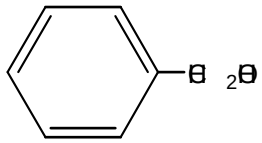
# BENZYL ALCOHOL

## SYNONYMS

Benzenemethanol
alpha-Hydroxytoluene
Phenyl carbinol
Phenylmethanol
Benzal alcohol
Benzene carbinol- toluenol
Phenyl carbinol-hydroxy toluene
Phenyl methyl alcohol

## CHEMICAL STRUCTURE



## CHEMICAL FORMULA

$C_7H_8O$

## IDENTIFIER DETAILS

| | | |
|---|---|---|
| CAS Number | : | 100-51-6 |
| CoE Number | : | 58 |
| FEMA | : | 2137 |
| EINECS Number | : | 202-859-9 |
| E Number | : | - |

## SPECIFICATIONS

Melting Point: -15.3 C

Boiling point: 205 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 |
|---|---|---|---|
| 0-5 | JECFA | 2001 | No safety concern at current levels of intake when used as a flavouring agent |

**FDA Status:**[CFR21]

| Section Number | Comments |
|---|---|
| 172.515 | Synthetic flavouring substances and adjuvants |

## HUMAN EXPOSURE

***Natural Occurrence:*** The free alcohol is often present in several essential oils and extracts of: jasmine, tobacco, tea, neroli, copaiba, *Acacia farnesiana* Wild., *Acacia cavenia* Hook. and Arn., *Robinia pseudacacia,* ylang ylang, *Pandanus laurocerasus,* tubercose, orris, castoreum, violet leaves, clove buds and others [Fenaroli, 1995].

Other natural occurrences include apricots, snap beans, cranberries, honey, mushrooms, strawberries, and tomatoes [Legacy, 2002].

Benzyl alcohol is found naturally in tobacco [Legacy, 2002].

***Reported Uses:*** Benzyl alcohol is reportedly used in baked goods at 401.8 ppm, frozen dairy at 244.6 ppm, meat products at 140 ppm, soft candy at 171.2 ppm, gelatine pudding at 175.7 ppm, non-alcoholic beverages at 47.93 ppm, alcoholic beverages at 95.24 ppm, gravies at 24 ppm, hard candy at 357.6 ppm, and chewing gum at 1254 ppm [Fenaroli, 2005].

Benzyl alcohol has been used at level up 1500 ppm in soap, creams and lotions, 250 ppm in detergents and 10000 ppm in fragrances [Legacy, 2002].

A possible average adult intake of benzyl alcohol has been listed as 41.9 mg, although the authors warn that this value may be an overestimate [FEMA, 1978; as cited by Legacy, 2002].

Benzyl alcohol is reportedly used as a local anaesthetic, pharmaceutical aid, and as a perfumery component. Benzyl alcohol has been in public use since the 1900's and is used in a wide variety of consumer products, foodstuffs, and fragrances as a flavouring, solvent, and preservative. As a solvent, it is used in injectible pharmaceuticals. It has also been used as a viscosity reducing agent. In 1994 the annual consumption of benzyl alcohol in food in the USA was 71,000 Kg [Legacy, 2002 SCF 2002].

## TOXICITY DATA

In a 2012 review in Food & Chemical Toxicology by McGinty *et al* , the available literature data for benzyl alcohol was evaluated and summarised . This included physical properties, acute toxicity, skin irritation, mucous membrane (eye) irritation, skin sensitization, elicitation, phototoxicity,

photoallergy, toxicokinetics, repeated dose, reproductive toxicity, genotoxicity, and carcinogenicity data. [McGinty *et al*., 2012]

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 benzyl alcohol at levels up to 1426 ppm, "did not increase the overall toxicity of cigarette smoke" [Carmines, 2002].

