CARAMEL COLOUR

SYNONYMS

AP 680
BC 420 (Colour)
Burnt sugar
Burnt sugar colouring
C.I. Natural Brown 10
Caramel
Caramel colour
Caramel colour dye
Caramel flavour
Caramel liquid
DS 400
HSDB 1920
Natural brown 10
P 255
RT 80
Sethness 858

CHEMICAL STRUCTURE

Undefined (mixture of components)

CHEMICAL FORMULA

Caramel is defined as the substance obtained by controlled heat treatment of food-grade carbohydrates. Food-grade acids, alkalis, and salts may be used to assist caramelisation. Food-grade antifoaming agents may be used in an amount not greater than that required to produce the intended effect. Consists essentially of colloidal aggregates that are dispersible in water but only partly dispersible in alcohol-water solutions. Depending upon the particular caramelising agent used, may have a positive or negative colloidal charge in solution.

IDENTIFIER DETAILS

CAS Number	:	8028-89-5
CoE Number	:	-
FEMA	:	2235
EINECS Number	:	232-435-9
E Number	:	E150 a,b,c & d

CLP CLASSIFICATION

Ingredient CLP Classification: No

Endpoint	Classification	Category
Acute Oral Toxicity	-	-
Acute Dermal Toxicity	-	-
Acute Inhalation Toxicity	-	-
Skin Corrosive/irritant	-	-
Eye Damage/Irritation	-	-
Respiratory Sensitisation	-	-
Skin Sensitisation	-	-
Mutagenicity/Genotoxicity	-	-
Carcinogenicity	-	-
Reproductive Toxicity	-	-
Specific Target Organ Toxicity	-	-
Aspiration Toxicity	-	-

SPECIFICATIONS

Melting Point: undefined (mixture of components)

Boiling point: undefined (mixture of components)

PURPOSE

Colour and flavour

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
Class I - NOT SPECIFIED Class II - 160 mg/kg Class III - 200 mg/kg Class IV – 200 mg/kg	JECFA	1985 2000 1985 1985	

FDA Status:[CFR21]

Section Number	Comments
182.1235	Caramel

HUMAN EXPOSURE

Natural Occurrence: Caramel colour is an amorphous, dark brown liquid, with a bitter, pleasant taste, made by heating sugar or glucose with added amounts of alkali or mineral acid [Fenaroli, 2005].

Reported Uses: Caramel colour is widely used as a colouring in food, confectionery, galenicals [*medicinal plant products*], and carbonated beverages. Gravies – 543 ppm, baked goods – 4.3 ppm, beverages – 2.5 ppm. [HSDB 2001; Fenaroli, 2005].

Caramel colours have been separated into 4 classes, based on their method of preparation. Class I is prepared by the heating of carbohydrates with alkali or acid; Class II is prepared by controlled heating of carbohydrates with sulphite containing compounds, Class III is prepared by controlled heating of carbohydrate with ammonium compounds; Class IV is prepared by controlled heating of carbohydrates with both ammonium and sulphite containing compounds [WHO, 1987].

TOXICITY DATA

Carmines *et al*., (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 caramel colour at levels up to 500 ppm, “did not increase the overall toxicity of cigarette smoke” [Carmines *et al*., 2002].

In Vivo Toxicity Status

The caramel colours are a complex mixture of compounds produced by heating carbohydrates under controlled heat and chemical processing conditions; they are divided into four classes (E150a (Class I), E150b (Class II), E150c (Class III), E150d (Class IV)) according to the manufacturing reactants used. The caramel colours were previously evaluated by the SCF and by JECFA, which concluded that a numerical ADI was not necessary for Class I, but established ADIs for the other classes of caramels, ranging from160-200 mg/kg bw/day. Given the consistency in the toxicological database, the Panel establishes a group ADI of 300 mg/kg bw/day for the caramel colours, by applying an uncertainty factor of 100 to a NOAEL of 30 g/kg bw/day (highest dose tested) identified in 13-week rat studies with Class IV and a similar NOAEL identified in a rat reproductive toxicity study, also with Class IV. Comparable NOAELS for Classes II, III and IV were reported in the SCF and JECFA evaluations. Within this group ADI, the Panel establishes an individual ADI of 100 mg/kg bw/day for Class III due to new information regarding the immunotoxicity of THI. The Panel concludes that the anticipated dietary exposure of child and adult populations may exceed the ADIs for

Classes I, III and IV caramels, but exposure estimates to Class II were below the ADI. Exposure estimates for the caramel constituents THI, 4-MEI and SO2 are not of concern, but the Panel welcomes additional studies to clarify remaining uncertainties regarding effects of THI on the immune system. The Panel notes that other constituents of caramel colours including 5-HMF and furan may be present at levels that may be of concern, and considers that the specifications should include maximum levels for these constituents [EFSA, 2011].

