SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of Brilliant Blue FCF (E 133) as a food additive[1]

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion re-evaluating the safety of Brilliant Blue FCF (E 133). Brilliant Blue FCF has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1970 and the EU Scientific Committee for Food (SCF) in 1975. Both committees established an ADI of 12.5 mg/kg bw/day. In 1984, the SCF revised the ADI to 10 mg/kg bw/day, based on new long-term studies. The Panel concluded that the present dataset on the absorption, distribution, metabolism and excretion, genotoxicity, subchronic, reproductive, developmental and long-term toxicity, and carcinogenicity give reason to revise the ADI of 10 mg/kg bw/day allocated by the SCF in 1984. The Panel considered that the NOAEL of 631 mg/kg bw/day from the chronic toxicity study in rat can be used to allocate a new ADI to Brilliant Blue FCF. By application of an uncertainty factor of 100, the Panel established a new ADI to Brilliant Blue FCF equal to 6 mg/kg bw/day. The Panel concluded that at the maximum reported levels of use of Brilliant Blue FCF, refined intake estimates (Tier 3) are lower than the ADI of 6 mg/kg bw/day. The Panel concluded that at Tier 2 the intake estimates are below the ADI at the mean for both adults and children and at the higher level for adults, but above the ADI at the higher level (95th percentile) for children.

KEY WORDS

Brilliant Blue FCF, E 133, CAS Registry Number 3844-45-9, EINECS number 223-339-8, N-ethyl-N(4-[(4-ethyl[(3-sulphophenyl)methyl]-amino]phenyl)(2-sulphophenyl)methylene]-2,5-cyclohexadien-1-ylidene)-3-sulphobenzenemet-hanaminium hydroxide inner salt, disodium salt, food colouring substance.

---

1 On request from the European Commission, Question No EFSA-Q-2008-233, adopted on 7 October 2010.

2 Panel members: F. Aguilar, B. Dusemund, P. Galtier, J. Gilbert, D.M. Gott, S. Grilli, R. Gürtler, J. König, C. Lambré, J-C. Larsen, J-C. Leblanc, A. Mortensen, D. Parent-Massin, I. Pratt, I.M.C.M. Rietjens, I. Stankovic, P. Tobback, T. Verguieva, R.A. Woutersen. Correspondence: ans@efsa.europa.eu

3 Acknowledgement: The Panel wishes to thank the members of the ANS Working Group B on Food Additives and Nutrient Sources: M. Bakker, D. Boskou, B. Dusemund, D. Gott, T. Hallas-Møller, A. Hearty, J. König, D. Marzin, D. Parent-Massin, I.M.C.M. Rietjens, G.J.A. Speijers, P. Tobback, T. Verguieva, R.A. Woutersen for the preparatory work on this scientific opinion.

Suggested citation: EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Scientific Opinion on the re-evaluation of Brilliant Blue FCF (E 133) as a food additive. EFSA Journal 2010; 8(11):1853. [36 pp.]. doi:10.2903/j.efsa.2010.1853. Available online: www.efsa.europa.eu

SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to provide a scientific opinion re-evaluating the safety of Brilliant Blue FCF (E 133) when used as a food colouring substance.

Brilliant Blue FCF (E 133) is a triarylmethane dye authorised as a food additive in the EU and has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1970 and the EU Scientific Committee for Food (SCF) in 1975. Both committees have established an ADI of 12.5 mg/kg bw/day. In 1984, the SCF revised the ADI to 10 mg/kg bw/day, based on new long-term studies.

The JECFA assigned an ADI of 12.5 mg/kg bw/day to Brilliant Blue FCF in 1970. This ADI was based on a No-Observed-Adverse-Effect Level (NOAEL) of 5% in the diet, equivalent to 2500 mg/kg bw/day. This value is probably derived from the chronic rat study, in which no effects were observed up to the highest dietary dose level of 5% (equivalent to 2500 mg/kg bw/day). An uncertainty factor of 200 was applied, probably because the study dossier was incomplete.

The SCF in 1975 established an ADI of 12.5 mg/kg bw/day. In 1984 the SCF had additional chronic toxicity data available which revealed a slight reduction in body weight of female mice and rats at the highest dose level. The NOAEL was set at 2% of the diet, equivalent to 1073 mg/kg bw/day in male and 1318 mg/kg bw in female, from which a revised ADI of 10 mg/kg bw/day was derived for Brilliant Blue FCF. An uncertainty factor of 100 seems to have been applied.

Data available on the absorption, distribution, metabolism and excretion of Brilliant Blue FCF, show that Brilliant Blue FCF is poorly absorbed and mainly excreted unchanged in faeces.

Subchronic toxicity studies are available in mouse, rat, and dog. The study in mouse has been carried out according to old protocols and is of limited value. One study in rat has been performed using two mixtures (A and B) of food colours. As Brilliant Blue FCF is not administered alone, these data can not be used for a specific safety evaluation of Brilliant Blue FCF. In a study performed on groups of rats, no gross lesions or microscopic pathology were attributed to ingestion of Brilliant Blue FCF. The NOAEL was considered to be 2%, equivalent to 1000 mg/kg bw/day. Beagle dogs were fed Brilliant Blue FCF in the diet for periods up to 1 year. The Panel considered that the NOAEL is 2% in the diet, equivalent to 500 mg/kg bw/day.

Brilliant Blue FCF showed no evidence of mutagenicity when tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 with or without metabolic activation. Brilliant Blue FCF has been tested *in vivo* in a mouse micronucleus assay and Comet assays. In all cases, no micronucleus induction nor DNA damage were observed. Based on these data, the Panel considered that Brilliant Blue FCF is not of concern with respect to genotoxicity.

Chronic toxicity studies available have been performed in mouse and rats before publication of OECD guidelines. One study in mouse did not show any carcinogenic effect of Brilliant Blue FCF. The Panel concluded that the NOAEL is 2000 mg/kg bw/day, the highest dose tested. In the second study in mouse, Brilliant Blue FCF was administered in the diet. The female of the highest dose group exhibited an increased incidence of haemangiomas of the spleen. The Panel agreed with the authors that the haemangiomas can be considered spontaneous and not related to the exposure to Brilliant Blue FCF. In this study, the NOAEL is considered to be 7354 mg/kg bw/day.

Two studies performed in rat were carried out according to old protocols, no treatment-related tumours were found, but these studies are of limited value, due to poor protocol description.

A chronic toxicity study in 30 Wistar rats was performed for 75 weeks. No treatment-related abnormalities were observed. The Panel considered that the NOAEL of this study is 1500 mg/kg bw/day, the highest dose tested.

A chronic toxicity study was also performed in Osborne-Mendel rats for 2 years. No relevant adverse effects were described. The Panel concluded that the NOAEL of this study is 5% Brilliant Blue FCF in the diet, equivalent to 2500 mg/kg bw/day, the highest dose tested.

A chronic toxicity study coupled with a reproductive study has been performed on Charles Rivers CD rats. This study was conducted with an *in utero* phase in which the compound was administered to the F0 generation. There was no dose-related trend in survival in either males or females. Survival was significantly decreased in the highest dose tested in females (1318 mg/kg bw/day) compared to controls. Histopathological evaluations revealed a variety of lesions, including neoplasm among treated and control rats. These were mainly isolated findings of commonly occurring tumours showing no dose-response relationship. Based on these arguments, the Panel concluded that these neoplastic lesions were not indicative of a carcinogenic effect. The Panel considered that the NOAEL established at this study is 631 mg/kg bw/day.

A reproductive study on three successive generations of male and female rats is available. The mean body weights of the high dose group were lower than the control group mean weights in nursing offspring and in males and females of the F1 and F2 generations; no other effects attributable to the colour were observed. The Panel noted that no further details are available. Animal number per group, way of administration, and statistical significance of the data are not specified. Consequently, no NOAEL can be derived from this study.

A reproductive toxicity study in rat was coupled with the chronic toxicity study. There were no compound-related effects on fertility, gestation, parturition, lactation, pup survival through weaning, or on number of live and stillborn pups. The Panel concluded that the NOAEL is 1073 mg/kg bw/day in males, the highest doses tested.

Two developmental studies are available. Brilliant Blue FCF was given orally to groups of mated female Long Evans rats. No signs of fetal toxicity or anomalies were observed which were attributable to the administration of the colour Brilliant Blue FCF orally to mated female rabbits (New Zealand White). No signs of fetal toxicity or anomalies were observed which were attributable to the administration of the colour. The Panel noted that no further details are available. Animal number per group, way of administration, and statistical significance of the data are not specified. Consequently, no NOAEL can be derived from these studies.

The Panel concluded that the present dataset on the absorption, distribution, metabolism and excretion, genotoxicity, subchronic, reproductive, developmental and long-term toxicity, and carcinogenicity give reason to revise the ADI of 10 mg/kg bw/day allocated by SCF in 1984. Subchronic toxicity studies are sufficiently documented to be used for the risk assessment. Chronic toxicity studies showed that Brilliant Blue FCF is not carcinogenic. Among the five chronic toxicity studies, the lowest NOAEL comes from the more recent chronic toxicity study. Observed adverse effects are decrease in terminal mean body weight and decreased survival in the highest dose tested (1318 mg/kg bw/day). The Panel agreed with the authors to a NOAEL of 631 mg/kg bw/day. In the oldest reproductive study the mean body weights of the higher dose group (1000 mg/kg bw/day) were lower than the control group mean weights in nursing offspring and in males and females of the F1 and F2 generations. However, in a more recent study in rat, no effects have been observed at all doses tested (62, 631 and 1318 mg/kg bw/day). The Panel considered that this second study invalidated the results of the first one. No adverse effects have been described in developmental studies in rat and rabbit.

The Panel considered that the NOAEL of 631 mg/kg bw/day derived from the chronic toxicity study in rat can be used to allocate a new ADI for Brilliant Blue FCF. By application of an uncertainty factor of 100, the Panel established a new ADI for Brilliant Blue FCF equal to 6 mg/kg bw/day.

The dietary exposure to Brilliant Blue FCF was estimated by the Panel based on the maximum permitted levels (MPLs) of use, by applying the Budget method (Tier 1) with the assumptions described in the report of the Scientific Cooperation (SCOOP) Task 4.2. The Panel calculated a theoretical maximum daily exposure of 8.1 mg/kg bw/day for adults, and 13.1 mg/kg bw/day for a typical 3 year-old child.

Refined exposure estimates have been performed both for children and the adult population according to the Tier 2 and Tier 3 approaches described in the SCOOP Task 4.2, which combines, respectively, detailed individual food consumption information from the population with the MPLs of use as specified in the Directive 94/36/EC on food colours (Tier 2), and with the maximum reported use levels of Brilliant Blue FCF listed in Table 3 (Tier 3), as identified by the Panel from the data made available by the UK Food Standards Agency (FSA), the Food Safety Authority of Ireland (FSAI), the Union of European Beverage Associations (UNESDA), and the Confederation of the Food and Drink Industries of the EU (CIAA). For children (1-10 years old), estimates have been calculated for the UK and for eleven European countries as part of the EXPOCHI consortium (Belgium, France, the Netherlands, Spain, Italy, Finland, Greece, Cyprus, Czech Republic, Sweden and Germany). For the adult population, the Panel has selected the UK population as representative of the EU consumers for Brilliant Blue FCF exposure estimates.

When considering MPLs (Tier 2), the mean dietary exposure to Brilliant Blue FCF for European children, including UK children (aged 1-10 years), ranged from 0.5 to 3.4 mg/kg bw/day, and from 1.2 to 7.2 mg/kg bw/day at the 95th percentile. Estimates reported for the UK adult population give a mean dietary exposure of 0.9 mg/kg bw/day and of 3.3 mg/kg bw/day for high level (97.5th percentile) consumers.

When considering the maximum reported use levels (Tier 3), the mean dietary exposure to Brilliant Blue FCF for European children, including UK children (aged 1-10 years) ranged from 0.2 to 2.1 mg/kg bw/day, and from 0.6 to 4.8 mg/kg bw/day at the 95th percentile. Estimates reported for the UK adult population give a mean dietary exposure to Brilliant Blue FCF of 0.6 mg/kg bw/day, and of 3.0 mg/kg bw/day for high level (97.5th percentile) consumers.

The Panel concluded that at the maximum reported levels of use of Brilliant Blue FCF, refined intake estimates (Tier 3) are lower than the ADI of 6 mg/kg bw/day. The Panel concluded that at Tier 2 the intake estimates are below the ADI at the mean for both adults and children and at the higher level for adults, but above the ADI at the higher level (95th percentile) for children.

