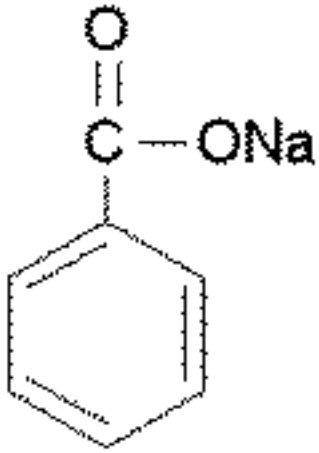
SODIUM BENZOATE

SYNONYMS

Benzoic acid, sodium salt.
Sobenate.
Natrium benzoicum.
Antimol
Benzoate of soda.

CHEMICAL STRUCTURE



CHEMICAL FORMULA

$C_6H_5CO_2Na$

IDENTIFIER DETAILS

CAS Number	:	532-32-1
CoE Number	:	-
FEMA	:	3025
EINECS Number	:	208-534-8
E Number	:	E211

SPECIFICATIONS

Melting Point: >300°C.

Boiling point:

PURPOSE

Preservative

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	1996 (maintained 2001)	The 1996 group ADI of 0-5 mg/kg bw for benzoic acid, the benzoate salts (calcium, potassium and sodium), benzaldehyde, benzyl acetate, benzyl alcohol and benzyl benzoate, expressed as benzoic acid equivalents, was maintained at the fifty-seventh meeting (2001).

FDA Status:[CFR21]

Section Number	Comments
184.1733 & 582.3733	FDA requirements: Substance added directly to human food affirmed as generally recognised as safe (GRAS). Current usage levels results in a max. level of 0.1 % (1000 ppm) in food. Quoted CFR 184.1733.

HUMAN EXPOSURE

Natural Oc currence: Benzoic acid occurs naturally at levels of 10-1000 mg/kg in many foodstuffs including blueberries, coffee beans, green peas, tea and milk [BIBRA, 1989]. Benzoic acid occurs naturally in many plants and in animals. The salt is not found to occur naturally [Fenaroli, 2005].

Reported Uses: Sodium benzoate is reportedly used (maximum levels) in baked goods at 1.10 ppm, breakfast cereals at 0.05 ppm, fats and oils at 0.98 ppm, milk products at 0.32 ppm, frozen dairy at 0.15 ppm, fruit ices at 0.50 ppm, fruit juice at 0.91 ppm, meat products at 0.08 ppm, processed vegetables at 1.0 ppm, condiment relish at 1.07 ppm, soft candy at 0.31 ppm, confection frosting at 0.94 ppm, jam and jelly at 1.0 ppm, sweet sauce at 2.92 ppm, gelatin pudding at 0.95 ppm, n on-alcoholic beverages at 0.60 ppm, alcoholic

beverages at 0.04 ppm, gravies at 0.73 ppm, imitation dairy at 0.80 ppm, hard candy at 0.01 ppm, and instant coffee and tea at 0.10 ppm [Fenaroli, 2005].

Although non-dissociated benzoic acid is the more effec tive antimicrobial agent for preservation purposes, sodium benzoate is used preferably, as it is approximately 200 times more soluble. About 0.1% is usually sufficient to preserve a product that has been properly prepared and adjusted to pH 4.5 or below [Chipley, 1983].

A major use for sodium benzoate is in the soft drink industry as a preservative, mainly due to the demand for high-fructose corn syrup in the beverages. Sodium benzoate is also widely used as a preservative in sauces, pickles and in fruit juices [Srour, 1998].

Sources other than foods: Used in various lotions and mouthwashes. It may also be used as a test for liver function . Sodium benzoate is also for preservation purposes in pharmaceuticals [up to 1.0% in liquid medicines] and for ther apeutic regimens in the treatment of patients with disrupted endogenous urea cycles [Srour, 1998].

The largest use of sodium benzoate, which accounts for 30-35% of the total output, is as an anticorrosive agent, particularly as an additive in the automotive engine and antifreeze coolants [Scholz *et al.,* 1991; Srour, 1998]. A relatively new use for sodium benzoate is in plastics such as polypropylene, to improve both the strength and clarity [BFGoodrich Kalama Inc., 1999].

