European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of Patent Blue V (E 131) 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

This scientific output, published on 25 April 2013, replaces the earlier version published on 01 March 2013[4].

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion re-evaluating the safety of Patent Blue V (E 131). Patent Blue V (E 131) is a triarylmethane dye permitted for use as a food additive in the EU, that has been previously evaluated by JECFA in 1970 and 1975 and the EU SCF in 1983; JECFA established a Temporary Acceptable Daily Intake (ADI) of 0-1 mg/kg bw/day in 1970, but withdrew it in 1975. Until now JECFA has not allocated an ADI to Patent Blue V (E 131). The SCF established an ADI of 0-15 mg/kg bw/day. 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 concluded that the present dataset provides a rationale for a re-definition of the ADI. Using the NOAEL of 500 mg/kg bw/day derived from a chronic toxicity study in mice and applying an uncertainty factor of 100 to this NOAEL, the Panel establishes an ADI of 5 mg/kg bw/day. The Panel noted that at the maximum permitted levels of use of Patent Blue V (E 131), exposure estimates for high consumers are above the ADI of 5 mg/kg bw/day in toddlers and children. At the maximum reported use levels of Patent Blue V (E 131), exposure estimates are below the ADI of 5 mg/kg bw/day for all groups of the population.

KEY WORDS

Patent Blue V, E 131, CAS Registry Number calcium salt: 3536-49-0, CAS Registry Number sodium salt: 129-17-9, food colouring substance.

[1] On request from the European Commission, Question No EFSA-Q-2011-00359, adopted on 4 July 2012.

[2] Panel members: Fernando Aguilar, Riccardo Crebelli, Birgit Dusemund, Pierre Galtier, John Gilbert, David Gott, Ursula Gundert-Remy, Juergen König, Claude Lambré, Jean-Charles Leblanc, Alicja Mortensen, Pasquale Mosesso, Dominique Parent-Massin, Ivan Stankovic, Paul Tobback, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen and Matthew Wright. 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 (mandate 2008-2011): Martine Bakker, Dimitrios Boskou, Birgit Dusemund, David Gott, Torben Hallas-Møller, Juergen König, Daniel Marzin, Dominique Parent-Massin, Iona Pratt, Ivonne Rietjens, Gerrit Speijers, Paul Tobback, Tatjana Verguieva, Rudolf Antonius Woutersen, and Pasquale Mosesso for the preparatory work on this scientific opinion.

[4] Where changes have been made to the opinion, footnotes have been included. The changes do not affect the overall conclusion of the opinion.

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SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety of Patent Blue V (E 131) when used as a food colouring substance.

Patent Blue V (E 131) is a triarylmethane dye authorised as a food additive in the EU, that has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1970 and 1975 and the EU Scientific Committee for Food (SCF) in 1983; JECFA established a Temporary Acceptable Daily Intake (ADI) of 0-1 mg/kg bw/day in 1970, but withdrew it in 1975. Until now JECFA has not allocated an ADI to Patent Blue V (E 131), whereas the SCF established an ADI of 0-15 mg/kg bw/day.

Data available on the absorption, distribution, metabolism and excretion of Patent Blue V after oral administration in rats and dogs show that Patent Blue V has low absorption, limited systemic availability and is mainly excreted unchanged in the faeces. In vitro data on the metabolism in humans and in rats showed that Patent Blue V is not metabolised.

Patent Blue V, in old studies, did not demonstrate any evidence of mutagenic activity in strains of tryptophan-requiring *Escherichia coli* and histidine-requiring *Salmonella typhimurium* (four strains), both with, and without metabolic activation. In contrast, in an Ames test performed in 2010 according to OECD Guidelines, high concentrations of Patent Blue V induced biologically significant increases in the number of revertants in the TA98 strain in the presence of metabolic activation. In the presence of low concentrations, there were no effects. This observation could suggest that an impurity present in the food colour could be responsible for this mutagenic effect.

A mouse lymphoma assay performed in 2011 according to OECD Guidelines with Patent Blue V in accordance with EU specifications, was negative.

Two in vivo micronucleus assays are available for Patent Blue V. The first one (in 1986), performed in mouse after intraperitonal injection, was evaluated as not reliable. In contrast, the second one (in 2011), performed in rat after i.v. injection according to OECD guidelines did not show any clastogenic effect.