Carcinogenicity and Mutagenicity

A recent mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including caramel colour at 5 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].

In an *in vivo* micronucleus assay, caramel colour III was administered by oral gavage to 5 males and 5 females at doses of 0, 1.05 or 3.5g/kg, in two doses, 24 hours apart. There was reported to be no increase in the incidence of micronuclei in polychromatic erythrocytes obtained from the bone marrow, and was considered to have no clastogenic activity under the Conditions of the assay [WHO, 1987].

In a study in which caramel was tested for its sub-chronic, chronic toxicity and carcinogenicity, in both F344 rats and $B6CF_1$ mice, it was determined that the NOAEL was 10 g/kg, with no evidence of carcinogenicity observed, even after two years [MacKenzie *et al.*, 1992b].

Reproductive and Developmental Toxicity

Teratogenicity studies were conducted with caramel colour III in groups of 22 or 23 pregnant mice per group, 21-24 pregnant rats per group or 11-12 pregnant rabbits per group, animals were dosed between days 6-15 of gestation (mice and rats) and days 6-18 in rabbits. All animals were dosed at 0, 16, 74.3, 345 or 1600 mg/kg/day. After examination of the foetuses visceral and skeletal abnormalities, there were found to be no differences between any of the abnormalities found in the treated groups and those that spontaneously occurred in control animals. Caramel colour III had no effect on maternal or foetal survival. There was no evidence of teratogenic activity in any of the animal species tested [WHO, 1987].

Teratogenicity studies were also conducted with caramel colour IV with pregnant rats, mice and rabbits at 0, 16, 74.3, 345 or 1600 mg/kg/day. Essentially following the same protocol as above there was found to be no differences between any of the abnormalities found in the treated groups and those that spontaneously occurred in control animals. Caramel colour IV had

no effect on maternal or foetal survival [WHO, 1987].

Groups of 15 male and 15 female Wistar rats were administered either 0 or 10 % caramel colour IV in solution for 100 days and then mated. Animals of the F_1 generation were then given 0 or 10% caramel solution until day 100. There was reported to be no effect upon any reproductive parameters, growth, food consumption, gross pathology or histology of the F_1 generation on Day 100 [WHO, 1987].

In a dose range finding study, groups of F334 rats were administered caramel colour IV at 0, 10, 15, 20 and 25 % in the drinking water for 21 days prior to mating throughout mating, gestation and lactation. At weaning, 2 pups per sex per litter were randomly chosen from those litters that had at least 2 rats of both sexes in them and were treated for another 13 weeks. There were reported to be soft stools from all animals treated with 20 and 25% in the drinking water. It was also reported that there was a dosage related reduced body weight gain in the F_0 and all animals in the F_1 generation. Mating pregnancy and fertility rate were reported to be similar between all groups. There were reported to be a reduction in the number of implantations and numbers of pups alive on Days 0, 4, 21 of lactation in the 20% dose group, compared to the control animals. At necropsy of the F_1 animals, there were reported to be dose-related increases in the absolute and relative weights of the liver, kidneys and caecum of animals in the 15% or higher dosage groups. The only reported treatment related changes were a green colouration of the content of the lower gut, mucous membrane and mesenteric lymph nodes [WHO, 1987].

Oral intubation of up to 1600 mg/kg bw caramel to pregnant mice and rats for 10 days was reported to have no effect on condition or on maternal or foetal survival [HSDB, 2001].

Inhalation Toxicity

When tested at 1617 ppm in cigarettes, in a 13-week inhalation study, the presence of caramel colour "…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).

A 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 caramel colour at 500 ppm, a level described as a multiple of its typical use in a US cigarette. The data from this study, along with that from a number of other biological and chemical studies, indicate that the addition of the combined ingredients "did not increase the inhalation

toxicity of the smoke, even at the exaggerated levels used" [Vanscheeuwijck *et al.*, 2002].