The Panel further noted that the specifications for Brilliant Blue FCF need to be updated with respect to the percentage of material not accounted for that may represent sodium chloride and/or sodium sulphate as the principal uncoloured components.

The Panel noted that the JECFA specification for lead is $\leq$ 2 mg/kg whereas the EC specification is $\leq$10 mg/kg.

The Panel noted that the aluminium lake of the colour could add to the daily intake of aluminium for which a TWI of 1 mg aluminium/kg bw/week has been established and that therefore specifications for the maximum level of aluminium in the lakes may be required.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

According to the framework Directive 89/107/EEC[4] on food additives, the Scientific Committee on Food (SCF) should be consulted before the adoption of provisions likely to affect public health, such as the drawing up of lists of additives and the conditions for their use. Accordingly, all food additives, prior to their authorization, have been evaluated for their safety by the SCF or by its successor, the European Food Safety Authority (EFSA).

Directive 89/107/EEC as well as Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives[5] which will apply as from 20 January 2010, require that food additives must be kept under continuous observation and must be re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. In addition Regulation (EC) No 1333/2008 requires that all food additives which were permitted before 20 January 2009 shall be subject to a new risk assessment carried out by EFSA.

In accordance with Regulation (EC) No 1333/2008, the Commission should, after consultation with EFSA, set up by 20 January 2010 an evaluation programme for EFSA to re-evaluate the safety of the permitted food additives. That programme will define the needs and the order of priorities according to which the approved food additives are to be examined.

Food colours were among the first additives to be evaluated therefore, many of the evaluations are old. For some of these colours many new studies have become available and the results of these studies should be included in the evaluation. Therefore, food colours should be evaluated with priority. The order of priorities for the re-evaluation of the remaining permitted food additives will be set in the Regulation for the re-evaluation program.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission asks the European Food Safety Authority to start a systematic re-evaluation of all authorised food additives and to issue scientific opinions on these additives, taking into account that colours as a group should be given the highest priority for the reasons outlined above.

[4] OJ L 40, 11.2.1989, p. 27
[5] OJ L 354, 31.12.2008, p. 16.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of Brilliant Blue FCF (E 133) when used as a food colouring substance.

Brilliant Blue FCF (E 133) is a dye authorised as a food additive in the EU and was previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1970, and the EU Scientific Committee for Food (SCF) in 1984.

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

2. Technical data

2.1. Identity of the substance

Brilliant Blue FCF (E 133) is a triarylmethane food colour. It consists essentially of *N*-ethyl-*N*-(4-[(4-ethyl[(3-sulphophenyl)methyl]-amino]phenyl) (2-sulphophenyl)methylene]-2,5-cyclohexadien-1-ylidene)-3-sulphobenzenemet-hanaminium hydroxide inner salt, disodium salt, soluble in water and slightly soluble in ethanol.

The CAS Registry Number is 3844-45-9, the EINECS number 223-339-8 and the Colour Index number 42090. The molecular formula is $C_{37}H_{34}N_2Na_2O_9S_3$, and its molecular weight is 792.85 g/mol. The structural formula is given in Figure 1:

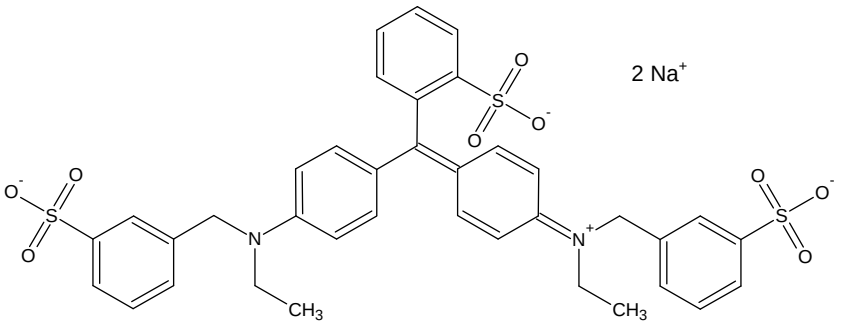

Figure 1: Structural formula of Brilliant Blue FCF

At least 29 synonyms are in use (ChemIDplus advanced, via internet, 2006). The most commonly used synonyms in published literature are Brilliant Blue FCF, FD&C Blue No. 1, and Food Blue No.1.

2.2. Specifications

Specifications have been defined in Commission Directive 2008/128/EC[6] and by JECFA (JECFA, 2006) (Table 1).

Brilliant Blue FCF is described as consisting essentially of disodium α-(4-(*N*-ethyl-3-sulphonatobenzylamino) phenyl)-α-(4-*N*-ethyl-3-sulphonatobenzylamino) cyclohexa-2,5-dienylidene) toluene-2-sulfphonate and its isomers and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components. Brilliant Blue FCF is described as the sodium salt. The calcium and the potassium salt are also permitted (2008/128/EC).

The purity is specified as not less than 85% total colouring matters, calculated as the sodium salt and including less than 6% subsidiary colouring matters. From the definition, it may be assumed that the missing 15% may be accounted for by sodium or calcium chloride or sodium or calcium sulphate (but this is never mentioned explicitly), not more than 0.2% water insoluble matter, not more than 6% subsidiary colouring matters, not more than 1.5% as the sum of 2-, 3- and 4-formylbenzene sulphonic acids, not more than 0.3% 3-((ethyl) (4-sulphophenyl)amino) methylbenzene sulphonic acid, not more than 5% leuco base and not more than 0.01% unsulphonated primary aromatic amines, originating from the manufacturing process.

Thus, if the existing specifications would be extended to include < 15% of sodium chloride and/or sodium sulphate as the principal uncoloured components, most of the material would be accounted for.

Table 1: Specifications for Brilliant Blue FCF according to Commission Directive 2008/128/EC and JECFA (JECFA, 2006)

Purity	Commission Directive 2008/128/EC	JECFA (2006)
Water insoluble matter	≤ 0.2%	≤ 0.2%
Subsidiary colouring matters	≤ 6.0%	≤ 6.0%
Sum of 2-,3- and 4-formylbenzene sulphonic acids	≤ 1.5%	≤ 1.5%
3-((ethyl)(4-sulphophenyl)amino) methylbenzene sulphonic acid	≤ 0.3%	≤ 0.3%
Leuco base	≤ 5.0%	≤ 5.0%
Unsulphonated primary aromatic amines	≤ 0.01% (calculated as aniline)	≤ 0.01% (calculated as aniline)
Ether extractable matter	≤ 0.2% (at pH 7)	≤ 0.2%
Arsenic	≤ 3 mg/kg	≤ 3 mg/kg
Lead	≤ 10 mg/kg	≤ 2 mg/kg
Mercury	≤ 1 mg/kg	-
Chromium	-	≤ 50 mg/kg
Cadmium	≤ 1 mg/kg	-
Heavy metals (as Pb)	≤ 40 mg/kg	-

Kusaka et al. (1999) detected a red subsidiary colour (magenta colour) in a commercial sample of Brilliant Blue FCF. This colour was identified (on the basis of MS and NMR spectroscopy), as the disodium salt of 2-{{4-{N-ethyl-N-(3-sulphophenylmethyl)amino}phenyl}{4-oxo 2,5-cyclohexa-dienylide acid. Twenty four commercial samples of Brilliant Blue FCF were found to contain this subsidiary colour at levels ranging from 0.1% to 0.8%. Matsufuji et al. (1998) reported five subsidiary colours, derivatives of benzenesulphonic acid, present in commercial Brilliant Blue FCF. Tsuji et al.(2001), reported that in a survey one sample of Brilliant Blue FCF aluminium lakes contained the violet subsidiary colour identified as 2-{{4-ethyl-N-(3-sulphophenylmethyl)amino}phenyl}{4-

[6] Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colours for use in foodstuffs. OJ L 6, 10.1.2009, p. 20.

hydroxyphenyl}methylio}benzenesulphonic acid with a relative content in relation to Brilliant Blue FCF of 39.5%.

The Panel noted that the specifications on the purity of Brilliant Blue FCF would permit concentrations of unsulphonated aromatic amines to be present in concentrations of up to 100 mg/kg Brilliant Blue FCF.

Given that the maximal allowed concentration of Brilliant Blue FCF that can be added to food is 500 mg/kg food, the concentration of these amines in food are allowed to be up to 50 µg/kg food.

The Panel noted that the JECFA specification for lead is ≤ 2 mg/kg whereas the EC specification is ≤ 10 mg/kg.

According to EU legislation (2008/128/EC), the above purity criteria for the pure substance also apply to the raw material from which the aluminium lake is produced. In addition, the aluminium lake should contain no more than 0.5% HCl-insoluble material, and no more than 0.2% ether-extractable material under neutral conditions. There are no additional specification requirements for the aluminium lake.

JECFA does not give specifications for aluminium lakes of Brilliant Blue FCF other than reference to the General Specifications for Aluminium Lakes of Colouring Matters (JECFA, 2004). The Brilliant Blue FCF used in the production process should comply with the specifications as given above, and the aluminium lake should contain not more than 2% water-soluble chlorides and sulphates calculated as sodium salts, not more than 0.5% HCl-insoluble matter, 0.2% ether-extractable matter, 3 mg arsenic/kg and 5 mg lead/kg. Unreacted aluminium oxide may also be present in the final product (not specified).

The Panel noted that the aluminium lake of the colour could add to the daily intake of aluminium for which a Tolerable Weekly Intake (TWI) of 1 mg aluminium/kg bw/week has been established (EFSA, 2008) and that therefore specifications for the maximum level of aluminium in the lakes may be required.

2.3. Manufacturing process

No data on the manufacture of Brilliant Blue FCF are available. Brilliant Blue FCF may be converted to the corresponding aluminium lake under aqueous conditions by reacting aluminium oxide with the colouring matter. Undried aluminium oxide is usually freshly prepared by reacting aluminium sulphate or aluminium chloride with sodium carbonate or sodium bicarbonate or aqueous ammonia. Following lake formation, the product is filtered, washed with water and dried (JECFA, 2004).

2.4. Methods of analysis in food

Methods described in the literature for the detection of Brilliant Blue FCF are based on spectrophotometry (Vidotti et al., 2005; Li, 2008), HPLC (Kirschbaum and co-workers, 2003, Pereira Alves et al., 2008; Vachirapatama et al., 2008), ion chromatography (Chen et al., 1998), cathodic stripping voltametry (Florian et al., 2002) and capillary electrophoresis (Ishikawa et al., 2004).

Li (2008) proposed a solid phase extraction followed by direct spectrophotometric determination of Brilliant Blue FCF in food samples. Kirschbaum and co-workers (2003) developed an HPLC–DAD method for the determination of permitted colorants including Brilliant Blue FCF. A similar technique was used by Pereira Alves et al. (2008) for the determination of Brilliant Blue FCF and other colorants in solid juice powders, solid jelly powders and soft drinks. HPLC on a reversed phase C18 column

was also proposed by Vachirapatama et al. (2008) for the analysis of Brilliant Blue FCF and six other synthetic colorants in samples of foodstuffs and soft drinks.

2.5. Reaction, stability and fate in food

No data on reaction and fate of Brilliant Blue FCF in food are available. The Panel considered that in general, the majority of colour additives are unstable in combination with oxidising and reducing agents in food. Since colour depends on the existence of a conjugated unsaturated system within the dye molecule, any substance which modifies this system (e.g. oxidising or reducing agents, sugars, acids, and salts) will affect the colour (Scotter and Castle, 2004).

2.6. Case of need and proposed uses

Currently, Brilliant Blue FCF is an allowed synthetic food colouring substance in the EU with a maximal allowed use level of 20 to 500 mg/kg food for various foodstuffs. Brilliant Blue FCF is also allowed in beverages at levels up to 200 mg/1. Table 2 summarizes those beverages and foodstuffs that are permitted to contain Brilliant Blue FCF up to specified maximum permitted levels (MPLs) set by Directive 94/36/EC[7] on colours for use in foodstuffs.

Table 2: Maximum Permitted Levels of use of Brilliant Blue FCF in beverages and foodstuffs according to the European Parliament and Council Directive 94/36/EC and maximum reported use levels of Brilliant Blue FCF in beverages and foodstuffs used for the refined exposure assessment (Annex A).