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/.

In vivo Toxicity Status

Species	Test Type	Route	Reported Dosage
Rat	LD_{50}	Oral	2.5-4.1g/kg [BIBRA, 1989]
Rat	LD_{50}	Intravenous	1714mg/kg
Dog	LD_{50}	Oral	2000mg/kg
Rabbit	LD_{50}	Oral	2000mg/kg
Rabbit	LD_{50}	Subcutaneous	2000mg/kg [Spector, 1956]

Sodium Benzoate was administered daily for 10 days to both male and female F444 rats at 0, 1.81, 2.09 or 2.40% of the diet and 0, 2.08, 2.50 or 3% to male

and female B6C3F1 mice. For male rats that received 2.4%, relative liver and kidney weights were increased, total protein and gamma gl utamyl transpeptidase were significantly increased and enlarged hepatocytes with glassy cytoplasm were noted at histological evaluation. For male mice that received 3% SB in the diet, absolute liver weights, serum phospholipids and cholesterol were elevat ed. Enlargement, vacuolisation and necrosis of liver hepatocytes were also evident [Fujitani, 1993].

Groups of three male and female rats were fed diets containing 0, 2 or 5% SB for 28 days. All animals exposed to 5% SB in the diet died during the firs t two weeks of the study, clinical signs prior to death included urinary incontinence, hyper excitability and convulsions. Males that received 2% in the diet had a statistically significant weight loss compared to the controls [Fanelli *et al.,* 1963]. Simil ar findings were seen for 28 rats given a diet containing 5% sodium benzoate in the diet for three weeks, with 19/28 rats having died during the first two weeks. Most animals developed severe diarrhoea, with treatment-related changes seen at necropsy incl uding haemorrhage in the gut and nasal blood crust [Kieckbusch *et al.,* 1960].

Groups of five male and five female rats were fed sodium benzoate at doses between 16-1090 mg/kg/bw for 30 days. There was reported to be no effect upon body weight, food consum ption and no histological changes in the organs examined [Smyth *et al.,* 1948].

Groups of 10 males and 10 female rats [4-5 weeks old] were fed diets containing SB at a level of 0, 0.5, 1, 2, 4, or 8 % for 6 weeks. All rats fed 8 % SB in the diet and 19 at 4% in the diet died within 4 weeks. Nineteen animals fed 2%, 18 at 1% and 17 at 0.5% SB in the diet survived for 6 weeks. Significant reductions in body weight gain were seen only in animals fed diets containing SB at 4 and 8%. The only acute toxicity observed was hypersensitivity. No morphological change at autopsy was seen, except for atrophy of the spleen and lymph nodes in rats fed at 4 and 8% SB in the diet [Sodemoto, 1980].

Repeated administration of benzoic acid or SB to rats in the diet at 3 % [1.5g/kg bw/day] or more has produced decreases in the growth rate, damage to the spleen and lymph nodes, liver and kidneys, brain and GI tract together with CNS effects. Decreased survival has also been recorded at dietary levels of 3-5% [BIBRA, 1989].

Groups of rats [8-10] were fed 1, 2, 4,or 8% sod ium benzoate in the diet for 13 weeks, four rats receiving 8% in the diet died during the first 13 days of the study, with the remaining rats having a bodyweight gain 2/3 of that of controls with an identic al food intake. Increased liver and kidney weights were recorded at necropsy [Deuel *et al.,* 1954].

Seventeen dogs receiving a diet containing sodium benzoate or benzoic acid at 1000 mg/kg/day for 35-36 weeks showed no effect on growth or appetite. Higher dose levels caused ataxia, epileptic convulsions and death [Rost *et al.,* 1913].

Administration of SB in the drinking water of Swiss albino rats in groups of four rats per sex per group at 0.5, 1, 2, 4 and 8% for 35 days, lead to the death of all the animals at 8% and 3/4 males and females receiving 4% [Toth, 1984].

Oral doses of up to 250 mg/kg bw SB given to infants to treat inherited urea cycle disorders was reported to be well tolerated. However accidental benzoate poisoning at 800 mg/kg/ over 24 hours lead to vomiting, hyperpnoea and irritability which had returned to normal after 24 hours [Batshaw 1981]. SB has also been used to treat metabolic disorders in three infants administered between 125-1000 mg/kg as four sub-doses per day. Repeated vomiting was reported for doses above 900 mg/kg/day [Wolf *et al.,* 1986].