The addition of caramel at 16,000 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 caramel 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]

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 caramel colour at levels up to 228 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other relevant studies

Caramel colour II (made from reactions with sulphite without ammonia) has a distinctive reddish colouration is used in liquor and ice-cream production and is reported to account for less than 1% of total caramel colour production. Caramel colour II was administered to Fischer-344 rats in groups of 20 rats per sex per group at concentrations of 0, 4, 8, 12, 16 g/kg in the drinking water for at least 13 weeks. All treated animals were reported to have soft faeces and reduced drinking water consumption. Reduced food consumption was noted for all treated groups except males receiving 4 g/kg/day, with significantly lower levels of bodyweight gain noted for males receiving 12 g/kg or more and females receiving 8 g/kg or more. There were reported to be no changes in either haematology or biochemistry parameters and there was reported to be no macroscopic or microscopic pathological findings related to treatment. The top dose of 16 g/kg was considered to be the NOAEL by the authors [Mackenzie *et al.,* 1992c].

Caramel (ammonia process) was given at levels of 0 (control), 1.25 and 5.0 % in the drinking-water to groups ef 40 lale and 50 fdmadd mice &or 96 7k, and then all the animals were maintained without caramel for a further 8 wk. Males given 5.0% caramel showed increased cumulative mortality from wk 100 to the end of the experiment. The white blood cell count in treated males was significantly elevated in a dose-related manner. However, these changes were not considered biologically significant. There were no treatment-related effects on clinical signs, body or organ weights, results of urine analyses, or histological features. Therefore, this study [the authors claim] did not

demonstrate any carcinogenic effect of caramel on mice at levels of up to 5.0% in the drinking water [Hagiwara *et al*., 1983].

Caramel colour I was reported to be free of heterocyclic compounds associated with depressed lymphocyte counts or convulsant activity that occurs with caramels prepared with ammonia or ammonia salts and has a low order of toxicity [WHO, 1987].

Administration of Caramel Colour III was associated with lymphopenia [deficiency of lymphocytes in the blood] in laboratory animals, especially if the animals are fed a vitamin B6-deficient diet. Functional immunological alterations in rats exposed to Caramel Colour III have been reported. The component of Caramel Colour III that is responsible for the immunological effects has been shown to be 2-acetyl-4-tetrahydroxybutyl imidazole (THI). In this study, female Balb/c mice fed a diet, with a relatively high vitamin B6 content, were exposed to 2 or 10% of a commercial Caramel Colour III preparation with a low THI content (less than 25 ppm) in the drinking water for 9 wk. Although this treatment did not induce a lymphopenia in the exposed mice, flow cytometric analysis of lymphocyte subpopulations demonstrated reductions in the CD4+ and CD8+ cell populations. In addition, the proliferative response of spleen cells to B and T cell mitogens was significantly reduced in the mice exposed to 2% Caramel Colour III. No changes were observed in natural killer cell activity or in the humoral antibody response to a viral antigen. The results indicate that Caramel Colour III that meets the specified limit of less than 25 mg THI/kg may, nevertheless, interfere with the lymphoid system in mice with an adequate vitamin B6 status [Thuvander *et al*., 1994]

Caramel colour III (ammonia treated) has been reported to cause reduced lymphocytes counts in rats, specifically fed a diet low in vitamin B6. This reduction was however reported to be reversed by the presence of THI in caramel colour III. No decrease in the number of lymphocytes was reported for humans, marginally deficient in vitamin B6, who consumed caramel colour III at the acceptable daily intake level of 200 mg/kg for a total of 7 days [Houben *et al.,* 1992].

Caramel colour IV was prepared with radio labelled glucose and was filtered to isolate the high molecular weight colour fraction (HMCF). Male rats were then administered either a single dose of 2.5 g/kg HMFC or administered repeat doses of 2.5 g/kg unlabelled HMFC in their drinking water for 13 days prior to receiving a single bolus dose of labelled HMCF on Day 14. On both regimes there was found to be predominantly excretion of HMCF in the faeces with less than 3% of the administered dose being excreted in the urine. There was no appreciable excretion of HMCF in to the air. There was reported to be in excess of 99% excretion of HMCF within 96 hours of dosing. The principal tissues that were found to contain radioactivity were reported to be the liver, mesenteric lymph nodes, kidneys and tissues of the gastro intestinal tract. It was reported that there were no differences in the absorption, distribution, metabolism and excretion of HMCF by either of the two regimes [Selim *et al.,* 1992].

The immunological toxicity associated with caramel colours has been reviewed by Houben *et al.* (1994). The toxicity of caramel is reported to be due to the presence of two minor contaminants: hydroxypyridines and hydroxypyrazines. One of these, 2-Acetyl-4(5)-tetra- hydroxybutylimidazole (THI), has been isolated and has been shown to be the major, if not only, compound responsible for the reduction in number of lymphocytes associated with high oral doses of caramel. In a study in which rats were exposed to a caramel colour containing low levels of THI, the NOAEL was found to be 20 g/kg, whilst the NOAEL for THI was found to be 0.38 mg/kg for males and 0.12 mg / kg for females [MacKenzie *et al.*, 1992a].