Beverages	**Maximum Permitted Level** (mg/l)	**Maximum reported use level** (mg/1)
Fruit wines, cider and perry	200	5[1]
Non-alcoholic flavoured drinks	100	65[1]
Liquid food supplements/dietary integrators	100	100[2]
Spirituous beverages	200	200[1]
Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails	200	200[2]
Foodstuffs	**Maximum Permitted Level** (mg/kg)	**Maximum reported use level** (mg/kg)
Complete formulae and nutritional supplements for use under medical supervision	50	20[1]
Complete formulae for weight control intended to replace total daily food intake or an individual meal Soups	50	50[2]
Flavoured processed cheese Fish paste and crustaceans paste Smoked fish Savoury snack products and savoury coated nuts Meat and fish analogues based on vegetable proteins	100	100[2]
Desserts including flavoured milk products	150	0.1[1]
Edible ices	150	145[1]
Fine bakery wares	200	200[1]
Candied fruit and vegetables, Mostarda di frutta	200	200[2]

[7] European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. OJ L 273, 10.9.94, p.13

Preserves of red fruits Extruded or expanded savoury snack products		
Pre-cooked crustaceans	250	250[2]
Confectionery	300	300[1]
Mustard Fish roe Solid food supplements/dietary integrators	300	300[2]
Sauces, seasonings, pickles, relishes, chutney and piccalilli Salmon substitutes Surimi	500	500[2]
Decorations and coatings	500	500[1]
Edible cheese rind and edible casings	*Quantum satis*	100[3]
Edible casings	*Quantum satis*	500[3]

[1] Maximum use level or maximum level determined by analysis.
[2] Maximum permitted level.
[3] *quantum satis* data.

2.6.1. Actual levels of use of Brilliant Blue FCF

More information on current use levels was made available to the Panel for several food categories in finished products.

2.6.1.1. Beverages

For non-alcoholic flavoured drinks, a usage survey conducted by the Union of European Beverage Associations (UNESDA) in 2005 suggested that the highest current use level of Brilliant Blue FCF in beverages was 40 mg/l (Tennant, 2006). The UK Food Standards Agency (FSA) conducted an *ad hoc* survey in which artificial colours were analytically determined in 201 ready-to-drink retail soft drinks selected for being distinctly coloured (FSA, 2003). Brilliant Blue FCF was found to be present at a level higher than 0.1 mg/l (Limit of Detection - LOD) in 14 products, with levels varying from 0.9 to 15 mg/l. In another survey, conducted in 2005-2006 by the Food Safety Authority of Ireland (FSAI), Brilliant Blue FCF was present at a level higher than 1.0 mg/l (Limit of Quantification - LOQ) in 2 of the 55 soft drinks; the concentration in these products varied from <1 to 8.2 mg/l (FSAI, 2009). In October 2009, the Confederation of the Food and Drink Industries of the EU (CIAA, 2009) reported typical use levels of 0.5 to 65 mg/l. For spirituous beverages, including products with less than 15% alcohol, the CIAA (2009) reported a maximum use level of 200 mg/l and in fruit wines, cider and perry, CIAA reported a maximum use level of 3 mg/l. Table 3 provides a review of the data provided on use levels of Brilliant Blue FCF in beverages.

Table 3: Review of data provided on use levels of Brilliant Blue FCF in beverages.

Beverages	**Data provided by**	**Reported typical use levels** mg/l	**Reported extreme use levels** mg/l
Non-alcoholic flavoured drinks	FSA, 2003[1]	0.3	0.9-15
	Tennant, 2006		40
	FSAI, 2009[1]		<1-8.2
	CIAA, 2009	0.5-65	65
Spirituous beverages	CIAA, 2009	0-50	200
Fruit wines, cider and perry	CIAA, 2009	1	3

[1] Levels determined by analysis

2.6.1.2. Foodstuffs

Within the *ad hoc* survey conducted by the FSA provided to the Panel, artificial colours were analytically determined in 195 retail samples of brightly coloured packaged sweets selected for being distinctly coloured (FSA, 2002). Brilliant Blue FCF was found to be present at level higher than 0.5 mg/kg (LOD) in 20 of the products, with levels varying from 0.2 to 27.1 mg/kg. According to the FSAI data, Brilliant Blue FCF was present at a level higher than 2.0 mg/kg or 5.0 mg/kg (LOQ) in 38 (23.6%) of 161 confectionery products, with levels varying from <1 to 37.9 mg/kg (FSAI, 2009). The Panel was also provided with data from the CIAA which reported typical use levels ranging from 0.85 to 300 mg/kg (CIAA, 2009).

For decorations and coatings, in the FSAI survey (2009) Brilliant Blue FCF was present in 1 out of 3 samples above the LOQ (2 mg/kg), at a level of 9.8 mg/kg. CIAA (2009) reported a range of typical levels from 4 to 500 mg/kg. For edible ices, the FSAI survey (2009) gave analytical values of Brilliant Blue FCF ranging from <1 to 10.3 mg/kg for 5 out of 30 retail samples, and CIAA reported a range of typical use values from 5-145 mg/kg. For sauces, seasonings, pickles, relishes, chutney and mustard, the FSAI survey (2009) gave a range of analytical values from < 2 to < 10 mg/kg for 2 out of 5 retail samples. For desserts, including flavoured milk products, the CIAA (2009) reported typical use levels of Brilliant Blue of 0.1 mg/kg. For fine bakery wares, the CIAA reported typical use levels of 2 to 200 mg/kg and for complete formulae and nutritional supplements for use under medical supervision CIAA reported typical use levels of 20 mg/kg.

For all other food groups where Brilliant Blue FCF is legally permitted, its levels were not found to be above the limit of detection or quantification in any survey from which data were provided to the Panel.

In order to refine the exposure assessment for children and adults to food colours, the Panel has defined some rules to identify maximum reported use levels based on maximum actual usage or maximum analytical data for Brilliant Blue from food uses. The rules followed in order to deal with *quantum satis* authorisation, with usage data or observed analytical data, for all regulated colours re-evaluated by the Panel, are given in Annex A. Table 2 summarises the maximum reported use levels of Brilliant Blue in beverages and foodstuffs used for the refined exposure assessment. They have been defined by applying the rules reported in Annex A, to the data available to EFSA. Table 4 provides a review of the data provided on use levels of Brilliant Blue FCF in foods.

Table 4: Review of data provided on use levels of Brilliant Blue in foodstuffs.

Foodstuffs	Data provided by	Reported typical use levels mg/l	Reported extreme use level mg/l
Edible ices	FSAI, 2009[1]	<2	<1-10.3
	CIAA, 2009	5-145	5-145
Sauces, seasonings, pickles, relishes, chutney and mustard	FSAI, 2009[1]		<2-<10
Desserts including flavoured milk products	CIAA, 2009	0.1	
Fine bakery wares	CIAA, 2009	2-200	
Complete formulae and nutritional supplements for use under medical supervision	CIAA, 2009	20	
Confectionery	CIAA, 2009	5-300	0.85-300
	FSAI, 2009[1]	<2	<1-37.9
	FSA, 2002[1]	0.5	0.2-27.1
Decorations and coatings	CIAA, 2009	22-120	4-500
	FSAI, 2009		9.8

[1] Levels determined by analysis

2.7. Information on existing authorisations and evaluations

Brilliant Blue FCF is authorised as a food additive in the EU under Directive 94/36/EC. Specific purity criteria on Brilliant Blue FCF have been defined in the EU Directive 2008/128/EC and JECFA (2006).

Brilliant Blue FCF has been evaluated previously by JECFA in 1970 and the SCF in 1979. Both committees have established an ADI of 12.5 mg/kg bw/day Brilliant Blue FCF. In 1984, the SCF revised the ADI to 10 mg/kg bw/day Brilliant Blue FCF, on the basis of new long-term studies. Brilliant Blue FCF has been recently evaluated and approved as cosmetic colorant by SCCP in cosmetics (2004).

2.8. Exposure

The Panel agreed to follow the principles of the stepwise approach, which were used in the report of the scientific cooperation (SCOOP) Task 4.2 (EC, 1998), to estimate additives' intakes. For each successive Tier, this involved a further refinement of intake estimates. The approach goes from the conservative estimates that form the first Tier of screening, to progressively more realistic estimates that form the Second and Third Tiers (Annex A).

2.8.2.1. Crude estimates (Budget Method)

The dietary exposure to Brilliant Blue FCF from the maximum permitted use levels was estimated using the Budget method (Tier 1) with the assumptions described in the report of the SCOOP Task 4.2 (EC, 1998).

In the case of Brilliant Blue FCF, the maximum permitted use level in beverages was 200 mg/l (Directive 94/36/EC). The maximum permitted level in solid foods was 500 mg/kg.

The default proportion (25%) of beverages and solid food that could contain the additive was considered adequate. In fact, even though Brilliant Blue FCF may be used in a variety of solid foods that could represent more than 25% of processed foods, it is unlikely that a person would systematically choose all processed foods with the same colour added even considering brand loyalty. This assumes that a typical adult weighing 60 kg consumes daily 1.5 litres of beverages and 375 grams of solid foods containing Brilliant Blue FCF.

The overall theoretical maximum daily exposure for adults would therefore be:

(200 x 0.1 x 0.25) + (500 x 0.025 x 0.25) = 5 + 3.12 = 8.1 mg/kg bw/day.

As recommended by SCOOP task 4.2 (EC, 1998) for children, it is assumed that 100% of beverages contain the additive. This conclusion was derived from UK data on consumption of soft drinks by children aged under 5 years, where the 97.5th percentile of consumption was between 70 and 80 ml/kg bw/day). This assumes that a typical 3 year-old child weighing 15 kg consumes daily 1.5 litres of beverages and 94 g of solid foods containing Brilliant Blue FCF. The overall theoretical maximum daily exposure to Brilliant Blue FCF in children would therefore be:

(100 x 0.1 x 1) + (500 x 0.025 x 0.25) = 10 + 3.12 = 13.1 mg/kg bw/day.

It was noted that Brilliant Blue FCF may be used *quantum satis* in edible cheese rind and edible casings. As this is a very specific food category, which is unlikely to be consumed in high amounts on a daily basis, if at all, it was excluded from the Budget Method calculation, since it is not expected to influence the outcome of this exposure calculation to any relevant extent.

2.8.2.2. Refined estimates

Refined exposure estimates have been performed for Tier 2 using national consumption data and maximum permitted use levels, presented in Table 2, and for Tier 3 using the maximum reported use levels presented in Table 2 for children and the adult population (see Annex A).

For adults, the Panel calculated the exposure based in the UK consumption survey as the UK population is considered to be one of the highest consumers of soft drinks in Europe and also because detailed individual food consumption data (UK NDNS, 2000-2001) are available from the UNESDA report (Tennant et al., 2006).

Exposure estimates for children (1-10 years old) have been performed by the Panel based on detailed individual food consumption data from eleven European countries (Belgium, France, the Netherlands, Spain, Italy, Finland, Greece, Cyprus, Czech Republic, Sweden and Germany) provided by the EXPOCHI ("Individual food consumption data and exposure assessment studies for children") consortium (Huybrechts et al., in press). As the UK is not part of the EXPOCHI consortium, estimates for UK children (aged 1.5 - 4.5 years) were made by the Panel with the use of detailed individual food consumption data (UK NDNS, 1992-1993) available from the UNESDA report (Tennant et al., 2006)

Table 5 summarises the anticipated exposure of children and adults to Brilliant Blue FCF.

Tier 2

In the case of Brilliant Blue FCF, when considering MPLs of use, estimates reported for the UK adult population give a mean dietary exposure to Brilliant Blue FCF ranging of 0.9 mg/kg bw/day and 3.3 mg/kg bw/day for high level consumers (mean consumption plus intake at the 97.5th percentile of 'Spirituous beverages'). The main contributor to the total anticipated mean exposure to Brilliant Blue FCF (>10%) were non-alcoholic flavoured drinks (47%).

The mean dietary exposure of European children (aged 1-10 years and weighing 16-29 kg) considered from the EXPOCHI consortium and UK children ranged from 0.5 to 3.4 mg/kg bw/day, and from 1.2 to 7.2 mg/kg bw/day at the 95th percentile. At Tier 2, the main contributors to the total anticipated exposure to Brilliant Blue FCF (>10% in all countries, these contributions differed per country), were non-alcoholic beverages (15 to 55%), fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (13 to 46%), desserts, including flavoured milk products (12 to 52%) and sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli (11 to 44%). Confectionery accounted for 13% of exposure in one country.