Carcinogenicity and Mutagenicity

Groups of 50 male and 52 female Fischer 344 rats were dosed with SB in the diet at 1 or 2 % [500 or 1000 mg/kg/day] for 18-24 months. There was reported to be no clinical signs attributable to treatment and tumours had the same incidence in both control and treated groups of each sex [Sodemoto *et al.,* 1980].

Fifty male and 50 female Swiss albino rats received Sodium Benzoate (2%) via the drinking wat er. The average intake of SB was found to be 6200 mg/kg/day for males and 5960 mg/kg/day for females. Treatment was reported as having had no effect on the incidence of tumours in the limited list of tissues examined histologically [Toth, 1984].

A mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including sodium benzoate at <0.1 ppm. The authors concluded that the study "did not indicate any substantive effe ct 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)].

Dermal toxicity

The application of benzoic acid to the skin in a study with 627 patients who were attending a contact dermatitis clinic, at a concentration of 5% benzoic acid in petrolatum for 24 or 48 hour using occluded patches was reported to be marginally irritating [De Groot *et al.,* 1986].

Open contact with 20% benzoic acid in acetone using either open or occluded patches, or 20% benzoic acid dissolved in water applied to the skin for 24 hours, did not irritate mouse or guinea pig skin [Gad *et al.,* 1986].

In a study with 2045 patients of dermatological clinics, only 5 people [approximately 0.2%] showed a positive reaction in patch tests [Brasch *et al.,* 1993], while 34 of 5202 patients [appro ximately 0.7%] with contact urticaria reacted positively [Broeckx *et al.,* 1987]. From the data above, it was

concluded that skin reactions caused by either sodium benzoate or benzoic acid in the general population are rare. However, it has been reported t hat in some patients suffering from asthma, rhinitis or urticaria, exposure to either food or beverages containing sodium benzoate have had attenu ated symptoms [Freedman, 1977].

Benzoic acid is not completely absorbed by the dermal route. In a study with six human subjects, Feldmann *et al.,* (1970) found an uptake of 36% of the applied dose [^{14}C-labelled benzoic acid dissolved in acetone; 4 µg/cm ^{2}; applied to an area of 13 cm ^{2}; on the forearm; non-occluded] within 12 hours. The total uptake of benzoic acid was calculated to be 43% within 5 days. In a second study with 6-7 subjects [a comparable method; application of 3, 400 or 2000 µg/cm^{2}], the percent absorption decreased from 35% to 14% within 24 h. However, the total uptake per cm ^{2} increased from 1 to 28 8 µg [Wester *et al.,* 1976].

The Addition of 100 mg of neat benzoic acid caused severe eye irritation in rabbits [BIBRA 1989].

Reproductive and Developmental toxicity

Groups of pregnant Sprague Dawley rats were administered SB intraperitoneally at 100, 315, or 1000 mg/kg on days 9-11 or 12-14 of gestation. The incidence of *in utero* deaths was 16% for rats given 1000 mg/kg on days 9-11 and 12% for rats given 1000 mg/kg on days 12-14 of gestation. Rats administered 1000 mg/kg on days 9-11 had foetuses wit h an increased incidence of gross anomalies [not specified]. All other treated groups and rats receiving 1000 mg/kg on days 12-14 of gestation had foetuses with abnormalities at the same incidence rate as the controls The no observed adverse effect level [NOAEL] was stated as being 350 mg/kg/day [Minor *et al.,* 1971].