Behavioural data

No data identified

***In vitro* toxicity status**

Carcinogenicity and Mutagenicity

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 caramel colour at levels up to 500 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 caramel at 16,000 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].

Caramel colour has been shown to produce equivocal results when tested in *in vitro* assay systems. It has been suggested that it is mutagenic in the Ames test [Ishidate *et al.,* 1984], although a more recent study demonstrated that caramel colours exhibited no genotoxic potential in a number of *Salmonella typhimurium* strains [Allen *et al.*, 1992; Brusick *et al.*, 1992]. Caramels have been shown not to be mutagenic in an *Escherichia coli* assay [Barabasz *et al.*, 1990], or in a *Saccharomyces cerevisiae* gene conversion assay [Brusick *et al.*, 1992].

Two samples of caramel colour I, caramel colour II, caramel colour III have been reported to be non mutagenic in the Ames assay with Salmonella typhimurium strains TA 98, TA 100, TA 1537, TA 1535 and TA1538 both with and without metabolic activation at concentrations up to 20 μl per plate [WHO, 1987].

Thirteen commercial caramels were examined for mutagenicity in strains of *Salmonella typhimurium* [TA 100 and TA 98] with and without S9 and for DNA damaging effects in *Escherichia coli*. No compound was genetically active against either *Salmonella typhimurium* or *Escherichia coli* [HSDB, 2001].
The genetic activity of 2 commercial caramel preparations, manufactured either by heating the malt sugar solution directly (non-ammoniated caramel) or by heating it with ammonia (ammoniated caramel) was studied in the *Salmonella* mutagenicity test and UDS assay in cultured mammalian cells. The non-ammoniated caramel was found to be mutagenic to *S. typhimurium* TA100, while the ammoniated preparation was genetically active in all the tester strains used, namely TA100, TA97 and TA98 [dilutions unspecified]. It was also reported that non-ammoniated caramel was capable of inducing UDS in cultured human amnion FL cells, but for the ammoniated preparation, no such activity was observed. Furthermore, based on the results obtained in the DNA synthesis inhibition assay, it was suggested that the DNA synthesis inhibition seen with the ammoniated caramel was probably not of DNA damage in origin. These data indicate that the mutagenic fractions formed during ammoniated and non-ammoniated caramelisation were quite different [Yu *et al*., 1984].

Although a report has been published on the positive clastogenicity effects of caramel colours in Chinese Hamster Fibroblast cells exposed for up to 48 hours to caramel at doses up to 10 mM [Ishidate *et al.,* 1984], other authors failed to find the same effects in CHO cells [Allen *et al.*, 1992; Brusick *et al.*, 1992]. Ishidate *et al*, (1984) did however comment [with regard to their study] that the results obtained for caramel were entirely based on a primary screening *in vitro* study and that such short-term tests do not necessarily reflect the long-term *in vivo* toxicity [Ishidate *et al*., 1984].

Caramelised sucrose samples were tested for mutagenic activity using *Salmonella typhimurium* strains TA98 and TA100 and tested for mitotic recombination and clastogenicity in Saccharomyces D7 yeast strain and Chinese hamster ovary cells (CHO), respectively. There was no mutagenicity reported in the TA98 strain and positive results were observed with the TA100 strain. Mitotic recombination and clastogenic activity were induced in yeast and CHO cells when exposed to caramelised sucrose. These mutagenic properties where reduced when pre-incubated with S9 fraction. Caramelised sucrose was fractionated into volatile and non-volatile components and tested for clastogenic activity in CHO cells. Volatile components were responsible for 10 % of clastogenicity, which was enhanced in the presence of S9 fraction, with non-volatile components causing the highest clastogenic activity (Kitts *et al*., 2006).

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 caramel colour at levels up to 196 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".

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 caramel colour at levels up to 228 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

PYROLYSIS AND TRANSFER STUDIES

In an extension to the Baker and Bishop (2004) study, a further 159 ingredients were analysed. Under pyrolysis of caramel colour breakdown product included acetic acid (42.7%), theobromine (25.6%), long chain hydrocarbons (12.2%), methylfurfural (6.6%), acetylfuran (3.2%) and furfural (2.5%) [Baker and Bishop, 2005].

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