In an unpublished study report, the DNA damaging capabilities of Patent Blue V (E 131) were assessed in the in vivo single cell gel/Comet assay in rats. The Panel concluded that Patent Blue V (E 131) did not induce any effect in DNA migration in rat liver, jejunum/ileum and peripheral blood after in vivo treatment under the reported experimental conditions. According to new studies provided by industry on request of EFSA, the Panel considered that Patent Blue V (E 131) (at purity level $\geq$ than 90 %) was not of concern with respect to genotoxicity.

There is one chronic toxicity study performed in mice. In the absence of the full study report, the Panel considered that the haematological effects observed at the highest dose tested were biologically significant. Therefore, the Panel considered that a NOAEL of 500 mg/kg bw/day (intermediate dose tested) can be derived from this study, based on growth reduction and alterations of haematological parameters reported at the highest dose tested mainly in males. Based on the data of this study, the Panel considers that Patent Blue V has no carcinogenic effects.

A reproductive toxicity study in mice did not reveal adverse effects. The NOAEL from this study is the highest dose tested, 1500 mg/kg bw/day.

No effects have been identified in a developmental study in rats. The NOAEL derived by the Panel is 500 mg/kg bw/day, the highest dose tested.

The Panel concluded that the present dataset provided a rationale for a re-definition of the ADI. Using the NOAEL of 500 mg/kg bw/day from a chronic toxicity study in mice and applying an uncertainty factor of 100 to this NOAEL, the Panel established an ADI of 5 mg/kg bw/day for Patent Blue V of purity at least 90 %.

Exposure to Patent Blue V from its use as a food additive has been calculated by using the Maximum Permitted Levels (MPLs) as indicated in the Commission Regulation No 1129/2011 and by using data on reported use levels provided by the food industry or data reported on analytical levels provided by national authorities or found in the literature. These data were combined with national consumption data for the population groups of toddlers, children, adolescents, adults and the elderly from the EFSA Comprehensive Food Consumption Database.

When considering MPLs, estimates calculated for toddlers, children, adolescents, adults and the elderly give dietary exposures at the mean in the range of 1-4.5, 1.1-3.6, 0.5-1.8, 0.3-1.4, and 0.2-0.6 mg/kg bw/day, respectively. High level exposures for these population groups were calculated to be in the range of 2.9-7.5, 2.4-7, 1.3-3.7, 0.9-2.9 and 0.6-1.5 mg/kg bw/day, respectively. The main contributors to the total anticipated exposure to Patent Blue V (>10 %) for adults were flavoured drinks (with sugar or sweeteners) (11-48 %), flavoured fermented milk products (14-29 %), fine bakery wares (11-34 %), and sauces (12-29 %). For children, main contributors were flavoured fermented milk products (13-32 %), fine bakery wares (11-45 %), and sauces (11-23 %).

When considering maximum reported use levels or maximum analytical levels, estimates calculated for toddlers, children, adolescents, adults and the elderly give dietary exposures at the mean in the range of 0.2-1.5, 0.4-1.2, 0.3-0.7, 0.1-0.5 and 0.04-0.3 mg/kg bw/day, respectively. High level exposures for these population groups were calculated to be in the range of 0.6-2.7, 1-2.4, 0.7-1.7, 0.4-1 and 0.1-0.8 mg/kg bw/day, respectively. The main contributors to the total anticipated exposure to Patent Blue V (>10 %) for adults were flavoured drinks with sweeteners (12-38 %), and fine bakery wares (15-64 %). For children, main contributors were fine bakery wares (10-70 %), and flavoured drinks with sugar (10-31 %).

The Panel noted that at the maximum permitted levels of use of Patent Blue V, exposure estimates for high consumers are above the ADI of 5 mg/kg bw/day in toddlers and children. At the maximum reported use levels of Patent Blue V, exposure estimates are below the ADI of 5 mg/kg bw/day for all groups of the population.

The Panel further noted that the specifications for Patent Blue V 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 chromium is < 50 mg/kg, whereas no specification for chromium is required in EC specifications.

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, and that therefore specifications for the maximum level of aluminium in the lakes may be required.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010[4]. This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[5] of 2001. The report "Food additives in Europe 2000[6]" submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with a highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010 the 2003 Terms of References are replaced by those below.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

[4] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.03.2010, p.19.

[5] Report from the Commission on dietary food additive intake in the European Union. COM(2001) 542 final.

[6] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002:560.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of Patent Blue V (E 131) when used as a food colouring substance.

Patent Blue V (E 131) is 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 1975, and the EU Scientific Committee for Food (SCF) in 1983.