Renne *et* al., (2006) ev aluated 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 ingredien ts which included benzyl alcohol at 260 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 re spiratory 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 gr oss 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

| Species | Test Type | Route | Reported Dosage |
|---|---|---|---|
| Rat | $LD_{50}$ | Oral | 1.2 – 3.2g/kg |
| Mouse | $LD_{50}$ | Oral | 1.4 – 1.6g/kg |
| Rabbit | $LD_{50}$ | Oral | 1.0 – 2.2g/kg |
| Guinea Pig | $LD_{50}$ | Oral | 2.5g/kg |
| Rat | $LD_{50}$ | Intravenous | 0.064g/kg |
| Rat | $LD_{50}$ | Intraperitoneal | 0.4 – 0.8g/kg |
| Mouse | $LD_{50}$ | Intraperitoneal | 0.4 – 0.8g/kg |
| Guinea Pig | $LD_{50}$ | Intraperitoneal | 0.4 - .08g/kg |
| Rat | $LC_{50}$ | Inhalation | 4.4g/kg |

[BIBRA, 1991]

### Carcinogenicity and Mutagenicity

The SCF [2002] committee concluded that the studies conducted on rats and mice did not show any evidence of carcinogenicity at dose levels up to 200 mg/kg/day in the mouse and 400 mg/kg/day in the rat which were also the highest dose levels tested. The 13-week studies conducted in both rats and mice indicated NOAELS of 400 mg/kg/day or more , and with the knowledge that benzyl alcohol is metabolised in to benzaldehyde and then on to benzoic acid, the committee confirmed the inclusion of benzyl alcohol to the group ADI of 0-5 mg/kg day for benzoic acid and benzoates [SCF 2002].

In a repeated oral rat (Fisher 344/n) study, rats were administered 0, 200 or 400 mg/kg/day in corn oil by oral gavage 5/7 days a week for 103 weeks, survival was reduced in females following 2 year exposure to an oral dose of 400 mg benzyl alcohol/kg bw/day, however, m any of the early deaths were considered to be related to the dosing procedure. No other treatment related abnormalities were found [NTP, 1989].

One hundred mice (B6C3F $_1$) receiving 0, 100 or 200 mg benzyl alcohol/kg bw/day, 5 days/wk for up to 2 years, did not generally suffer any treatment related clinical abnormalities, or tissue changes. The exception to this was an increase in a 'commonly-occurring brain lesion in mice' (anterior pituitary gland neoplasms), in male mice dosed at 400 mg/kg/day [NTP 1989].

Based on the studies described above, the NTP concluded that there was no evidence of carcinogenic activity of benzyl alcohol in mice or rats [NTP, 1989].

In assessing the use of benzyl alcohol in parenteral medication intended for neonates, the Euro pean Commission was of the opinion that insufficient information was provided to them. They requested that where available, recent toxicity data concerning the suspected carcinogenicity and teratogenicity of benzyl alcohol should be submitted to commissio n. [EC, 1999].

A recent mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including benzyl alcohol at 3674 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**

Eighteen out of 614 subjects receiving 24/48 hour covered patches containing 0.05% benzyl alcohol in ethanol or a cream showed signs of irritation [BIBRA, 1991].

Similarly, an uncovered 45-minute exposure to a higher application of 5% benzyl alcohol in petrolatum produced contact urticaria in 32 out of 50

volunteers. [It should be noted that the volunteers consisted of 19 health y controls, 15 eczema patients and 16 with a previous history of contact dermatitis.] The authors suggest that the reactions caused were not a result of an immune response [BIBRA, 1991].

In a skin irritation study, neat benzyl alcohol was applied to unco vered rabbit skin for 24 hours. An irritant reaction was observed [no other details] [BIBRA, 1991].

A 30% concentration applied to the uncovered guinea-pig was again irritant after a 24-hour exposure period. However, a lower concentration of 10% benzyl alcohol was considered non-irritant [no details given regarding solvents]. In contrast to this later result, the same exposure time and concentration applied to mouse skin [covered] caused severe irritation [BIBRA, 1991].

A 1% benzyl alcohol formulation [solvent unspecified] applied daily, for 21 days, to guinea pig skin [uncovered] failed to induce any signs of irritancy. However, at a concentration of 3%, benzyl alcohol was irritant [BIBRA, 1991]. Applied neat to the eye of the rabbit [5 mg instillatio ns] produced irritation, although a 4% concentration of benzyl alcohol in aqueous solution was non-irritant [BIBRA, 1991].