Tier 3

Further data suggest that current use levels of Brilliant Blue FCF in some food categories are lower than the MPLs. Therefore, it was decided that concentration data made available to the Panel by the CIAA, UNESDA, FSA and the FSAI, would be used to refine the estimate of dietary exposure to Brilliant Blue FCF (Tier 3).

When considering the maximum reported use levels from Table 2, estimates reported for the UK adult population give a mean dietary exposure to Brilliant Blue FCF of 0.6 mg/kg bw/day and of 3.0 mg/kg bw/day for high level (97.5th percentile) consumers of 'Spirituous beverages'.

The mean dietary exposure of European children (aged 1-10 years and weighing 16-29 kg) considered by the EXPOCHI consortium and UK children, ranged from 0.2 to 2.01 mg/kg bw/day, and from 0.6 to 4.8 mg/kg bw/day at the 95th percentile. At Tier 3, the main contributors to the total anticipated mean exposure to Brilliant Blue FCF (>10% in all countries), were non-alcoholic beverages (13 to 53%), fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (12 to 64%) and sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli (14 to 60%). Confectionery

accounted for 19 to 24% of exposure in two countries, while extruded or expanded savoury snack products accounted for 17% of exposure in one country.

Table 5: Summary of anticipated exposure to Brilliant Blue FCF using the tiered approach (EC, 2001) in children and the adult population.

	Adult UK population (>18 years old)	**Children UK & EXPOCHI population** (1-10 years old, 15.8-29 kg body weight
	mg/kg bw/day	mg/kg bw/day
Tier 1. Budget method	8.1	13.1
Tier 2. Maximum Permitted Level		
• Mean exposure	0.9	0.5 – 3.4
• Exposure 95th*or 97.5th percentile**	3.3	1.2 – 7.2
Tier 3. Maximum reported use levels		
• Mean exposure	0.6	0.2 – 2.1
• Exposure 95th*or 97.5th percentile**	3.0	0.6 – 4.8

* For EU children, estimates are based on the EXPOCHI report, which gives the 95th percentile intake.
** For UK, estimates are based on the UNESDA report which gives the 97.5th percentile intake from beverages plus *per capita* average from the rest of diet (Tennant, 2006).

3. Biological and toxicological data

3.1. Absorption, distribution, metabolism and excretion

In female Sprague-Dawley rats, 0.27% of the ^{14}C Brilliant Blue FCF radiolabelled on 7-C methylene position (sodium salt) given by gavage at a dose of 1.5 mg/kg bw was absorbed through the gastrointestinal tract. The faeces were the major route of excretion (91.14%) with total recoveries being 92%. Intestinal absorption was estimated by urinary ^{14}C excretion, expired $^{14}CO_2$ and residual radioactivity in internal organs and tissues 96 hours after oral administration. In bile duct ligated rats, intestinal absorption (on the same basis) was 2.05%. The mean faecal excretion was 97.28%, and total recoveries averaged 99.38%. Biliary excretion in bile-cannulated animals amounted to 1.32% of the dose in 96 hours. The level of $^{14}CO_2$ excreted in the expired air was 0.04% of the dose in intact and 0.01% in bile-cannulated animals. Thin layer chromatography of 24-hour urine and bile samples showed that about 95% of the excreted radioactivity was unaltered Brilliant Blue FCF with about 5% being an unidentified metabolite or degradation product. A minor peak of radioactivity with similar chromatographic characteristics was also detected in the dose solution standard at levels from 2 to 4% (over a 4-month storage period) and may represent a decomposition product (Brown et al., 1980).

Following administration of either 30 µg/kg bw or 3 mg/kg bw of ^{14}C-Brilliant Blue FCF by gavage to male or female rats, substantially all of the dose was excreted unchanged in the faeces within 72 hours (99.9% at low dose, 95.4% at high dose). No radioactivity was detected in the expired air and less than 0.5% was detected in the urine. Phillips et al. (1980) considered that this probably represented contamination of the urine from contact with faeces in the metabolism cage. Similar excretion kinetics were seen in pregnant rats given 3 mg Brilliant Blue FCF/kg on day 8 of pregnancy. Pre-treatment with 30 mg/kg/day given in the diet for 21 days prior to gavage dosing with the labelled material had no effect on the route of excretion or time taken to eliminate all the radioactivity. Male mice and guinea pigs excreted practically all of a single dose of Brilliant Blue FCF in the faeces, the urinary content of radioactivity being less than 1% at a dose of 30 µg/kg bw or 3 mg/kg bw. There was some indication that the rate of excretion in the guinea pig and mouse was slower than that seen in the rat. Thin layer chromatography of the radioactive material extracted from the faeces of rats and guinea

pigs receiving a single dose of the colour indicated that the dye was not metabolised during its passage through the gastrointestinal tract.

The lack of absorption and metabolism in the gastrointestinal tract was confirmed by studies in guinea pigs and mice using isolated loops of small intestine.

Less than 0.05% of a labelled dose of 3 mg/kg bw was excreted in the bile of rats over a 5-hour period. Very little radioactivity (0.004 – 0.006% of dose) was detected on day 11 in the fetuses of pregnant rats given ^{14}C-labelled Brilliant Blue FCF orally on day 8 of gestation. In all these studies Brilliant Blue FCF was labelled with ^{14}C in the central methane ring and had a radioactive purity of greater than 95%. The unlabelled material was food-grade complying with the specification given in the 1973 UK regulations (Phillips et al., 1980).

Earlier studies suggested there was neither significant absorption nor biotransformation of Brilliant Blue FCF given at doses of 200 mg per animal in the rat, rabbit and dog. No dye was detected in the urine. A rat study used an unlabelled material and the recovery of the administered dose was 96% in 36 hours. Some absorption was indicated by the fact that small amounts of unchanged Brilliant Blue FCF were excreted in the bile of all three species. Data were reported only for the dogs where 0.7 and 2.8% of the administered dose was recovered in the bile of two animals (Hess and Fitzhugh, 1953; 1954; 1955).

Kuno and Mizutani (2005) investigated the influence of Brilliant Blue FCF on the activities of phase I and phase II drug-metabolizing enzymes (CYP2A6, UGT1A6, and UGT2B7). Their findings indicate that Brilliant Blue FCF was neither a substrate, nor an inhibitor of the enzymes studied.

3.2. Toxicological data

3.2.1. Acute oral toxicity

The JECFA reported one oral acute toxicity test. In this study Lu and Lavallée (1964) found that the LD_{50} in rats was higher than 2000 mg/kg bw.

3.2.2. Short-term and subchronic toxicity

Mouse

The JECFA reported a study in mice after feeding (no detail on number of treated animals) 1200 mg Brilliant Blue FCF over 19 days (approximately 60 mg Brilliant Blue FCF/animal/day); no adverse effects were observed.

Rat

Brilliant Blue FCF caused severe growth retardation in Wistar rats when added to a low fibre diet at a level of 5%. The concurrent addition of dietary fibre from the roots of edible burdock completely protected against this toxic effect (Tsujita et al., 1979).

Male rats received in the diet two mixtures containing Tartrazine, Brilliant Blue FCF, Sunset Yellow and Carmoisine (mixtures A and B) for 30- and 60-day periods at a dose level of 800 mg mixture/kg bw/day (Aboel-Zahab et al., 1997). A third mixture, mixture C, contained Brown HT and Indigo Carmine. The compositions of mixtures A, B, and C were not specified as they were stated to be company secrets and so the concentration of each colour is not reported. The effects on body weight, blood picture, liver and kidney functions, blood glucose, serum and liver lipids, liver nucleic acids

(DNA and RNA), thyroid hormones (T3 and T4) and growth hormone, and histopathological examinations of liver, kidney and stomach sections were evaluated at the end of the treatment period. These parameters were also investigated 30 days after the end of exposure.

Rats fed diets supplemented with both mixture A and B showed significant increases in serum total lipids, cholesterol, triglycerides, total protein, globulin and serum transaminases. Haematological investigations demonstrated selective neutropenia and lymphocytosis (no significant alterations of total white blood cell counts), and significantly decreased haemoglobin concentrations and red blood cell counts. Eosinophilia was noted only in rats receiving mixture A. Histopathological studies showed brown pigment deposition in the portal tracts and Kupffer cells of the liver as well as in the interstitial tissue and renal tubular cells of the kidney. Congested blood vessels and areas of haemorrhage in both liver and renal sections were revealed in rats receiving mixture B. No histopathological effects were recorded in the stomach tissue.

The Panel concluded that the results of this study (Aboel-Zahab et al., 1997) cannot be used as the basis for a re-assessment of the ADI for Brilliant Blue FCF, as the exposure of the experimental animals has been to a mixture of food colours in which the dose level of each colour has not been specified and it is not clear what were the amounts/percentage of the individual colours added in the diet to achieve the cited level of 0.8 g of mixture/kg bw/day.

Dog

Five beagle dogs received a dietary level of 2% Brilliant Blue FCF and 3 dogs a 1% Brilliant Blue FCF dietary level for one year. One dog at the top dose died after 17 days and another died after 46 weeks at the low dose, due, in the view of the authors, to intercurrent virus infections. No clinical signs, gross lesions or histological abnormalities observed were attributable to treatment, but no more details were available (Hansen et al., 1966).

The Panel considered that the NOAEL is 2% in the diet, equivalent to 500 mg/kg bw/day.

3.2.3. Genotoxicity

Brilliant Blue FCF (from commercial sources and complying with FDA specifications, or Australian legislative standards) showed no evidence of mutagenicity when tested at concentrations of 0 to 10000 µg/plate in a number of standard Ames tests in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 with or without metabolic activation (Bonin and Baker, 1980; Bonin et al., 1981; Brown et al., 1978; Auletta et al., 1977; Hayashi et al., 1988; Ishidate et al., 1984; Kawachi et al., 1980; Haveland-Smith and Combes, 1980). Kawachi et al., 1980 reported a negative result in the rec assay in *Bacillus subtilis*.

Brilliant Blue FCF (10 mg/ml and liver S9 fraction) did not exhibit any ability to induce mutation when tested in *Escherichia coli* (Haveland-Smith and Combes, 1980).

In the Ishidate et al. study (1984), 190 synthetic food additives, including Brilliant Blue FCF were tested in an Ames *Salmonella*/microsome test and in a chromosomal aberration test (Chinese hamster fibroblast cell line, CHL). This study gave positive results for Brilliant Blue FCF. However these results were accompanied by excessive variations in the osmolality of the culture medium and were therefore not considered relevant. Kawachi et al., 1980 reported a positive result in a chromosome aberration test, while the authors classified Brilliant Blue FCF as a non-mutagenic carcinogen. However, since no information about the protocol and the dose levels or on the details of the results were reported, this study could not be considered relevant.

Brilliant Blue FCF has been tested *in vivo* in a mouse micronucleus assay. The compound was administered by one to four *i.p* injections (0, 500, 1000, 2000 mg/kg bw). In all cases, no micronucleus induction was observed in bone marrow (Hayashi et al., 1988).

Sasaki et al. (2002) studied the genotoxicity of 39 chemicals currently in use as food additives including Brilliant Blue FCF. They treated groups of four male ddY mice once orally up to the limit dose of 2000 mg/kg bw and performed Comet assays on glandular stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow, 3 and 24 hours after treatment. Brilliant Blue FCF did not yield a statistically significant increase in DNA damage in any organs. Although in this study, several deviations from the standard protocol can be observed, the study seems to be sufficiently reliable not to be disregarded.

Based on the available data, the Panel considered that Brilliant Blue FCF is not of concern with respect to genotoxicity.

3.2.4. Chronic toxicity and carcinogenicity

Chronic toxicity studies in mice and in rats were available. Most of them were performed before the publication of OECD guidelines.

Subcutaneous injections of 10 doses of 4 mg followed by 50 doses of 6 mg Brilliant Blue FCF showed no tumour production after 78 weeks in mouse (ICI, 1962).

Groups of 48 male and 50 female ASH/CS1 mice were given diets containing 0, 0.015, 0.15 or 1.5% of Brilliant Blue FCF (equivalent to 0, 20, 200, 2000 mg/kg bw/day) for up to 80 weeks. There was evidence of a significantly (Students t-test) reduced body weight in female animals in the highest dose group, but the experiment did not establish whether this was a toxic effect or due to unpalatability of the diet resulting in restriction of food intake. Standard haematological examinations conducted on groups of 10 males and 10 females at week 13, 26 and 52 and on all surviving mice at week 80 revealed no consistent treatment related effects.