The Panel on Food Additives and Nutrient Sources added to Food (ANS) 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[7,8,9]. 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

Patent Blue V (E 131) is a triarylmethane food colour that exists under two inner salt (calcium and sodium) forms.

The systematic name for the calcium salt is *N*-(4-((4-(diethylamino)phenyl)(5-hydroxy-2,4-disulfophenyl)methyl-ene)-2,5-cyclohexadien-1-ylidene)-*N*-ethylethanaminium, hydroxide, inner salt, calcium salt (2:1). The molecular formula is $C_{27}H_{32}N_2O_7S_2.1/2Ca$, the molecular weight is 579.72 g/mol, the CAS Registry Number is 3536-49-0, the EINECS number is 222-573-8 and the Colour Index number is 42051 (ChemIDplus, 2012).

The systematic name for the sodium salt is *N*-(4-((4-(diethylamino)phenyl)(2,4-disulfophenyl)methylene)-2,5-cyclohexadien-1-ylidene)-*N*-ethylethanaminium, hydroxide, inner salt, sodium salt. The molecular formula is $C_{27}H_{32}N_2O_7S_2{\cdot}Na$, the molecular weight is 582.67 g/mol, the CAS Registry Number is 129-17-9, the EINECS number is 204-934-1 and the Colour Index number is 42045 (ChemIDplus, 2012).

The structural formula for the two salt forms is depicted in Figure 1:

[7] Call for scientific data on food colours to support re-evaluation of all food colours authorised under the EU legislation. Published on 8 December 2006. Available at: http://www.efsa.europa.eu/en/dataclosed/call/afc061208.htm

[8] Call for scientific data on Patent Blue V (E 131). Published on 23 July 2010. Available at: http://www.efsa.europa.eu/en/dataclosed/call/ans100723.htm

[9] Call for scientific data on Patent Blue V (E 131). Published on 6 June 2011. Available at: http://www.efsa.europa.eu/en/dataclosed/call/110606.htm

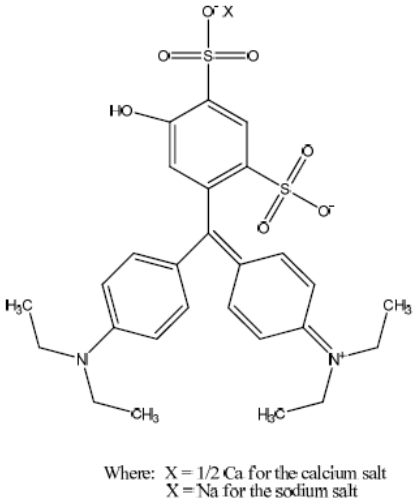

Figure 1: Structural formula of Patent Blue V

Patent Blue V (E 131) is a dark blue powder, or in the form of granules. The melting point is >300°C. The substance is soluble in water and slightly soluble in ethanol.

Several synonyms are in use. The most commonly used synonyms in published literature are for the calcium salt: C.I. Acid Blue 3, C.I. Food Blue 5, Carmine Blue V, C.I 42051; for the sodium salt: C.I Acid Blue 1, Acid Blue V, C.I. Food Blue 3, Carmine Blue VF, C.I 42045, Sulphan Blue, Brilliant Acid Blue VS, Blue VRS.

2.2. Specifications

Specifications have been defined in the Directive 2008/128/EC[10] and new specifications according to Commission Regulation (EU) No 231/2012[11] will apply from 1 December 2012. Specifications have also been defined by JECFA (JECFA, 2008) (Table 1).

According to Commission Regulation (EU) No 231/2012, Patent Blue V (E 131) consists essentially of the calcium or sodium compound of [4-(α-(4-diethylaminophenyl)-5-hydroxy-2,4-disulphophenyl-methylidene)-2,5-cyclohexadien-1-ylidene] diethyl-ammonium hydroxide inner salt and subsidiary colouring matters, together with sodium chloride and/or sodium sulphate and/or calcium sulphate as the principal uncoloured components. The potassium salt is also permitted.

The purity is defined as content not less than 85 % total colouring matters, calculated as the sodium salt. The remaining 15 % may be accounted for by sodium chloride or sodium sulphate (but this is never mentioned explicitly), ≤ 2 % subsidiary colouring matters and total ≤ 0.5 % (3-hydroxy benzaldehyde, 3-hydroxy benzoic acid, 3-hydroxy-4-sulphobenzoic acid and *N*,*N*-diethylamino benzene sulphonic acid), ≤ 4 % leuco base, ≤ 0.01 % unsulphonated primary aromatic amines and ≤ 0.2 % ether extractable matter, originating from the manufacturing process (Commission Regulation (EU) No 231/2012).