Various sensitisation reactions have been noted in patients with skin complaints following patch tests with 5-10% benzyl alcohol in petrolatum. In the largest study, the incidence of sensitisation reactions was around 1% of 2261 patients. One in 63 dermatitis patients also reacted to benzyl alcohol following administration of 1% benzyl alcohol in petrolatum [BIBRA assume 24/48 hour covered contact] [BIBRA, 1991].

In a sensitisation study, 25 volunteers were first initiated by five 48-hour closed patch tests over 10 days with 10% benzyl alcohol in petrolatum. Following challenge 10-14 days later, with a final 48 hour closed patch of 10% benzyl alcohol solution, no evidence of sensitisation was given [BIBRA, 1991].

Of 8 Guinea-pigs initiated by 21 daily applications of 30% benzyl alcohol [presumably to the skin], and subsequently challenged on days 21 and 35 with 10% benzyl alcohol, only 2 animals gave a positive reaction [BIBRA, 1991].

Local anaesthesia of the skin has been noted in humans following the application of neat benzyl alcohol to the skin, or following intradermal injection at a concentration of 1%. Oral exposure to 5g of 1% benzyl alcohol in saline resulted in raised salivation and a ‘prickly’ sensation. Applied to the eye at 1%, benzyl alcohol produced a ‘smarting’ sensation followed by complete local anaesthesia [BIBRA, 1991].

Benzyl alcohol (5%) injected into the side of a cat’s face resulted in local nerve degeneration. As revealed by microscopic examination. At 10%, benzyl alcohol produced local anaesthesia [BIBRA, 1991].

The anaesthetic action of benzyl alcohol in rabbits has been found pH dependent. The effect is increased at higher pH levels [Ritchie *et al*., 1965 cited in Legacy, 2002].

The pure alcohol has been reported as irritating & corrosive "but much less toxic than phenol" in a standard textbook. Ingestion of large volumes [unspecified] is followed by vomiting, diarrhoea, & central nervous depression. A human fatality has been ascribed to the rectal administration of 45 ml benzyl alcohol [Gosselin, 1984].

**Reproductive and Developmental Toxicity**

Reduced birth weight and decreased growth were seen in mice and pups post natal growth rate, administered 750 mg/kg bw/day by stomach tube on days 7-14 of pregnancy. There was reported to be 18 maternal deaths with clear signs of toxicity in most survivors [SCF 2002].

As part of the preliminary screening tests on CD-1 mice described above, 50 dams received 550 mg/kg bw per day benzyl alcohol by gavage on days 6-15 of gestation did not show any increased signs of mortality. Mice and pups were observed up to 3 days *post partum*. One treated mouse died. There were no significant differences in pup development compared with control animals [JECFA, 1996].

Injections of 0.01-0.02 ml benzyl alcohol into the yolks of fertile eggs, either before incubation or from the first through the seventh day of their incubation, gave rise to meningoceles, limb deformities, beak defects such as arched upper beaks, localised blebs, and generalised edema [Duraiswami *et al*., 1954; as cited in Legacy, 2002].

**Inhalation Toxicity**

The addition of benzyl alcohol at 1990 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 benzyl alcohol 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]

When Sherman rats (3 groups of 6) were exposed to 2000 ppm benzyl alcohol vapours for 4 hours, 9 of the 18 animals died within 14 days of exposure [Carpenter *et al*., 1949; as cited in Legacy, 2002].

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 benzyl alcohol at 1426 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 3674 ppm in cigarettes, in a 13-week inhalation study, the presence of benzyl alcohol "…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 cigarett es 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].

All rats exposed to 2000 mg benzyl alcohol/kg bw/day for 12 days, died [this dose level is within the $LD_{50}$ range stated above] [BIBRA, 1991].