At week 13, the reticulocyte counts in both sexes given 1.5% Brilliant Blue FCF were significantly lower than those of the controls, and in males there was a similar difference at the 0.15% group; the lowest dose group was not examined at this time point (BIBRA, 1982).

The relative stomach weight of male mice receiving 1.5% Brilliant Blue FCF was significantly reduced. In the females of the highest dose group, the relative brain weight was significantly increased, but this was probably merely a reflection of the reduced body weight, as the absolute weight of this organ was unaffected by treatment.

Full histopathological examination was performed on a wide range of tissues from mice of the control and those given the 1.5% diet (approximately 2000 mg/kg bw/day). At the lower dietary levels, histopathological examination was confined to the liver, kidney and tissues seen to be abnormal at autopsy. The incidence of glomerulonephritis was significantly higher in each of the three groups of male animals when compared individually to the incidence in control group, but with no consistent dose-response (incidence: 14/44 in controls, 24/34 low dose, 17/30 mid dose, 24/44 high dose). No comparable effect was seen in the female animals. An increased incidence of mild liver changes occurred in the male mice given the highest dietary level of Brilliant Blue FCF (incidence of foam cells: 2/44 in controls, 13/44 high dose, incidence of fatty change: 3/44 controls, 11/44 high dose). Eight of the 44 high dose males examined had tumours of the reticulolymphatic system (lymphosarcoma and reticulum cell neoplasm), but this was not a statistically significant increase over the control incidence of 4 out of 44. Tumour pathology seen in the treated mice but not in the controls were squamous cell carcinoma of the stomach (one of the 39 females receiving the highest dose, none in the 44 controls), thyroid adenoma (a single occurrence in the 30 mid-dose males), adrenal tumours

(one medullary tumour in each of the mid and top dose males, and one cortical nodule in the low dose males), a mesenteric lipoma (a single occurrence in the low dose males) and squamous cell carcinoma of the skin (one in the 26 mid-dose females and one in the 39 high dose females). None of these tumour incidences in treated animals were individually statistically significantly higher than the control. The authors stated that stomach and skin carcinoma occur spontaneously in mice, but provided no data specific to the ASH/CS1 strain. It was also stated that adrenal tumours and lipomas are frequently reported in mice. Thyroid tumours were said not to be common in mice, but "that their spontaneous occurrence has been reported". Kidney tumours (6 adenomas and 1 adenocarcinoma) developed in 7 of the 30 male mice examined in the 0.15% dietary group, as compared with a single tumour seen in the 44 controls examined, a statistically significant increase ($P < 0.05$). Only a single kidney tumour was observed in 44 high dose males, and none at all in the low dose male or the 103 females of all treatment groups. The authors stated that kidney tumours are unusual in most strains of mice but are consistently found in the ASH/CS1 strain (BIBRA, 1982).This study did not show any carcinogenic effect of Brilliant Blue FCF.

Brilliant Blue FCF was administered in the diet to 360 mice 4 weeks old (60/sex/group) at dietary levels of 0.5, 1.5 or 5% (equivalent to 661, 2064 and 7354 mg/kg bw/day in male and 819, 2562 and 8966 mg/kg bw/day in female). Mice CD-1, COBS (ICR derived) from Charles Rivers Breeding Laboratories have been used in this study. Brilliant Blue FCF used in this study was certified by US FDA and was found to contain 90% pure colouring. The remaining 10% consisted of subsidiary colourings, volatile chlorides and sulphates, and uncombined intermediates. Both male and female mice received Brilliant Blue FCF for 104 weeks. Clinical observations were conducted twice daily with at least 5 hours between observations. Individual body weight and food consumption were measured weekly for the first 14 weeks, biweekly for week 16-26, and monthly thereafter. Intake was calculated from body weight, food consumption and dietary concentration and was expressed in mg/kg bw/day. Detailed physical examination for signs of toxicity and palpation for masses were conducted weekly. Ten animals from each group were randomly selected for haematology tests after 3, 6, 12, 18 and 24 months of the study. The haematological parameters examined were haemoglobin, haematocrit, erythrocyte and total and differential leukocyte counts. Autopsies were conducted on all animals that died spontaneously, were sacrificed in a moribund condition or as scheduled. Histology was conducted on all animals from the two control groups and the highest dose group (5%). The same tissues, in addition to the gall bladder, were examined histologically in mice as in rats. Organ weights were recorded for the brain, gonads, kidneys, liver, spleen and thyroid and relative organ weight were calculated. Relevant statistical tests were performed. There were enough animals alive at the end of the study to allow the detection of late appearing-tumours. Blue staining of the hair, exposed skin and faeces was noted in all treatment groups.

Mean food consumption throughout the study remained similar for control and treated mice. Statistically significant decreases in body weight ($P < 0.01$) occurred at some intervals for males and females in the 1.5% and 5% group. Haematological values were similar among treated and control animals throughout the study. Occasional statistically significant decreases in mean haemoglobin, haematocrit and leukocytes values of treated mice were noted, but they were not considered as toxicologically significant by the authors. The lifetime exposure of mice to Brilliant Blue FCF as a dietary admixture did not demonstrate consistent biologically significant, compound-related adverse effects on behaviour, morbidity, mortality, haematology, general physical observations or tumour incidence according to the authors. Decreases in mean body weight of treated mice were most likely due to the non-nutritive nature of Brilliant Blue FCF.

Incidence of neoplasms in mice which received Brilliant Blue FCF in the diet was evaluated throughout their lifetimes (60 mice per sex per group). Only grossly detected masses and lesions from animals in the groups receiving 0.5%, 1.5% Brilliant Blue FCF were examined microscopically. A variety of microscopic lesions was observed at similar incidence in control and treated animal. The 5% female group exhibited an increased incidence of haemangiomas in the spleen which was only significant with the Fisher test with unadjusted trend (IRCD, 1981a; Borzelleca et al., 1990).

The Panel agreed with the authors that the haemangiomas were considered spontaneous and not related to the exposure to Brilliant Blue FCF.

The Panel considered that the NOAEL for this study is 7354 mg/kg bw/day for male and 8966 mg/kg bw/day for female, the highest doses tested.

Rat

Five studies were performed in rat, two of which are relatively old.

In the first one, 5 males and 5 females received 4% Brilliant Blue FCF in their diets for 600 days (Willheim and Ivy, 1953). In the second one, 85 rats were fed a diet containing 0.1% of the colour for their life span. No treatment related tumours were found (Klinke, 1955).

Four groups of 30 Wistar rats (15/sex) were administered Brilliant Blue FCF through their diets at dose levels of 0, 0.03, 0.3 or 3% (equivalent to 15, 150, or 1500 mg/kg bw/day) for 75 weeks. Weekly reports of food consumption and food efficiency were kept during the first year, while body weights were recorded weekly until the end of the experiment. Red blood cell counts, haemoglobin estimations and haematocrit reading were done at intervals throughout the test. At the end of the test, the surviving rats were sacrificed and gross examination was made of all organs and tissues. Microscopic examinations were performed on lung, heart liver, spleen, thyroid and pancreas, stomach, small intestine and kidneys, urinary bladder, adrenal, testis, prostate, coagulating gland, ovary, uterus and thymus. No treatment-related abnormalities were observed concerning growth, food consumption, food efficiency, mortality, or haematology (Mannel et al., 1962).

The Panel considered that the NOAEL of this study is 1500 mg/kg bw/day, the highest dose tested.

Groups of 24 weanling Osborne-Mendel rats (12 per sex) were fed 0, 0.5, 1, 2 or 5% Brilliant Blue FCF in the diet (equivalent to 250, 500, 1000 or 2500 mg/kg bw/day) for 2 years (Hansen et al., 1966). During the study, the animals were weighed weekly and mortalities and abnormalities were recorded. Blood counts (white blood cell count, haemoglobin, haematocrit and differential cell count) were performed on 10 animals at each level at 3, 11, 17 and 22 month intervals. Survivors were autopsied and organ weights were recorded for heart, liver, spleen, kidney and testes. There were mainly isolated findings, of commonly occurring tumours showing no dose-response relationship.

The Panel concluded that these neoplastic lesions were not indicative of a carcinogenic effect.

The Panel concluded that the NOAEL of this study is 5% Brilliant Blue FCF in the diet, equivalent to 2500 mg/kg bw/day, the highest dose tested.

A chronic toxicity study coupled with a reproductive study has been performed on Charles Rivers CD rats (IRCD, 1981b; Borzelleca et al., 1990). This study was conducted with an *in utero* phase in which the compound was administered to the F0 generation (see reproductive and developmental toxicity section). At the start of the chronic phase, there were a total of 700 rats (70/sex/group, including 2 control groups). A maximum of two rats per sex from each litter were randomly selected for the chronic phase following the completion of the *in utero* phase. Offspring were exposed to 0.1, 1 or 2% Brilliant Blue FCF, corresponding to 50, 514 and 1073 mg/kg bw/day for males respectively and 62, 631 and 1318 mg/kg bw/day for females respectively (same dietary levels as their parents). The protocol required exposure for 30 months or to the point where survival decreased to 12 rats/sex in any group, at which point of animals were sacrificed.

Observation of mortality, morbidity and gross signs of toxicity were conducted twice daily with at least 5 hours between observations. Individual body weight and food consumption of the F1 were measured weekly for the first 14 weeks, biweekly for the next 12 weeks, and every 4 weeks thereafter. Intake was calculated from body weight, food consumption and dietary concentration, and was expressed in mg/kg bw/day.

Detailed physical examination for signs of toxicity and palpation for masses were conducted weekly.

Ten animals from each group were randomly selected for haematology, clinical chemistry and urine analysis tests after 3, 6, 12, 18 and 24 months of the chronic phase and at the end of the study.

The haematological parameters examined were haemoglobin, haematocrit, erythrocyte and total and differential leukocyte counts. The following clinical chemistry tests were performed: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, fasting glucose, total protein and creatinine. Urine was examined for gross and microscopic appearance, specific gravity, pH, and for the presence of protein, glucose, ketones, bilirubin and occult blood.

Gross examination was conducted on all animals that died spontaneously, were sacrificed in a moribund condition or were sacrificed on schedule. Organ weights were measured in 10 rats/sex/group at the interim killing, and all survivors at the end of the study for brain, gonads, kidneys, liver, spleen, and thyroid. Complete histology was conducted on all animals from the two control groups and the highest dose (2%) and from ten rats of each sex from each group that were randomly selected for an interim killing at 12 months. The following tissues were examined histologically: adrenal, aorta (abdominal), bone and marrow (femur), blood smear, brain, oesophagus, eye, heart, intestine, kidneys, liver, lung, lymph nodes, mammary gland, ovaries, pancreas, pituitary gland, nerve (sciatic), prostate, salivary gland, seminal vesicles, skeletal muscles, skin, spinal cord, spleen, stomach, testes with epididymides, thymus, thyroid, trachea, urinary bladder, uterus, any tissue with gross change of an uncertain nature. Animals from middle and low dose groups were examined grossly. Histology was conducted on any animals with gross lesions or masses. Relevant statistical tests have been performed.

Male and female rats received Brilliant Blue FCF for 116 and 111 weeks respectively. At the end of the study, 186 rats were sacrificed, and 414 were sacrificed *in extremis* or died spontaneously or accidentally during the study.

Group mean body weights at the end of the study were generally similar for control and treated male and female rats with the exception of the females in the 2% group. After 90 weeks, mean body weight of the 2% females began a steady downward trend that was statistically significant ($P < 0.01$) from week 102 until the end of the study.

There was no dose-related trend in survival in either males or females. Survival was significantly decreased ($P < 0.01$) in 2% females compared to 2 control groups (number of surviving at the end of the study 10/70, 28/70, 24/70 respectively) from week 80.

Blue staining of the hair, exposed skin and faeces was noted in all treatment groups. Few of the haematological clinical chemistry and urinanalysis parameters showed significant differences between control and treated animals, and none of the differences appeared to be compound related according to the authors.

Food consumption was slightly greater for males and females in the 2% group (♂ 54 ± 19 versus 51 ± 18/51 ± 19, ♀ 66 ± 17 versus 61 ± 16 /61 ± 17 g/kg bw/day). These differences were statistically significant ($P > 0.05$) for male between week 27 and 66 and female between week 27 and 38, and between week 79 and 90.

No compound related gross and histological changes were revealed after complete autopsies and histological evaluation of the ten rats per sex from each group that were sacrificed after 1 year.