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

[10] 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. 20–63.

[11] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p 1-295.

Table 1: Specifications for Patent Blue V according to Commission Regulation (EU) No 231/2012 and JECFA (JECFA, 2008)

Purity	Commission Regulation (EU) No 231/2012	JECFA (2008)
Assay	≥ 85 %	≥85 %
Water insoluble matter	≤ 0.2 %	≤ 0.5 %
Subsidiary colouring matters	≤ 2.0 %	≤ 2.0 %
Organic compounds other than colouring matters : - 3-hydroxy benzaldehyde - 3-hydroxy benzoic acid - 3-hydroxy-4-sulphobenzoic acid - *N*,*N*-diethylamino benzene sulphonic acid	total ≤ 0.5 %	total ≤ 0.5 %
Leuco base	≤ 4.0 %	≤ 4.0 %
Unsulphonated primary aromatic amines	≤ 0.01 % (calculated as aniline)	≤ 0.01 % (calculated as aniline)
Ether extractable matter	≤ 0.2 % (from a solution pH 5)	≤ 0.2%
Arsenic	≤ 3 mg/kg	-
Lead	≤ 2 mg/kg	≤ 2 mg/kg
Mercury	≤ 1 mg/kg	
Cadmium	≤ 1 mg/kg	-
Chromium	-	≤ 50 mg/kg

The Panel noted that the JECFA specification for chromium is < 50 mg/kg whereas no specification for chromium is required in EC specifications.

The Panel noted that the specifications on the purity of Patent Blue V (E 131) would permit concentrations of unsulphonated aromatic amines to be present in concentrations of up to 100 mg/kg Patent Blue V (E 131). Given the maximal allowed concentration of Patent Blue V (E 131) that can be added to food (500 mg/kg food), the concentration of these unidentified unsulphonated primary aromatic amines in food could be 50 µg/kg food.

According to Commission Regulation (EU) No 231/2012, aluminium lakes of Patent Blue V (E 131) may be used and the above purity criteria for the pure substance also apply to the raw material from which the aluminium lake is produced. In addition, under neutral conditions the aluminium lake should contain no more than 0.5 % HCl-insoluble material and no more than 0.2 % ether extractable material. There are no additional specification requirements for the aluminium lake.

JECFA does not give specifications for aluminium lakes of Patent Blue V (E 131), other than reference to the General Specifications for Aluminium Lakes of Colouring Matters (JECFA, 2006). The Patent Blue V (E 131) 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

According to information provided by industry, Patent Blue V (E 131) is manufactured, in a one-step reaction, by condensation and sulphonation of *N*,*N*-diethylaniline and 3-hydroxybenzaldehyde in acidic conditions (sulphuric acid) to produce the substance under leuco form. The leuco form is tested by thin layer chromatography (TLC). The leuco form is then solubilised in ammonia and oxidised with manganese dioxide in phosphoric acid. This reaction is controlled by high pressure liquid chromatography (HPLC). The oxidised substance is neutralised with sodium hydroxide and precipitated using either hydrochloric acid and sodium chloride (to produce the sodium salt) or hydrochloric acid and calcium chloride (to produce the calcium salt). This reaction is UV controlled. The precipitate is filtrated and the wet press cake so obtained is dried at 180°C. The dried product is further grinded or milled to obtain the product under powdered or granulated form. The substance is further standardised at a titre of 90 % with either sodium sulphate or sodium chloride (Fiorio Colori, 2012b).

Patent Blue V (E 131) 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 (Commission Regulation (EU) No 231/2012; JECFA, 2004).