A similar oral study with a dose of 325 mg benzyl alcohol/kg bw/day for 8 -12 days did not produce any signs of overt toxicity in mice. However, at 400 mg/kg bw/day, 5 days/wk for 13 weeks resulted in growth reduction and some deaths. At 500 mg/kg bw/day for 12 days over a 16 day period, benzyl alcohol produced lethargy and co at deterioration in mice. Muscle incoordination, a 'hunched appearance' and CNS depression occurred in mice receiving 645 mg/kg bw/day for 8 days. At 800 mg/kg bw/day for 5 days/wk, for 13 weeks, benzyl alcohol produced 'staggering' as well as reduced gro wth and some deaths. Breathing difficulties and eye discharge, followed by death resulted from oral doses of 1300-2000 mg/kg bw/day for 8-12 days [BIBRA, 1991].

Other effects on rats include the condition of the fur coat, which was stated to have deteriorated in a number of rats following oral exposure of 250 mg benzyl alcohol/kg bw/day or more for 12 days [BIBRA, 1991].

A man with lung disease developed severe bronchitis and haemoptysis when benzyl alcohol was present as a preservative in his nebulizer; he was exposed to approximately 18 mg benzyl alcohol four times a day [BIBRA, 1991].

BIBRA (1991) report an incident where seven workers exposed to vapours from a lacquer containing 10% benzyl alcohol, 5% benzene and other solvents for 2 months developed headaches, vertigo, nausea, gastric pains, vomiting, diarrhoea and weight loss. The cause of which was believed to be benzene and benzyl alcohol [BIBRA, 1991].

High doses on benzyl alcohol has been reported to cause convulsions followed by paralysis of the respiratory centre, however at lower concentrations it has been used successfully as a local anaesthetic for minor surgical applications. The rectal administration of 45 ml has been reported to be fatal [HSDB 2003].

**Other relevant studies**

Following intraperitoneal injection of 700-1100 mg/kg bw benzyl alcohol in peanut oil, benzyl alcohol was detectable in the plasma of CD-1 mice within 5 minutes [McCloskey *et al*., 1986; as cited by JECFA, 1996].

In animals and humans benzyl alcohol is readily oxid ised to benzoic acid, which after conjugation with glycine, is readily eliminated as hippuric acid in the urine [Legacy, 2002].

JECFA state that evidence provided by Chapman *et al*. (1990), suggests that cytochrome P450 and not catalase or alcohol dehydrog enase is responsible for the metabolism of benzyl alcohol [JECFA, 1996].

Short term intake of benzyl alcohol (2%) solution in the drinking water was found to result in the inhibition of hepatic alcohol dehydrogenase and mitochondrial aldehyde dehydrogenas e isoenzymes activity in female rats only, there was no effect in males rats. Benzyl alcohol non competitively inhibited the activity of hepatic alcohol dehydrogenase for rats treated with 5% ethanol in the short term compared to control rats [SCF 2002].

Dogs receiving acute oral doses of 1g/Kg bw by stomach tube suffered vomiting and diarrhoea [BIBRA, 1991].

Unspecified high oral doses of benzyl alcohol in rabbits caused them to become ‘prostrate’ within 30 minutes [BIBRA, 1991].

Application of 5.2 g/k g bw of benzyl alcohol to the skin of guinea-pigs for 24 hours resulted in some deaths. The same result occurred in cats exposed to 6.9 g/kg bw for 22 hours [BIBRA, 1991].

Acute intravenous injection of 480 mg/kg bw of 0.9% benzyl alcohol in saline to 10 mice caused transient respiratory arrest although no deaths occurred. Other unspecified acute intravenous doses in mice resulted in tremors, gasping respiration, and convulsions. Death occurred in mice receiving 90 mg/kg bw by *i.v.* injection [BIBRA, 1991].

In a similar acute study, intravenous injections of benzyl alcohol were administered to CD2F1, B6D2F1, and C57BI/6, at doses of 0.05-0.2, 0.05-0.4, and 0.025-0.1 ml/kg bw. Within 24 hours, convulsions were seen at the highest doses, and dyspnoea and reduced motility were seen at all but the lowest dose. A decrease in body weight gain or a slight decrease in body weight was noted in B6D2F1 and C57BI/6 nice at all doses except the lowest. Haemolytic and precipitation potential in blood samples from t he mice indicated was strong at benzyl alcohol concentrations of 0.4-3.0 l/ml [Montaguti *et al*., 1994; as cited by JECFA, 1996].