Complete autopsies on animals that died on test or were sacrificed at the end of the study did not reveal any compound-related changes.

Histological evaluations revealed a variety of lesions, including neoplasm among treated and control rats. These lesions were present in similar incidences in control and treated rats and appeared to be spontaneous. Incidences of neoplasms in rat receiving Brilliant Blue FCF in the diet *in utero* and

throughout their lifetimes (70 rats/group) were considered. Only grossly detected masses and lesions from animals in the groups receiving 0.1% or 1% were examined microscopically.

In this rat study, lifetime exposure of rats to Brilliant Blue FCF as a dietary mixture did not demonstrate carcinogenic effects.

The Panel considered as NOAEL in this study to be the dietary concentration of 2%, equivalent to 1072 mg/kg bw/day for male rats and 1%, 631 mg/kg bw/day for female rats, based on a 15% decrease in terminal mean body weight and decreased survival in the high dose females.

3.2.5. Reproductive and developmental toxicity

Reproductive toxicity studies

Rat

Brilliant Blue FCF was administered in the diet to three successive generations of male and female rats at dose levels of 0, 10, 100, 300 or 1000 mg/kg bw/day. The first generation of parents was mated twice, the second generation of parents was mated three times, and the third generation of parents was mated twice. Dams were allowed to deliver their offspring and raise them to weaning for all matings except the third mating of the F1b generation, when dams were sacrificed on day 19 of gestation and their uterine contents examined. Offspring from the various matings were either autopsied at weaning or selected to become the parents of the next generation. Records of parental body weights and food consumption and offspring survival and growth were maintained. The parents (F1b) of the second generation and the offspring of the last generation (F3a), five rats/sex/dietary level were autopsied, certain tissues preserved, and selected tissues from animals of the control and high dose groups examined histopathologically. The mean body weights of the high dose group were less than the control group mean weights in nursing offspring and in males and females of the F1 and F2 generations. No effects attributable to the colour were observed in adult mortality, mating, pregnancy and fertility rates, lengths of gestation period, offspring survival or sex, litter survival, or necropsy findings in the animals which were sacrificed on day 19. There were no macroscopic or microscopic tissue alterations of either the F1b or F3a generation rats considered attributable to the administration of Brilliant Blue FCF (no further details available) (Bio/dynamics Inc., 1973).

The Panel noted that no further details are available. Animal number per group and statistical significance of the data are not specified. Consequently, no NOAEL can be derived from this study.

A reproductive toxicity study in rat was coupled with the chronic toxicity study (Borzelleca et al., 1990). Brilliant Blue FCF was administered at dose levels of 0.1, 1 and 2% (corresponding to 50, 514 and 1073 mg/kg bw/day for males, and 62, 631 and 1318 mg/kg bw/day for females, respectively) to 60 males and 60 females of the F0 generation at each dosage level. Rats received their respective control or treated diets for 62 days at approximately 100 days of age. The F0 generation was housed, one male with one female within the same treatment group for period of 15 consecutive days to produce F1 litters. Females were weighed on gestation days 0, 4, 14 and 21. There were no compound-related effects on fertility, gestation, parturition, lactation, pup survival through weaning, or on number of live and stillborn pups. Three females (1 from 0.1%, 1 from 1%, and 1 from 2% group) died. These deaths were not related to treatment.

The Panel concluded that the NOAEL for this study is 1318 mg/kg bw/day for females, and 1073 mg/kg bw/day for males.

Developmental toxicity study

Brilliant Blue FCF was given orally to groups of mated female Long Evans rats from day 6 to 15 of gestation at 0, 200, 600 and 2000 mg/kg bw/day. Rats which survived the duration of the experiment

were sacrificed on day 20 and the number of *corpora lutea* and uterine contents recorded. All fetuses were examined for malformations. Approximately two thirds of the fetuses were selected for visualization of skeletal ossification, variations and anomalies. No signs of fetal toxicity or anomalies were observed which were attributable to the administration of the colour (Bio/dynamics Inc., 1972).

The Panel noted that no further details are available. Animal number per group, way of administration, and statistical significance of the data are not specified. Consequently, no NOAEL can be derived from this study.

Brilliant Blue FCF was administered orally to mated female rabbits (New Zealand White) from day 6-18 of gestation. The dose levels were 0, 20, 60 or 200 mg/kg bw/day and were administered in volumes of 1 ml/kg for all treatment groups. Rabbits which survived the duration of the study were sacrificed on day 20 and the number of *corpora lutea* and uterine contents recorded. All fetuses were weighed, examined grossly for externally visible defects, and carefully dissected and examined for visceral anomalies. All fetuses were cleared and stained with alizarin red for visualization of skeletal ossification and variation and anomalies. No signs of fetal toxicity or anomalies were observed which were attributable to the administration of the colour (Bio/dynamics Inc., 1972).

The Panel noted that no further details are available. Animal number per group, way of administration, and statistical significance of the data are not specified. Consequently, no NOAEL can be derived from this study.

In vitro study

A study by Lau et al. (2006) has focussed on synergistic interactions between commonly used food additives in an *in vitro* developmental neurotoxicity test using neuroblastoma cells. Developmental neurotoxicity was measured as an inhibition of neurite outgrowth in 24 hours. It was found that Brilliant Blue FCF (0.05-500 nM) in combination with L-glutamic acid (0.5-100 μM), which are themselves relatively weak inhibitors of neurite outgrowth, synergistically reduced neurite outgrowth length.

3.2.6. Human studies

In two recent studies by Gaur et al. (2003) and Lucarelli et al. (2004) three cases of refractory shock and metabolic acidosis in critically ill patients are reported. The patients received Brilliant Blue FCF added to enteral nutrition formulations in order to facilitate the detection of aspiration. According to Lucarelli et al. (2004), the dose did not exceed 0.7 mg/kg bw/day for the first patient and 2 mg/kg bw/day for the second one. The dose was not mentioned by Gaur et al. (2003). Although in healthy subjects absorption of Brilliant Blue FCF appears to be limited, critically ill patients have increased gastrointestinal permeability to Brilliant Blue FCF secondary to enterocyte death and stress induced release of neuro-endocrine factors in the intestinal epithelium.

4. Discussion

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

Brilliant Blue FCF (E 133) is a triarylmethane dye authorised as a food additive in the EU and has been previously evaluated by JECFA in 1970 and the SCF in 1979. Both committees have established an ADI of 12.5 mg/kg bw/day. In 1984, SCF revised the ADI to 10 mg/kg bw/day, based on new long-term studies.

The JECFA assigned an ADI of 0-12.5 mg/kg bw/day to Brilliant Blue FCF in 1970. This ADI was based on a NOAEL of 5% in the diet, equivalent to 2500 mg/kg bw/day. This value is probably derived from the chronic rat study by Hansen et al. (1966), in which no effects were observed up to the highest dietary dose level of 5% (equivalent to 2500 mg/kg bw/day). An uncertainty factor of 200 was applied, probably because the study dossier was incomplete.

The SCF in 1975 established an ADI of 12.5 mg/kg bw/day. In 1984 the SCF had additional chronic toxicity data available which revealed a slight reduction in body weight of female mice and rats at the highest dose level (IRCD, 1981). The NOAEL was set at 2% of the diet, equivalent to 1073 mg/kg bw/day in male and 1318 mg/kg bw in female, from which a revised ADI of 10 mg/kg bw was derived for Brilliant Blue FCF. An uncertainty factor of 100 seems to have been applied.

Data available on the absorption, distribution, metabolism and excretion of Brilliant Blue FCF, show that Brilliant Blue FCF is poorly absorbed and mainly excreted unchanged in faeces.

Subchronic toxicity studies are available in mouse rat, and dog. The study in mouse has been carried out according to old protocols and is of limited value. One study in rat has been performed using two mixtures (A and B) of food colours. The composition of mixture A and B, containing Sunset Yellow, Tartrazine, Carmoisine and Brilliant Blue was not specified (unknown concentration of each colour). As Brilliant Blue FCF is not administered alone, these data can not be used for a specific safety evaluation of Brilliant Blue FCF.

In a study performed on groups of 20 young rats (10/sex) no gross lesions or microscopic pathology were attributed to ingestion of Brilliant Blue FCF. The NOAEL was considered to be 2%, equivalent to 1000 mg/kg bw/day. Beagle dogs were fed Brilliant Blue FCF in the diet for periods up to 1 year. No gross lesions or microscopic pathology were attributed to ingestion of Brilliant Blue FCF. The Panel considered that the NOAEL is 2% level in diet, equivalent to 500 mg/kg bw/day.

Brilliant Blue FCF showed no evidence of mutagenicity when tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 with or without metabolic activation and in *Escherichia coli.* Some *in vitro* studies gave conflicting data. Brilliant Blue FCF has been tested *in vivo* in a mouse micronucleus assay and Comet assays. The compound was administered by one to four *i.p* injections (0, 500, 1000, 2000 mg/kg bw). No micronucleus induction or DNA damage was observed. Based on these observations, the Panel considered that *in vivo* tests are negative.

Based on the available data, the Panel concluded that Brilliant Blue FCF is not of concern with respect to genotoxicity.

Chronic toxicity studies available have been performed, in mouse and rats before publication of OECD guidelines. One study in mouse did not show any carcinogenic effect of Brilliant Blue FCF. The Panel concluded that the NOAEL is 2000 mg/kg bw/day, the highest dose tested. In the second study in mouse, Brilliant Blue FCF was administered in the diet to mice (60/sex/group, CD-1 mice) at dietary levels of 0.5, 1.5 or 5% (equivalent to 661, 2064 and 7354 mg/kg bw/day in male and 819, 2562 and 8966 mg/kg bw/day in female). The lifetime exposure of mice to Brilliant Blue FCF as a dietary admixture did not demonstrate consistent biologically significant, compound-related adverse effects on behaviour, morbidity, mortality, haematology, general physical observations. Incidence of neoplasms in mice receiving Brilliant Blue FCF in the diet were evaluated throughout their lifetimes (60 mice per group). A variety of microscopic lesions was observed at similar incidence in control and treated animals. The 5% female group exhibited an increased incidence of haemangiomas of the spleen. The Panel agreed with the authors that the haemangiomas were considered spontaneous and not related to the exposure to Brilliant Blue FCF. The NOAEL is considered to be 7354 mg/kg bw/day. Two studies in rat were carried out according to old protocols, no tumours were found.

Four groups of 30 Wistar rats (15/sex) were administered Brilliant Blue FCF through their diets at dose levels of 0, 0.03, 0.3 or 3% (equivalent to 15, 150, or 1500 mg/kg bw/day) for 75 weeks. No treatment-related abnormalities were observed concerning depression of growth, food consumption,

food efficiency, mortality, or haematology. The Panel considered that the NOAEL of this study is 1500 mg/kg bw/day, the highest dose tested.

Groups of 24 weanling Osborne-Mendel rats (12 per sex) were fed 0, 0.5, 1, 2 or 5% Brilliant Blue FCF in the diet (equivalent to 250, 500, 1000 or 2500 mg/kg bw/day) for 2 years. No relevant adverse effects were described. The Panel concluded that the NOAEL of this study is 5% Brilliant Blue FCF in the diet, equivalent to 2500 mg/kg bw/day, the highest dose tested.

A chronic toxicity study coupled with a reproductive study has been performed on Charles Rivers CD rats. This study was conducted with an *in utero* phase in which the compound was administered to the F0 generation (see reproductive and developmental toxicity section). Group mean body weights at the end of the study were generally similar for control and treated male and female rats, with the exception of the females in the 2% group. There was no dose-related trend in survival in either males or females. Survival was significantly decreased ($P < 0.01$) in 2% females compared to controls (10/70 versus 28/70 and 24/70 in two control groups) from week 80 onwards. Histological evaluations revealed a variety of lesions, including neoplasm among treated and control rats. These lesions were present in similar incidences in control and treated rats and appeared to be spontaneous. In this rat study, lifetime exposure of rats to Brilliant Blue FCF as a dietary mixture did not demonstrate carcinogenic effects. The Panel considered that the NOAELs established in this study are dietary concentrations of 2%, equivalent to 1072 mg/kg bw/day for male rats, and 1%, equivalent to 631 mg/kg bw/day for female rats, based on a 15% decrease in terminal mean body weight and decreased survival in the high dose females.