Groups of 27-30 pregnant Wistar rats were given SB in the diet at 0, 1, 2, 4 or 8 % on Days 1-20 of gestation. Those females receiving 4% in the diet of SB in the diet did not gain weight an d those receiving 8% in the diet lost weight, with associated poor food consumption. Two dams at 4% SB in the diet and three dams at 8% SB in the diet died after convulsions and depressed motor activity. The rate of consumption of SB was calculated to be a t 1 % in the diet equivalent to 700 mg/kg /day, 2% level 1310 mg/kg/day, 4% 1875 mg/kg/day and 8% 965 mg/kg/day. On day 20 of gestation there was found to be a significant increase in the number of reabsorbed or dead foetuses for animals at 4 and 8% of SB in the diet. Significant abnormalities and pathological findings were only seen in the foetuses of rats that had received 4 or 2% SB in the diet. The authors suggested that the effects seen in the dams and foetuses at 4 and 8% SB in the diet, were reduce d due to reduced maternal feed intake. The authors suggested that the NOAEL was 1310 mg/kg/day [Onodera *et al.,* 1978].

The Food and Drug Research Labs Inc. (1972) [As cited in BIBRA , 1989] conducted a series of studies on the developmental toxicity of SB

administered by gavage to multiple species [CD1 mice, Wistar rats, Golden hamsters and Dutch belted rabbits]. There were no teratogenicity effects of significance in any of the treated groups and none occurred over excess of those seen in the control gro ups. The NOAEL values established were; Mice & Rats 175 mg/kg bw / day, Hamsters 300 mg/kg bw /day, and Rabbits 250 mg/kg bw/day

Administration of SB to chick embryos of up to 5mg of per egg failed to induce any teratogenicity [Verrett *et al.,* 1980].

In a safety assessment, Sodium benzoate was considered not to be a reproductive or developmental toxin at doses that are non-toxic to the mother (Andersen, 2006).

Tsay et al., (2007) reports on a study conducted to test the toxicity and teratogenicity of sodium benzoate in zebrafish. Sodium benzoate (SB) is a commonly used food preservative and anti-microbial agent in many foods from soup to cereals. However, little is known about the SB-induced toxicity and teratogenicity during early embryonic developme nt. After low dosages of SB (1-1000 ppm) treatment, the zebrafish embryos exhibited a 100% survival rate. As the exposure dosages increased, the survival rates decreased. No embryos survived after treatment with 2000 ppm SB. The 50% lethal dose (LD(50)) o f zebrafish is found to be in the range of 1400-1500 ppm. Gut abnormalities, malformation of pronephros, defective hatching gland and edema in pericardial sac were observed after treatment with SB. Compared to untreated littermates (vehicle-treated control), SB-treated embryos exhibited significantly reduced tactile sensitivity frequencies of touch-induced movement (vehicle-treated control: 27.60+/-1.98 v.s. 1000 ppm SB: 7.89+/-5.28; N=30). Subtle changes are easily observed by staining with specific monocl onal antibodies F59, Znp1 and alpha6F to detect morphology changes in muscle fibers, motor axons and pronephros, respectively. Our data showed that the treatment of SB led to misalignment of muscle fibers, motor neuron innervations, excess acetyl-choline r eceptor cluster and defective pronephric tubes. On the basis of these observations, the authors suggest that sodium benzoate is able to induce neurotoxicity and nephrotoxicity of zebrafish larvae.

Inhalation Toxicity

Ten CD rats per sex per group were ex posed to 0, 25, 250, or 1200 mg benzoic acid dust aerosol/m ^{3} for 6 hours per day and 5 days per week for a total of 4 weeks. After this time, various serum biochemical, haematological, organ weight, and histopathological examinations were conducted. At $\geq$25 mg/m^3, an increased incidence of interstitial inflammatory cell infiltrate and interstitial fibrosis in the trachea and lungs was reported in treated animals compared to the controls, however there was no clear dosage dependency. At a concentration of $\geq$250 mg/m ^{3}, this resulted in upper respiratory tract irritation, as indicated by inflammatory exudate around the nares, and significantly decreased absolute kidney weights in females. In the highest dose group [1200 mg/kg], one rat per sex died, and the bo dy weight gain was significantly decreased in males and females compared with controls. In

addition, a significant decrease in platelets [males and females], absolute/relative liver weights [males], and trachea/lung weights [females] were noted [Velsicol Chemical Corp., 1981].

When tested at <0.1 ppm in cigarettes, in a 13-week inhalation study, the presence of sodium benzoate "…had no discernible effect on the character or 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)].

The addition of sodium benzoate at 12 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 sodium benzoate 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 ingredients, which included sodium benzoate 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].