2.4. Methods of analysis in food

The most widely applicable methods to identify and quantify Patent Blue V (E 131) in foods and beverages employ high pressure liquid chromatography (HPLC) with diode array detection, after varying degrees of sample preparation depending on the matrix. Patent Blue V can be quantified in soft drinks from plant extracts and fruit juices, energy drinks, and vegetable nectars by degassing, filtering and direct injection into the HPLC with diode array detection. Recoveries range from 96.3 to 98.5 % and the LOQ is 0.4 mg/l (Serdar and Knezevic, 2009). Beverages and powder mixes, fruit jellies, and hard candies, are extracted into hot water, filtered and concentrated. Cookies, cereal, wafers, chips, noodles, and soft candies are extracted into 25 % glacial acetic acid, centrifuged and chloroform defatted before being concentrated. After clean-up on a C18-Sep Pak cartridge, the extracts are evaporated to dryness and re-dissolved in HPLC mobile phase. Analysis again is by reverse phase HPLC with diode array detection giving an LOD of 0.12 mg/l for Patent Blue V (Harp et al., 2012). Similar HPLC methods have been reported by others (Kirschbaum et al., 2003; Minioti, et al., 2007; Yoshioka and Ichihashi, 2008; Dixit et al., 2010). Other less widely applicable approaches to the analysis of Patent Blue V have employed thin layer chromatography (Baranowska, 2004; Tuzimski and Woźniak, 2008), high performance thin layer chromatography (Tuzimski, 2011) and capillary electrophoresis (Patsovskii, 2004; Perez-Urquiza, 2000).

2.5. Reaction and fate in food

No data on reaction and fate of Patent Blue V (E 131) in food are available. 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) may affect the colour (Scotter and Castle, 2004).

Data on stability of the Patent Blue V (E 131) in the dry state were provided by industry. Three different batches from three different productions were stored for up the 5 years at 25 °C and 60 % relative humidity and at 40 °C and 65 % relative humidity. The data show that the substance was fully stable under these conditions (Fiorio Colori, 2012b).

2.6. Case of need and proposed uses

Maximum Permitted Levels (MPLs) of Patent Blue V have been defined in the Commission Regulation (EU) No 1129/2011[12] on food additives for use in foodstuffs.

Currently, Patent Blue V (E 131) is an authorised food colour in the EU with MPLs ranging from 50 to 500 mg/kg in foods. For edible cheese rind and edible casings, Patent Blue V is authorised at quantum satis levels.

Table 2 summarises foods that are permitted to contain Patent Blue V (as part of group III food colours with combined maximum limit) and the corresponding MPLs as set by Commission Regulation (EU) No 1129/2011.

Table 2: MPLs of Patent Blue V in foods according to the Commission Regulation (EU) No 1129/2011[13]

Category number	**Category name**	**Maximum Permitted Level** (mg/kg or mg/l)
01.4	Flavoured fermented milk products including heat-treated products	150
01.6.3	Other creams (only flavoured creams)	150
01.7.1	Unripened cheese excluding products falling in category 16 (only flavoured unripened cheese)	150
01.7.3	Edible cheese rind	QS
01.7.6	Cheese products (excluding products falling in category 16) (only flavoured unripened products)	100
03	Edible ices	150
04.2.1	Dried fruit and vegetables (only preserves of red fruit)	200*
04.2.2	Fruit and vegetables in vinegar, oil, or brine (only preserves of red fruit)	200*
04.2.3	Canned or bottled fruit and vegetables (only preserves of red fruit)	200*
04.2.4.1	Fruit and vegetable preparations excluding compote (only mostarda di frutta and preserves of red fruit)	200*
05.2	Other confectionery including breath freshening microsweets (only candied fruit and vegetables)	200
05.2	Other confectionery including breath freshening microsweets (except candied fruit and vegetables)	300
05.3	Chewing gum	300
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4 (only decorations, coatings and sauces, except fillings)	500
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4 (only fillings)	300
06.6	Batters (only batters for coating)	500
07.2	Fine bakery wares	200
08.2.3	Casings and coatings and decorations for meat (only decorations and coatings except edible external coating of pasturmas)	500
08.2.3	Casings and coatings and decorations for meat (only edible casings)	QS
09.2	Processed fish and fishery products including molluscs and crustaceans (only surimi and similar products and salmon substitutes)	500

[12] Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council establishing a Union list of food additives. The Panel noted that the Commission Regulation (EC) No 1129/2011 of 11 November 2011 will enter into force on June, 1st 2013 but confirm the approved uses of Patent Blue V as a food additive as described in previous Council Directive No 94/36/EC of 30 June 1994 on colours for use in foodstuffs.

[13] The description of the permitted uses of Patent Blue (E 131) has been amended to reflect the exact food category names reported in Commission Regulation (EU) No 1129/2011 (food categories 01.6.3, 01.7.1, 01.7.6, 05.2 and 06.6). The MPL value of category number 05.3 has been corrected from 100 mg/kg to 300 mg/kg, as in Commission Regulation (EU) No 1129/2011.