Brilliant Blue FCF was administered in the diet to three successive generations of male and female rats at dose levels of 0, 10, 100, 300 or 1000 mg/kg bw/day. The mean body weights of the high dose group were lower than the control group mean weights in nursing offspring and in males and females of the F1 and F2 generations. No effects attributable to the colour were observed in mortality, mating, pregnancy and fertility rates, lengths of gestation period, offspring survival or sex, litter survival, or necropsy findings in the animals which were sacrificed on day 19. There were no macroscopic or microscopic tissue alterations of either the F1b or F3a generation rats considered attributable to the administration of Brilliant Blue FCF. Due to lack of details on this study, no NOAEL can be derived from it.

A reproductive toxicity study in rat was coupled with the chronic toxicity study. Brilliant Blue FCF was administered at dietary level of 0.1, 1 and 2% (corresponding to 50, 514 and 1073 mg/kg bw/day for males, and 62, 631 and 1318 mg/kg bw/day for females, respectively). There were no compound-related effects on fertility, gestation, parturition, lactation, pup survival through weaning, or on number of live and stillborn pups. The Panel concluded that the NOAEL for reproductive toxicity is 1318 mg/kg bw/day for females and 1073 mg/kg bw/day in males, the highest doses tested.

Two developmental studies are available. Brilliant Blue FCF was given orally to groups of mated female Long Evans rats from day 6 to 15 of gestation at 0, 200, 600 and 2000 mg/kg bw/day. No signs of fetal toxicity or anomalies were observed which were attributable to the administration of the colour. Due to lack of details on this study, no NOAEL can be derived from it. Brilliant Blue FCF was administered orally to mated female rabbits (New Zealand White) from day 6-18 of gestation. The dose levels were 0, 20, 60 or 200 mg/kg bw/day. No signs of fetal toxicity or anomalies were observed which were attributable to the administration of the colour. Due to lack of details on this study, no NOAEL can be derived from it.

The Panel concluded that the present dataset on the absorption, distribution, metabolism and excretion, genotoxicity, subchronic, reproductive, developmental and long-term toxicity, and carcinogenicity give reason to revise the ADI of 10 mg/kg bw/day allocated by SCF in 1984. Subchronic toxicity studies are not sufficiently documented to be used for the risk assessment. Chronic toxicity studies showed that Brilliant Blue FCF is not carcinogenic. Among the five chronic toxicity studies, the lowest NOAEL comes from the more recent chronic toxicity study. Observed adverse effects are

decrease in terminal mean body weight and decreased survival in the highest dose tested (1318 mg/kg bw/day). The Panel agreed with the authors NOAEL of 631 mg/kg bw/day. In the oldest reproductive study the mean body weights of the higher dose group (1000 mg/kg bw/day) were lower than the control group mean weights in nursing offspring and in males and females of the F1 and F2 generations. However, in a more recent study in rat, no effects have been observed at all doses tested (62, 631 and 1318 mg/kg bw/day). The Panel considered that this second study invalidates the results of the first one. No adverse effects have been described in developmental studies in rat and rabbit.

The Panel considered that the NOAEL of 631 mg/kg bw/day from the chronic toxicity study in rat can be used to derive a new ADI for Brilliant Blue FCF. By application of an uncertainty factor of 100, the Panel established a new ADI for Brilliant Blue FCF equal to 6 mg/kg bw/day.

The exposure assessment approach goes from the conservative estimates that form the First Tier of screening, to progressively more realistic estimates that form the Second and Third Tier. The dietary exposure to Brilliant Blue FCF from the MPLs of use was estimated by the Panel using the Budget method (Tier 1) with the assumptions described in the report of the SCOOP Task 4.2. The Panel calculated a theoretical maximum daily exposure of 8.1 mg/kg bw/day for adults, and 13.1 mg/kg bw/day for a typical 3 year-old child.

Refined exposure estimates have been performed both for children and the adult population according to the Tier 2 and Tier 3 approaches described in the SCOOP Task 4.2, which combines detailed individual food consumption information from the population with the MPLs of use as specified in the Directive 94/36/EC on food colours (Tier 2), and with the maximum reported use levels of Brilliant Blue FCF, as identified by the Panel from the data by the FSA, FSAI, UNESDA and CIAA (Tier 3).

For children (1-10 years old), estimates have been calculated for the UK and for eleven European countries as part of the EXPOCHI consortium (Belgium, France, the Netherlands, Spain, Italy, Finland, Greece, Cyprus, Czech Republic, Sweden and Germany). For the adult population, the Panel has selected the UK population as representative of the EU consumers for Brilliant Blue FCF exposure estimates.

When considering MPLs (Tier 2), the mean dietary exposure to Brilliant Blue FCF for European children, including UK children (aged 1-10 years), ranged from 0.5 to 3.4 mg/kg bw/day, and from 1.2 to 7.2 mg/kg bw/day at the 95th percentile. Estimates reported for the UK adult population give a mean dietary exposure of 0.9 mg/kg bw/day and of 3.3 mg/kg bw/day for high level (97.5th percentile) consumers.

When considering the maximum reported use levels (Tier 3), the mean dietary exposure to Brilliant Blue FCF for European children, including UK children (aged 1-10 years) ranged from 0.2 to 2.1 mg/kg bw/day, and from 0.6 to 4.8 mg/kg bw/day at the 95th percentile. Estimates reported for the UK adult population give a mean dietary exposure to Brilliant Blue FCF of 0.6 mg/kg bw/day, and of 3.0 mg/kg bw/day for high level (97.5th percentile) consumers.

The Panel concluded that at the maximum reported levels of use of Brilliant Blue FCF, refined intake estimates (Tier 3) are lower than the ADI of 6 mg/kg bw/day The Panel concluded that at Tier 2 the intake estimates are below the ADI at the mean for both adults and children and at the higher level for adults, but above the ADI at the higher level (95th percentile) for children.

CONCLUSIONS

Brilliant Blue FCF (E 133) is a triarylmethane dye authorised as a food additive in the EU and has been previously evaluated by JECFA in 1970 and the SCF in 1975. Both committees have established an ADI of 12.5 mg/kg bw/day. In 1984, the SCF revised the ADI to 10 mg/kg bw/day, according to new long-term studies.

The Panel concluded that the present data set on the absorption, distribution, metabolism and excretion, genotoxicity, subchronic, reproductive, developmental and long-term toxicity, and carcinogenicity give reason to revise the ADI of 10 mg/kg bw/day allocated by SCF in 1984.

The Panel considered that the NOAEL of 631 mg/kg bw/day from the chronic toxicity study in rat can be used to allocate a new ADI to Brilliant Blue FCF. By application of an uncertainty factor of 100, the Panel established a new ADI to Brilliant Blue FCF equal to 6 mg/kg bw/day.

The Panel concluded that at the maximum reported levels of use of Brilliant Blue FCF, refined intake estimates (Tier 3) are lower than the ADI of 6 mg/kg bw/day. The Panel concluded that at Tier 2 the intake estimates are below the ADI at the mean for both adults and children and at the higher level for adults, but above the ADI at the higher level (95th percentile) for children.

The Panel further noted that the specifications for Brilliant Blue FCF need to be updated with respect to the percentage of material not accounted for that may represent sodium chloride and/or sodium sulphate as the principal uncoloured components.

The Panel noted that the JECFA specification for lead is $\leq$ 2 mg/kg whereas the EC specification is $\leq$ 10 mg/kg.

The Panel noted that the aluminium lake of the colour could add to the daily intake of aluminium for which a TWI of 1 mg aluminium/kg bw/week has been established and that therefore specifications for the maximum level of aluminium in the lakes may be required.

DOCUMENTATION PROVIDED TO EFSA

1. Pre-evaluation document prepared by the Dutch National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.

REFERENCES

Aboel-Zahab H, el-Khyat Z, Sidhom G, Awadallah R, Abdel-al W and Mahdy K, 1997. Physiological effects of some synthetic food colouring additives on rats. Bollettino Chimico Farmaceutico 136, 615-627.

Auletta A, Kuzava JM and Parmar AS, 1977. Lack of mutagenic activity of a series of food dyes for *Salmonella typhimurium*. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 56(2), 203-206.

BIBRA (British Industrial Biological Research Association), 1982. A review of the toxicology of nine food colours. BIBRA Information Services Ltd. Surrey, UK.

Bio/dynamics Inc., 1972, 1973. (Unpublished reports cited in catalogue of Food Colours Volume 2. Colour Committee of the International Life sciences Institute).

Bonin AM and Baker RSU, 1980. Mutagenicity testing of some approved food additives with the *Salmonella*/microsome assay. Food Technology Australia 32, 608-610.

Bonin AM, Farquharson JB and Baker RSU, 1981. Mutagenicity of arylmethane dyes in *Salmonella*. Mutation Research/Genetic Toxicology, 89(1), 21-34.

Borzelleca JF, Depukat K and Hallagan JB, 1990. Lifetime toxicity/carcinogenicity studies of FD & C Blue No. 1 (Brilliant blue FCF) in rats and mice. Food and Chemical Toxicology. 28, 221-234.

Brown JP, Roehm GW, Brown RJ, 1978. Mutagenicity testing of certified food colors and related azo, xanthene and triphenylmethane dyes with the *Salmonella*/microsome system. Mutation Research. 56(3), 249-71.

Brown, JP, Dorsky A, Enderlin FE, Hale RL, Wright VA and Parkinson TM, 1980. Synthesis of C-14-labeled DF and C Blue No-1 (Brilliant Blue FCF) and its intestinal-absorption and metabolic-fate in rats. Food and Cosmetic Toxicology 18, 1-5.

ChemIDplus Advanced (via internet, 2006). Accessible via: http://chem.sis.nlm.nih.gov/chemidplus/

Chen QC, Mou SF, Hou XP, Riviello JM, Ni ZM, 1998. Determination of eight synthetic food colorants in drinks by high-performance ion chromatography. The Journal of Chromatography A. 827(1), 73-81.

CIAA (Confederation of the Food and Drink Industries of the EFSA re-evaluation of some food colours. December 2009.

EC (European Commission), 2001. Report from the Commission on Dietary Food Additive Intake in the European Union. Available at: http://ec.europa.eu/food/fs/sfp/addit_flavor/flav15_en.pdf.

EC (European Commission), 1998. Report on Methodologies for the Monitoring of Food Additive Intake Across the European Union. Final Report Submitted by the Task Coordinator, 16 January 1998. Reports of a Working Group on Scientific Cooperation on Questions Relating to Food, Task 4.2. SCOOP/INT/REPORT/2 (Brussels: European Commission Directorate General I11 Industry).

EFSA, 2008. Safety of aluminium from dietary intake. Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC).

Florian M, Yamanaka H, Carneiro PA, Zanoni MV, 2002. Determination of Brilliant Blue FCF in the presence and absence of erythrosine and quinoline yellow food colours by cathodic stripping voltammetry. Food Additives and Contaminants 19(9), 803-9.

FSA (Food Standard Agency), 2002. Survey of colours in sweets. Food Survey Information Sheets. No. 23/02 April 2002. Available at: http://www.food.gov.uk/multimedia/pdfs/23sweets.pdf

FSA (Food Standard Agency), 2003. Survey of colours in soft drinks. Food Survey Information Sheets. No.37/03 May 2003. Available at: http://www.food.gov.uk/multimedia/pdfs/fsiscoloursinfoods032003.pdf

FSAI (Food Safety Authority of Ireland), 2009. A Surveillance Study on Levels of Artificial Colours and Sweeteners in Irish Retail Products. Available at: http://www.fsai.ie/assets/0/86/204/7f60074b-56eb-4fa2-ac6c-e013e20b9e7d.PDF

Gaur S, Sorg T and Shukla V, 2003. Systemic absorption of FD&C blue dye associated with patient mortality. Postgraduate Medical Journal 79 (936), 602-603.

Hansen WH, Fitzhugh OG, Nelson AA and Davis KJ, 1966. Chronic toxicity of two food colors, Brilliant blue FCF and Indigotine. Toxicology and Applied Pharmacology 8, 29-36.

Haveland-Smith RB and Combes RD, 1980. Screening of food dyes for genotoxic activity. Food and Cosmetics Toxicology 18(3), 215-21.

Hayashi M, Kishi M, Sofuni T, Ishidate JR, 1988. Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. Food and Chemical Toxicology 26, 487-500.

Hess SM and Fitzhugh OG, 1953. Metabolism of coal-tar dyes. I. Triphenylmethane dyes (Abstract No. 1090), Fed. Proc. 12, 330-331.