Other relevant studies

SB was also reported not induce mutations in the fruit fly *Drosophila* or silkworms but produced chromosomal effects in the bean *Vicia faba* [BIBRA, 1989].

After oral uptake benzoic acid and SB are rapidly absorbed from the gastrointestinal tract. In the acid conditions of the stomach, the equilibrium moves to the undissociated benzoic acid molecule, which is absorbed rapidly. Benzoate from SB would change from the ionised form to the undissociated benzoic acid molecule. As a result, the metabolism and systemic effects of benzoic acid and SB can be evaluated together. The benzoic acid molecule is then conjugated with glycine and glucuronic acid in the liver in man and most experimental animals except the dog in which the kidney is the main site of biosynthesis. In man, rat and rabbit benzoic acid conjugate is then almost entirely excreted as hippuric acid [JECFA, 1996].

In humans the availability of glycine is the rate-limiting step in the formation of hippuric acid. The factor limiting the biosynthesis of hippuric acid is the

availability of glycine, resulting in a reduction in the glycine levels of the body. Therefore, the ingestion of benzoic acid or sodium benzoate affects any body function or metabolic process in which glycine is involved; leading for example to a reduction in glutamine, creatinine, urea, and uric acid levels [Kubota *et al.,* 1991; JECFA, 1996]. The primary effect from ingestion of moderate amounts of benzoate is gastrointestinal irritation. Benzoic acid is more irritating than benzoate salts [JECFA, 1996].

Human female volunteers were given 175 ml or 350 ml of a soft drink containing 190 mg of SB and 3 males given 20 mg/kg of SB. In both treatments the peak rate of excretion of hippuric acid in the urine was rapid being during the first 30 minutes in the females and the first hour for the males [Fujii *et al.,* 1991]. JECFA concluded that the rapid metabolism and elimination of SB in the urine, meant that an accumulation of SB in the body was not anticipated [JECFA, 1996].

Hypersensivity cause by exposure to food additives such as sodium benzoate was assessed in 226 patients (76 males and 150 females) aged 12-60 years (mean age 40.2+/-16.3 years). Patients were fed an additive free diet for 1 month followed by an additive rich diet for two weeks. 8.8% of patients reported an improvement in rhinitis symptoms (with additive free diet), 2.6% were symptom free and 6.2% showed an improvement in their symptoms with the additive free diet. The double blind placebo-controlled study (20 challenges with sodium benzoate) induced sneezing, rhinorrhoea, nasal blockage and itching with reduction of nasal peak inspiratory flow>/=20% (of basal value) (19 with monosodium benzoate). The authors concluded that nonatopic persistent rhinitis may be caused by infrequent, daily; ingestion of small doses of a non-tolerated substances (food additives) and some patients with chronic vasomotor rhinitis may be intolerant to a particular food additive (Pacor *et al.,* 2004).

250 mg each of SB and sodium phenylacetate is reportedly administered in 3 to 6 equally divided doses as adjuvant therapy to prevent and treat hyperammonemia [waste nitrogen accumulation in the body], by providing an alternative pathway for waste nitrogen disposal. The total daily dose should not exceed 10 g of either SB and sodium phenylacetate. In three patients that received excessive doses, two developed and died from cerebral oedema [Praphanphoj *et al.,* 2000].

Behavioural data

The effects of artificial food colours and additives (AFCA) were investigated in a randomised, double blind, crossover test of 153 3-year-old and 144 8/9-year-old children. The children were given a drink containing AFCA mixes: Mix A [containing Tartrazine (E102), Ponceau 4R (E124), Sunset yellow FCF (E110) and Carmosine (E122)], Mix B (containing Sunset yellow FCF (E110), Carmosine (E122), Quinoline yellow (E104) and Allura red AC (E129)] and sodium benzoate (E211), or a placebo. The behaviour and activity was scored based on observations by parents and teachers to give a global hyperactivity aggregate (GHA). An attention test was also completed by the 8/9-year-olds.