Similar CNS effects and cardiac arrests were seen in rats and dogs receiving large intravenous injections upwards of 50 mg/kg bw. The severity of these effects were dependent upon dose concentration and speed of injection [BIBRA,1991].

Monkeys receiving 9 mg/kg bw benzyl alcohol as a 9% solution [in saline] via intravenous injection, did not suffer from alterations in blood pr essure, heart rate, respiration or blood chemistry [BIBRA, 1991].

Subcutaneous injections of 660-686 mg/kg bw resulted in CNS effects in cats [no other details given]. A second injection 7 hours later resulted in death. At a lower dose level of 392 mg/k g bw, two injections 10 hours apart produced a loss of muscular co-ordination followed by coma and death [BIBRA, 1991].

Rats receiving benzyl alcohol via intraperitoneal injection at just below the $LD_{50}$ value (approx. 0.3 g/kg bw) were unable to co-ordinate muscular movement [BIBRA, 1991].

CNS effects, along with respiratory arrest were seen in mice at intraperitoneal doses within the $LD_{50}$ range [BIBRA, 1991].

Five infants, preterm, received multiple injections of heparinized bacteriostatic sodium chloride for flushing the catheters, and medications reconstituted with bacteriostatic water, both containing 0.9% benzyl alco hol. Daily quantities of benzyl alcohol equalled 99 to 234 mg/kg of body weight. They then developed gradual neurological deterioration, severe metabolic acidosis, a striking onset of gasping respirations, hematologic abnormalities, skin breakdown, hepat ic and renal failure, hypotension, and cardiovascular collapse [Ellenhorn, 1988]. A matched control group of infants that had received 27-99 mg/kg did not develop the symptoms [SCF 2002].

Human subjects eliminated 75-85% of a 1.5 g of orally administered benzoic acid within 6 hours. The uptake of benzyl alcohol uptake via the skin was reported to be relatively low, with 1.42 percent for adults and 0.73 percent for full term infant skin within 6 hours of an unspecified dose [HSDB 2003].

'Gasping baby synd rome' developed in preterm infants as a result of intravenous catheter exposure to large volumes of fluids containing 0.95% benzyl alcohol. The estimated exposure was 99-405 mg benzyl alcohol/kg bw/day for 2-28 days. Severe metabolic acidosis, gasping, n eurological deterioration, blood abnormalities, skin breakdown, liver and kidney failure, lowered blood pressure, heart failure and death were also observed. At lower doses of 27-99 mg benzyl alcohol/kg bw/day, 'gasping syndrome ' did not occur, although a single report showed a similar dose range produced breathing difficulties after 7 days. BIBRA note that other researchers have attributed increased incidence of degenerative changes in the brain, cerebral palsy and death in preterm infants to similar exp osure to benzyl alcohol [BIBRA, 1991].

In a repeated oral study, the growth and survival of 10 rats was unaffected following administration of 125 mg benzyl alcohol/kg bw/day, by stomach tube, for 12 days [over a 16 day period]. At 125 mg benzyl alcohol/ kg bw/day, for 2 years, female rats were considered to have a decreased survival rate. Microscopic examination failed to identify any changes in tissues [no other details given concerning tissues examined] [BIBRA, 1991].

A 13-week repeated oral gavage study where rats and mice received 0, 50,100,200, 400 or 800 mg benzyl alcohol/kg bw/day produced 'some deaths in rats at 800 mg/kg, 'staggering respiratory difficulty and lethargy which was indicative of neurotoxicity were seen, reduced growth, bloody discharge around the mouth and nose, with histological tissue changes in the brain, thymus, skeletal muscle and kidneys also reported. Reduced bodyweight gain was reported for male rats dosed 800 mg/kg/day and female rats dosed 200 mg/kg/day or more. There was reported to be reduced bodyweight gain in male mice dosed 400 or 800 mg/kg/day and female mice dosed at 200 mg/kg/day or more [NTP 1989].

