological significance can be inferred [McGregor *et al.,* 1991].

The genotoxicity of 4 benzyl derivatives including benzaldehyde, JECFA state "None of the four compounds were mutagenic in the Ames test, either with or without metabolic activation. The compounds all induced gene mutations in the mouse lymphoma assay at the thymidine kinase locus. Some weak clastogenic activity was noted for *in vitro* assays, but not *in vivo* assays" [JECFA, 2002].

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 benzaldehyde at levels up to 12 ppm (a multiple of its typical use in a US cigarette) [Roemer *et al.*, 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 benzaldehyde at 351 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].

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 benzaldehyde at levels up to 308 ppm.

In a study conducted by Demir *et al.* (2010), different concentrations of four benzyl derivatives (benzyl alcohol, benzyl acetate, benzoic acid and benzaldehyde) used as flavour ingredients were investigated for genotoxicity in *in vitro*. By taking blood from two healthy people the comet assay was carried on to investigate the potential health damages of benzyl derivatives. For the evaluation of genotoxic effects, the tail moment and % tail DNA in the treated chemicals were compared to the solvent control (distilled water). The % tail DNA was statistically increased at 10 mM and higher concentrations, tail moment has significant difference at 10 and 25 mM concentrations of benzaldehyde. Only the highest concentration of benzoic acid increased both tail moment and % tail DNA [Demir *et al.* 2010].

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 benzaldehyde at levels up to 596 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 Benzaldehyde at levels up to 279 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other relevant studies

Disulfiram has been found to inhibit benzaldehyde oxidation, by 24% in rat liver slices exposed to a concentration of 25 μmol/litre benzaldehyde, and by 13% at a concentration of 250 μmol/litre. At this latter concentration, only a small portion of the benzaldehyde was metabolised to benzyl alcohol by the action of aldehyde dehydrogenase [JECFA, 1996].

Increasing concentrations of benzaldehyde were tested on cultured human lymphocytes using lactate dehydrogenase assay, cell proliferation (water-soluble tetrazolium salts-1) assay and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). The cytotoxicity increased when cells were treated with 10, 25 and 50 μg/mL concentrations of benzaldehyde. TUNEL assay results also show that the concentration of benzaldehyde at 10, 25 and 50 μg/mL caused DNA damage significantly [Ulker *et al*., 2013].

PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of Benzaldehyde 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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