The 8/9-year-olds' behaviour was adversely affected by both Mix A and Mix B, when compared to placebo. The 3-year-olds behaviour was adversely affected by Mix A but not by Mix B, when compared to placebo. It was concluded that artificial colours or preservatives, such as sodium benzoate, in the diet caused adverse behaviour in a significant proportion of the 3 and 8/9-year-old children included in the study. It was not determined whether sodium benzoate alone would also have had adverse effects upon behaviour (McCann *et al*., 2007).

The effects of food colourings and benzoate preservatives on hyperactivity in 3-year old children (277 selected from 1873 based on p resence of hyperactivity and atopy) was investigated. Children received a diet free from artificial colours and benzoate preservatives for one week. For the subsequent three weeks within a subject double blind cross over study children received in random o rder periods dietary challenge with a drink containing 20mg of artificial colourings and 45mg of sodium benzoate daily or a placebo supplementary to their diet. Results were assessed by a tester blind to the dietary status and also parental observation. A significant reduction in hyperactivity was observed during the withdrawal period. Parents reported a significant increase in hyperactive behaviour during the active compared to the placebo period. No significant differences were observed based on objectiv e testing in the clinic (Bateman *et al.,* 2004).

In vitro Toxicity Status

Carcinogenicity and mutagenicity

Baker *et al.,* [2004]; examined the effects of the addition of 482 tobacco ingredients upon the biological activity and chemistry of mainstream smok e. The ingredients, essentially different groups of flavourings and casings, were added in different combinations to reference cigarettes. The addition of sodium benzoate at 12 ppm was determined not to have affected the mutagenicity of the total particul ate 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].

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 additio n of the ingredients, which included sodium benzoate at levels up to 3000 ppm.

Mpountoukas *et al*., (2008) report on a study conducted on Potassium sorbate, sodium benzoate and potassium nitrate to assess their cytotoxicity and genotoxicity potential in i n human peripheral blood cells in vitro. Potassium nitrate has shown no activity in the test system. When potassium sorbate and sodium benzoate were used at concentrations of 2.0, 0.2 and 0.02 mM, no

cytostatic activity was detected. However, concentrati ons of 4 and 8 mM have shown to be weakly cytostatic. Additionally, a genotoxic activity using the SCE methodology has been observed at 8 mM of sodium benzoate and at 4 and 8 mM of potassium sorbate. No cytotoxic activity has been induced by the three preservatives. The authors suggest that the data presented demonstrate that the preservatives at low concentrations can be considered as non-genotoxic under the conditions tested.

Sodium benzoate was negative in the Ames test in the presence and absence of ra t liver S9 fraction in the following strains of *Salmonella typhimurium* [TA92, TA94, TA98, TA100, TA1535 TA1537] at concentrations up to 3000 µg/plate Ishidate *et al.,* [1984]; in strains [TA98, TA 100, TA 1535,TA 1537, and TA1538] at concentrations between 33-10000 µg/plate , Prival *et al.,* (1991); and in strains TA97, TA98, TA100, TA1535 TA1537 at 33-10000 µg/plate [Zeiger *et al.,* 1988].

Sodium benzoate was also negative in the *E.coli* WP2 test system in the presence and absence of rat liver S9 fraction at concentrations between 33-10,000 µg/plate, [Prival *et al.,* 1991].

A positive result was obtained in the *Bacillus subtilis* assay using H17 and M45 strains both with S9 at 16 mg/disc and without metabolic activation at 20 mg/disc [Ishizaki *et al.,* 1989].

Sodium benzoate was found to be positive in the chromosome aberration assay using Chinese hamster cells, without metabolic activation up to 2000 µg/ml [Ishidate *et al.,* 1984, 1988].

A slightly positive result for sister chromatid exchange was noted for C hinese hamster cells at 1-10 mmol/litre, with out metabolic activation [Abe *et al.,* 1977]. It has also been reported to have induced sister chromatid exchanges in human lymphocytes when dosed at 10 mmol/litre [Xing *et al.,* 1990]. SB has also been reporte d to be negative in the induction of sister chromatid exchange in human lymphoblastoid cells at 1-30 mmol/litre, both with and without metabolic activation [Tohda *et al.,* 1988] and in human lymphocyte cells 2.0 mmol/litre [Jansson *et al.,* 1988].

In Rec assays [which measures DNA damage indirectly] benzoic acid gave no evidence of activity while both negative and positive results have been reported for SB [BIBRA, 1989].