Benzyl alcohol administered by gavage at a dose of 10, 100, or 1000 mg/kg bw per day for 5 days to female mice (3 groups of 3) resulted in 1 death and 1 mouse with a 'hunched' posture at the highest dose level. Animals were examined daily for 7 days and no other clinical signs of toxicity were found. In a follow on study to estimate the $LD_{10}$ of benzyl alcohol, groups of four pregnant mice were given a dose of 200, 380, 720, 1370, or 2605 mg/kg bw per day on days 6-15 of gestation. All surviving animals were sacrificed on day 17 and examined. Clinical signs were observed in the 3 lower doses but were not considered to be related to treatment. On day 4, animals receiving 1370 mg/kg bw day showed unsteady gait, languid behaviour, hunched posture, tremors, rapid respiration, squinted eyes, convulsions, hyperactivity, prostration, and laboured breathing, and 2 animals died (1 was sacrificed). A dose-dependent reduction in body weight gain was also demonstrated [although no statistical analysis was presented]. All dams at 200 mg/kg bw per day, 2 of 3 at 380 mg/kg bw per day, and 2 of 4 at 720 mg/kg bw per day had surviving foetuses. The remainder of the dams resorbed their litters at these doses. The $LD_{10}$ was calculated at 550 mg/kg bw per day. The NOAEL was 550 mg/kg bw per day [York *et al*., 1986; as cited by JECFA, 1996].

In a similar series of preliminary screening tests on CD-1 mice, no NOAEL was set, but the LOAEL was 750 mg/kg bw per day [JECFA, 1996].

It has been suggested that whilst intraperitoneal benzyl alcohol caused significant increases in reactive oxygen species (ROS) formation in hepatic mitochondrial fractions, it had no effect on the brain. Similarly, it had no effect on glutathione levels in the brain, whilst reducing levels in the liver. It seems likely that benzyl alcohol does not cross the blood brain barrier, although small amounts must diffuse into the central nervous system. The authors concluded that glutathione may play an important role in the protection against ROS generation in the liver [Mattia *et al.*, 1993].

## BEHAVIOURAL DATA

No data identified

### *In Vitro* Toxicity Status

### Carcinogenicity and Mutagenicity

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 *benzyl alcohol* at levels up to 973 ppm.

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 benzyl alcohol at 1990 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].

Benzyl alcohol was negative in a number of *in vitro* genotoxicity tests. It failed to induce mutations in the Ames test [TA92, TA1535, TA100, TA1537, TA94 and TA98], at concentrations up to 10 mg/plate, and failed to induce chromosome aberrations in a Chinese hamster fibroblast cell line [1 mg/ml] [Ishidate *et al.*, 1984].

Similarly, it was negative in a *Salmonella* mutagenicity assay, in a number of strains in the absence and presence of a rat liver activation fraction, when tested up to 50000 ml/plate [Heck *et al.*, 1989].

BIBRA (1991) reference several Ames studies with *Salmonella typhimurium* and *Escherichia coli* all of which showed that benzyl alcohol was not mutagenic in the strains tested, with or without a metabolic activation system [BIBRA, 1991].

Yoo (1985) demonstrated benzyl alcohol to be negative in a mutagenicity test with *E. coli* [WP2] within the dose range used [1-8 mg/plate]. In an anti-mutation test with the same bacterial strain, benzyl alcohol exhibited unequivocal anti-mutagenic activity [Yoo, 1985].

A rec assay involving *Bacillus subtilis* [M45 & H17 strains; dose of 20 l/disk] gave indirect evidence that benzyl alcohol was able to induce DNA damage [no further details given] [BIBRA, 1991; Yoo, 1985].

Benzyl alcohol produced chromosomal damage in hamster cells in culture in the presence of a metabolic activation system, but not in its absence. A weekly positive result was also found for the induction of sister chromatid exchange, with and without an activation system [BIBRA, 1991].

A mouse cell culture mutagenicity assay produced negative results in the presence of a metabolic activation system. However, in its absence, results were found to be equivocal [BIBRA, 1991].

An NTP mouse lymphoma assay (L5178y/TK $^{+/-}$) found that benzyl alcohol induced trifluorothymidine resistance in the absence, but not in the presence of Aroclor 1254 [Legacy, 2002].