Hess SM and Fitzhugh OG, 1954. Metabolism of coal-tar colours. II. Bile studies (Abstract No. 1201), Fed. Proc. 13, 365

Hess SM and Fitzhugh OG, 1955. Absorption and excretion of certain triphenylmethane colors in rats and dogs. Journal of Pharmacology and Experimental Therapeutics 114, 38-42.

Huybrechts I, Sioen I, Boon PE, Ruprich J, Lafay L, Turrini A, Amiano P, Hirvonen T, De Neve M, Arcella D, Moschandreas J, Westerlund A, Ribas-Barba L, Hilbig A, Papoutsou S, Christensen T, Oltarzewski M, Virtanen S, Rehurkova I, Azpiri M, Sette S, Kersting M, Walkiewicz A, Serra-Majem L, Volatier JL, Trolle E, Tornaritis M, Busk L, Kafatos A, Fabiansson S, De Henauw S, Van Klaveren J, 2010. Dietary Exposure Assessments for Children in Europe (the EXPOCHI 159 project): rationale, methods and design. Archives of Public Health. In press.

ICI (Industrial Chemical Industries), 1962. Unpublished report (as referred to by JECFA, 1970).

IRCD (International Research & Development Corporation), 1981a. Long-term dietary toxicity/carcinogenicity study in rats. Unpublished report.

IRCD (International Research & Development Corporation), 1981b. Long-term dietary toxicity/carcinogenicity study in mice. Unpublished report.

Ishidate MJ, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M and Matsuoka A, 1984. Primary mutagenicity screening of food additives currently used in Japan. Food and Chemical Toxicology 22, 623-63.

Ishikawa F, Oishi M, Kimura K, Yasui A, Saito K, 2004. [Determination of synthetic food dyes in food by capillary electrophoresis][Article in Japanese]. Shokuhin Eiseigaku Zasshi 45(3), 150-5.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1970. 13th Report. Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances. WHO Food Additives Series, 70.36.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2004. 63rd meeting. Aluminium Lakes of Colouring Matters, General Specifications. Accessible via: http://www.fao.org/ag/agn/jecfa-additives/specs/Monograph1/Additive-013.pdf

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. Combined compendium of food additive specifications - all specifications monographs from the 1st to the 65th meeting (1956-2005). FAO JECFA Monographs Series, No. 1 Volume 1-3.

Kawachi T, Yaha GIT, Kada T, Tazima Y, Ishidate M, Sasaki M, Sugiyama, 1980. Cooperative programme on short-term assays for carcinogenicity in Japan. IARC Sci Publ 27, 323-330.

Kirschbaum J, Krause1 C, Pfalzgraf S and Brückner H, 2003. Development and evaluation of an HPLC-DAD method for determination of synthetic food colorants. Chromatographia 57(1), 115-119.

Klinke, 1955. Referred to by JECFA but not included in reference list (as referred to by JECFA, 1970).

Kuno N and Mizutani T, 2005. Influence of synthetic and natural food dyes on activities of CYP2A6, UGT1A6, and UGT2B7. Journal of Toxicology and Environmental Health A 68(16), 1431-1444.

Kusaka T, Matsufuji H, Chino M, Kato Y, Nakamura M, Goda Y, Toyoda M, Takeda M, 1999. Isolation, identification and determination of a magenta subsidiary colour in food blue no. 1 (brilliant blue FCF). Food Addit Contam 16(12), 501-7.

Lau K, McLean WG, Williams DP and Howard CV, 2006. Synergistic interactions between commonly used food additives in a developmental neurotoxicity test. Toxicological Sciences 90, 178-187.

Li R, Jiang Z-T and Wang R-X, 2008. Solid Phase Extraction Combined Direct Spectrophotometric Determination of Brilliant Blue in Food Using β-Cyclodextrin Polymer. Food Analytical Methods 2, 264–270.

Lu FC and Lavallée A, 1964. Canadian Pharmaceutical Journal. 97, 30 (as referred to by JECFA, 1970).

Lucarelli MR, Shirk MB, Julian MW and Crouser ED, 2004. Toxicity of Food Drug and Cosmetic Blue No. 1 dye in critically ill patients. Chest 125(2), 793-795.

Mannel WA, Grice HC and Allmark MG, 1962. Chronic toxicity studies on food colours. V. Observations on the toxicity of Brilliant Blue FCF, Guinea Green B and Benzyl Violet 4B in rats. Journal of Pharmacy and Pharmacology 14, 378-384.

Matsufuji H, Kusaka T, Tsukuda M, Chino M, Kato Y, Nakamura M, Goda Y, Toyoda M and Takeda M, 1998. Structural determination of subsidiary colors in commercial Food Blue No. 1 (Brilliant Blue FCF) product. Journal of the Food Hygienic Society of Japan 39, 7- 12.

Pereira Alves S, Mares Brum D, Castello Branco de Andrade E and Duarte Pereira Netto A, 2008. Determination of synthetic dyes in selected foodstuffs by high performance liquid chromatography with UV-DAD detection. Food Chemistry 107, p. 489.

Phillips JC, Mendis D, Eason CT and Gangoli SD, 1980. The metabolic disposition of ^{14}C-labelled Green S and Brilliant Blue FCF in the rat, mouse and guinea pig. Food Cosmetics Toxicology 18(1), 7-13.

Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniguchi K and Tsuda S, 2002. The Comet assay with 8 mouse organs: results with 39 currently used food additives. Mutation Research 519, 103-119.

SCCP (Scientific Committer on Cosmetic Products), 2004. Opinion of the scientific committee on cosmetic products and non-food products intended for consumers concerning Acid Blue 9. COLIPA n° C40, 27 pages.

SCF (Scientific Committee for Food), 1975. Reports from the Scientific Committee for Food (1st series), opinion expressed in 1975, p. 17.

SCF (Scientific Committee for Food), 1979. Reports from the Scientific Committee for Food (8th series), opinion expressed in 1979, p. 11.

SCF (Scientific Committee for Food), 1984. Reports of the Scientific Committee for Food (14th series), opinion expressed in 1983, 47-61.

Scotter MJ and Castle L, 2004. Chemical interactions between additives in foodstuffs: a review. Food Additives and Contaminants, Part A, 21:2, 93–124.

TemaNord, 2002. Food additives in Europe 2000; Status of safety assessments of food additives presently permitted in the EU. TemaNord 560, 122-125.

Tennant D, 2006. Screening of Colour Intakes from Non-Alcoholic Beverages. Report prepared for the Union of European Beverages Associations (UNESDA), December 2006.

Tsuji S, Amakura Y, Umino Y, Nishi M, Nakanishi T, Tonogai Y, 2001. Structural determination of the subsidiary colors in food blue No. 1 (brilliant blue FCF) aluminum lake detected by paper chromatography. Shokuhin Eiseigaku Zasshi 42(4), 243-8.

Tsujita J, Takeda H, Ebihara K and Kiriyama S, 1979. Comparison of protective activity of dietary fiber against the toxicities of various food colors in rats. Nutrition Reports International 20(5), 635-642.

Vachirapatama N, Mahajaroensiri J and Visessanguan W, 2008. Identification and Determination of Seven Synthetic Dyes in Foodstuffs and Soft Drinks on Monolithic C18 Column by High Performance Liquid Chromatography. Journal of Food and Drug Analysis Vol. 16, No. 5, 77-82.

Vidotti EC, Cancino JC, Oliveira CC, Rollemberg Mdo C, 2005. Simultaneous determination of food dyes by first derivative spectrophotometry with sorption onto polyurethane foam. Analytical Sciences 21(2), 149-53.

Willheim R and Ivy AC, 1953. A preliminary study concerning the possibility of dietary carcinogenesis. Gastroenterology 23, 1-19.

ANNEX A

Rules defined by the Panel to deal with *quantum satis* (QS) authorisation, usage data or observed analytical data for all regulated food additives to be re-evaluated and procedures for estimating intakes using these rules.

1. **Decision rules taken to deal with QS authorisations for MPL: (see the decision tree in Figure 1)**

    a. If the category 'All other foodstuff' is QS, the highest observed MPL value should be used, which is 500 mg/kg

    b. At the food category level, if a colour is authorised QS in a food category for one or more colours

        i. If a value is available for only one colour, this value is used for all the colours

        ii. If many values are available for more than one colour, the highest value is used

        iii. If there is no available value, the available value for a similar food group for the same colour is used. If there is no similar food group, the highest MPL of 500 mg/kg is used.

Particular cases:

- **Edible casings QS**: If available use the pork-based products use level; if there is no value available, the highest MPL of 500 mg/kg is used.
- **Edible cheese rinds**: The MPL of 100 mg/kg (from the flavoured processed cheese category) is used, except for E 120 (Cochineal) whose level is 125 mg/kg for red marbled cheese.

2. **Rules to identify the maximum reported use levels to be used for the refined exposure assessment:**

    A maximum reported use level is the maximum value selected from reported usage by industry and analytical data provided to the Panel:

    a. If the identified maximum reported use level is greater than or equal to the actual MPL, then the actual MPL is used by default.

    b. If both maximum analytical and maximum current use level data are available, priority is given to the use level data, even if analytical values are lower or higher; the selected value is rounded to the nearest whole number.

    c. If no use level data are available, because either no uses were reported or industry was not asked to provide them, the choice is made between the highest analytical value or the MPL:

        i. if more than10 analytical data are available, the highest quantified reported value is used;

        ii. if less than10 analytical data are available, the MPL is used.

d. If the highest use level or the highest analytical data are higher than the proposed adjusted QS values for MPL, priority is given to the highest use level/analytical data.

3. Tiered approach to intake estimation

The basic principles of the stepwise approach for the estimation of additives' intakes involve, for each successive Tier, a further refinement of intakes from the conservative estimates for screening (Tier 1) to more realistic estimates (Tier 2 and 3) (EC, 2001). Depending on the information on use levels data available, the three screening tiers approach must be adapted (see Figure 2 for the decision rules).

The three screening tiers performed both for children and adult population are:

Tier 1: Estimates are based on the MPLs, as specified in the Directive 94/36/EC on food colours and the Budget method.

Tier 2: Estimates are based on the MPLs, as specified in the Directive 94/36/EC on food colours with adjustment for quantum satis usages, and national individual food consumption data.

Tier 3: Estimates are based on maximum reported use levels and national individual food consumption data.

In Tier 2 and 3, the following approach is used to calculate the high percentile consumption: The high consumption should be calculated by examining the 97.5th percentile of food additive intake per food group, and selecting the highest intake* and then adding this value to the sum of the mean intakes for the remaining food groups. This approach is slightly different to the usual approach, in which the two highest food group intakes at the 97.5th percentile of additive intakes are added to the mean consumption of the other food groups. The approach was modified based on evaluation of the Expochi study, as it provides a more realistic estimate of exposure.

*High consumption value of Fruit wines (still or sparkling), Cider (except cidre bouche) and perry, Aromatized fruit wines, cider and perry from UK adult data is not taken into account for the calculation of high percentile exposure when this food category appeared to be the highest P95 exposure. In this case, the second highest contributor is taken in the calculation.

GLOSSARY AND/OR ABBREVIATIONS

ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism, and Excretion
Aluminium lakes	Aluminium lakes are produced by the absorption of water soluble dyes onto a hydrated aluminium substrate rendering the colour insoluble in water. The end product is coloured either by dispersion of the lake into the product or by coating onto the surface of the product
ANS	Panel on Food Additives and Nutrient Sources added to Food
BIBRA	British Industrial Biological Research Association
CAS	Chemical Abstract Service
CIAA	Confederation of the Food and Drink Industries of the EU
DNA	Deoxyribonucleic Acid
EC	European Commission
EFSA	European Food Safety Authority
EINECS	European Inventory of Existing Commercial Chemical Substances
EU	European Union
FAO/WHO	Food and Agriculture Organization/World Health Organization
FSA	UK Food Standard Agency
FSAI	Food Safety Authority of Ireland
HPLC	High-Performance Liquid Chromatography
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50% i.e. dose that causes death among 50% of treated animals
LOD	Limit of Detection
LOQ	Limit of Quantification
MPL	Maximum Permitted Level
NDNS	UK National Diet and Nutrition Survey
NOAEL	No-Observed-Adverse-Effect Level
OECD	Organisation for Economic Co-operation and Development
SCF	Scientific Committee for Food
SCOOP	A scientific cooperation (SCOOP) task involves coordination amongst Member States to provide pooled data from across the EU on particular issues of concern regarding food safety
TWI	Tolerable Weekly Intake
UNESDA	Union of European Beverage Associations