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".

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 sodium benzoate 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 addit ion of high levels of an ingredient caused a small reduction in toxicity findings, probably due to displacement of burning tobacco [Gaworski *et al*., 2011].

In this study, the genotoxic effects of sodium benzoate was investigated in cultured human peripher al lymphocytes using chromosomal aberrations (CA), sister chromatid exchange (SCE), and micronuclei (MN). The level of nuclear DNA damage of Sodium benzoate was also evaluated using the comet assay. The lymphocytes were incubated with different concentrati ons of Sodium benzoate (6.25, 12.5, 25, 50, and 100 µg/ml). A significant increase was observed in CA, SCE, and MN, in almost all treatments compared to negative controls. Sodium benzoate significantly decreased the mitotic index (MI) in all the treatments, compared to the negat ive controls. However, the replication index (RI) was not affected. The authors conclude the results indicate that sodium benzoate is clastogenic, mutagenic and cytotoxic to human lymphocytes *in vitro* [Zengin *et al*., 2011].

Reproductive and Developmental Toxicity

In the hydra regeneration assay, it was predicted that SB was a teratogen under the conditions of the assay [Cheng *et al.,* 1997].

Other Relevant Studies

Eberlein-Koenig *et al.,* (1993) failed to find any photohemolysis effects when sodium benzoa te was tested using suspensions of human erythrocytes irradiated with various light sources [Eberlein-Koenig *et al.,* 1993].

The toxicity of the food colour tartrazine, the preservatives sodium nitrate and sodium benzoate, and the antioxidant BHT, was st udied using the protozoan Tetrahymenapyriformis as a toxicological model. The 4 food additives were added to Tetrahymena cultures and DNA content of the protozoan nuclei measured by an image analysis system. These food additives caused a statistically sign ificant increase in DNA content suggesting stimulation of the mitotic process. This system may contribute to the investigation of the cellular action of food additives, since mitogenic stimuli substantially alter susceptibility to chemical carcinogenesis [Stefanidou *et al*., 2003].

Using a primary culture of hepatocytes, the activities of both ornithine transcarbamylase [an indicator of mitochondrial function] and tyrosine aminotransferase [a marker of the cytosol] were decreased by sodium benzoate [SB] at concentrations in excess of 500 micrograms/ml. Intracellular protein synthesis and DNA synthesis was found to be decreased by SB at 100 g/ml [Oyanagi *et al.,* 1987].

Sodium benzoate can be used as a free radical scavenger to provide additional protectio n against alveolar epithelial cell asbestos induced

apoptosis characterised by caspase 9 activation and DNA fragmentation of A549-rho(omicron) cells (which lack mitochondrial DNA and functional electron transport)(No further details provided) (Panduri *et al.,* 2004).

Leptin, IL-6 and nitrite concentrations were analysed in the supernatants of murine 3T3-L1 adipocytes after co-incubation with LPS and the food preservatives, sodium sulphite (SS), sodium benzoate **(**SB) and the spice and colourant, curcumin, for 24 h. In addition, the kinetics of leptin secretion was analysed. A significant and dose-dependent decrease in leptin was observed after incubating the cells with SB and curcumin for 12 and 24 h, whereas SS decreased leptin concentrations after 24 h of tr eatment. Moreover, SS increased, while curcumin decreased LPS-stimulated secretion of IL-6, whereas SB had no such effect. None of the compounds that were investigated influenced nitrite production. The food additives SS, SB and curcumin affect the leptin release after co-incubation with LPS from cultured adipocytes in a dose- and time-dependent manner. Decreased leptin release during the consumption of nutrition-derived food additives could decrease the amount of circulating leptin to which the central ner vous system is exposed and may therefore contribute to an obesogenic environment. [Ciardi *et al.* , 2012]

PYROLYSIS AND TRANSFER STUDIES

In an extension to the Baker and Bishop (2004) study, a further 159 ingredients were analysed. Under pyrolysis of sodiu m benzoate, breakdown product included benzene (64.0%), biphenyl (10.4%), benzoic acid (5.7%), benzophenone (3.8%) and other minor compounds [Baker and Bishop, 2005].

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