In cytogenetic assays with Chinese hamster ovary (CHO) cells, treatment with benzyl alcohol produced an increase in sister chromatid exchanges (SCEs) which was judged to be equivocal both with and without S9; a significant increase in chromosomal aberrations was observed after exposure to benzyl alcohol in the presence, but not the absence, of S9 [NTP, 1989].

No damage to cell membranes was seen when human diploid lung fibroblasts [MRC-5] were incubated with 25 mM benzyl alcohol for 30 minutes. Damage was measured by the release of the intracellular marker, $H^3$-uridine [Thelestam *et al*., 1980; as cited in Legacy, 2002].

Human cell cultures exposed to unspecified amounts of benzyl alcohol did not suffer any DNA single-strand breaks or chromosomal aberrations [no other details given] [Legacy, 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 sm oke 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 includ ed benzyl alcohol at levels up to 1426 ppm [a multiple of its typical use in a US cigarette] [Roemer *et al.,* 2002].

Demir *et al.,* (2010) investigated the genotoxicity of a number of benzyl derivatives, including benzyl alcohol in the Comet assay. Human lymphocytes were derived from the blood of two healthy people. The Comet assay has the ability of detecting both single and double strand breaks in DNA. For evaluation of genotoxic effects, the tail moment and % tail DNA in the treated chemicals were comp ared with the solvent control. In this study, the Comet assay showed significantly increased tail moment and % tail DNA at 25 and 50 mM concentrations of benzyl alcohol. The authors do state in the discussion that these effects at the highest concentrati ons tested could be due to

cytotoxic effects rather than genotoxic and that further studies are required to clarify these results.

**Reproductive and Developmental Toxicity**

There was no visible effect on the ciliary activity of embryo chicken tracheal organ cultures exposed for 1 hour to 5mM benzyl alcohol [Pettersson *et al*., 1982; cited in Legacy, 2002].

**Other relevant studies**

Benzyl alcohol is reported to be a membrane 'fluidiser' that affects the lipid bilayer of cell membranes including hepatocytes and erythrocytes [SCF 2002]. Benzyl alcohol was reported to induce dose, time and temperature dependent haemolysis of erythrocytes at concentrations above 500 nmoles/mg protein (54 g/mg/protein) [SCF 2002].

Benzyl alcohol was reported to have both increase the activity of Ca2+ - dependent enzymes such as adenylate cyclase and inhibit the activity of various glycosyltransferases in the rat liver Golgi membrane [SCF 2002].

In a study conducted by Chang *et al.,* (2011) the molecular mechanisms and signaling pa thways underlying benzyl alcohol (BA) toxicity in human retinal pigment epithelial (RPE) cells were studied. Cultured human RPE cells from the ARPE-19 cell line were exposed to culture medium alone (control) or with BA (0.0225, 0.225, 0.9, 3, or 9 mg/mL) f or up to 6 hours. BA toxicity was assessed by TUNEL assay, propidium iodide/annexin V-FITC staining and flow cytometry, caspase activation assay, caspase and apoptosis inhibition assays, mitochondrial transmembrane potential by rhodamine staining and flow cytometry, reactive oxygen species by chemiluminescence, and apoptosis-inducing factor staining. BA caused RPE cell death not only by necrosis but also by apoptosis, evidenced by exposure to 9 mg/mL BA for 6 hours leading to 19.0% early apoptotic cells and 64.2% apoptotic necrotic cells. Apoptotic signalling involved the immediate production of reactive oxygen species, activation of caspase-8, impairment of the mitochondrial transmembrane potential, and further activation of caspase-9 and -3. In addition, B A induced translocation of apoptosis-inducing factor into the nucleus, indicating caspase-independent apoptosis. BA leads to necrosis of RPE cells and triggers mitochondrial apoptosis through both caspase-dependent and - independent pathways. Extreme cauti on is suggested in the intraocular use of TA suspensions and meticulous evaluation before adoption of BA as a preservative in the future development of ophthalmic formulations.

## PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of benzyl alcohol 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 dev ice 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.

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 benzyl alcohol 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 tox icity findings, probably due to displacement of burning tobacco [Gaworski *et al*., 2011].

## REACH Statement

This ingredient has been registered under REACH. Under REACH, registrants have an obligation to provide information on substances they manufacture o r 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/.

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