Category number	Category name	Maximum Permitted Level (mg/kg or mg/l)
09.3	Fish roe (only Sturgeons´ eggs (Caviar))	300
12.2.2	Seasonings and condiments (only seasonings, for example curry powder, tandoori)	500
12.4	Mustard	300
12.5	Soups and broths	50
12.6	Sauces (including pickles, relishes, chutney and piccalilli; excluding tomato-based sauces)	500
12.9	Protein products, excluding products covered in category 1.8 (only meat and fish analogues based on vegetable proteins)	100
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	50
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	50
14.1.4	Flavoured drinks (excluding chocolate milk and malt products)	100
14.2.3	Cider and perry (excluding cidre bouché)	200
14.2.4	Fruit wine and made wine	200
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008 (except: spirit drinks as defined in article 5(1) and sales denominations listed in Annex II, paragraphs 1-14 of Regulation (EC) No 110/2008 and spirits (preceded by the name of the fruit) obtained by maceration and distillation, London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà)	200
14.2.7.1	Aromatised wines (except americano, bitter vino)	200
14.2.7.2	Aromatised wine-based drinks (except bitter soda, sangria, claria, zurra)	200
14.2.7.3	Aromatised wine-product cocktails	200
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol (only alcoholic drinks with less than 15 % of alcohol)	200
15.1	Potato-, cereal-, flour- or starch-based snacks (excluding extruded or expanded savoury snack products)	100
15.1	Potato-, cereal-, flour- or starch-based snacks (only extruded or expanded savoury snack products)	200
15.2	Processed nuts (only savoury-coated nuts)	100
16	Desserts excluding products covered in categories 1, 3 and 4	150
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	300
17.2	Food supplements supplied in a liquid form	100
17.3	Food supplements supplied in a syrup-type or chewable form (only solid food supplements)	300
17.3	Food supplements supplied in a syrup-type or chewable form (only liquid food supplements)	100

* Maximum individually or for the combination of E 120, E 122, E 124, E 129, E 131, E 133

2.7. Reported use levels or data on analytical levels of Patent Blue V

Summarised data on reported use levels in foods from industries and other sources

Table 3 provides data on the use levels of Patent Blue V in foods as reported by industries and on analysed levels. Table 3 also shows the levels used for the refined exposure assessment identified by the Panel and based on data for several food categories in finished products reported by industries or analytical data from other sources (member states, scientific literature) or from the rules followed to deal with quantum satis (QS) authorisation as indicated in Annex A, figures 1 and 2.

Table 3: Summary of use levels of Patent Blue (E 131) as reported by industries or analytical data and levels used in the refined exposure assessment (mg/kg food or mg/l beverage)[14]

Matching FoodEx Food codes	Food items	MPL	INDUSTRY		ANALYTICAL				Level used for calculation
			CIAA[(a)]	CIAA[(a)]	Ireland[(b)]	UK[(c)]	Cyprus[(d)]	Slovakia[(e)]	
			typical	max					
14.1.4	Non-alcoholic beverages	**100**	8-10	22*	<LOQ (1 mg/l) - 3	<LOD (0.1 mg/l) - 10	<LOD (0.2 mg/l) - 1.3	1.3	25
14.2.3, 14.2.4	Fruit wines, cider and perry	**200**	3-10	10	<LOD (1 mg/l)				10
14.2.6	Spirituous beverages	**200**	0-20	60	<LOQ (1 mg/l)				60
04.2.1, 04.2.2, 04.2.3, 04.2.4.1	Preserves of red fruits	**200**	10-100	150					150
05.4	Decorations and coatings	**500**	10-100	136				12.3-27.4 mg/kg	140
12.4	Mustard	**300**	5	5					5
01.4, 01.6, 16	Desserts including flavoured milk products	**150**	10	10					10
12.2, 12.6	Sauces, seasonings, pickles, relishes, chutney and piccalilli	**500**	6	6	<LOD (2-10 mg/kg)			20.66**	10
05.2	Confectionary	**300**	5-200	300	<LOD (1 mg/kg) - 158	<LOD (0.5 mg/kg) - 70	<LOD (0.2 mg/kg) - 8	1.4-27.1 mg/kg	300
03	Flavoured ices	**150**			<LOD (1-2 mg/kg)		<LOD (0.2 mg/kg) - 19.9		20

* Tennant reported 24 mg/l for this category (Tennant, 2006)

** Tabasco Green Pepper Sauce

[(a)] CIAA, 2009; [(b)] FSAI, 2009; (c) FSA, 2002, 2003; [(d)]Cyprus State General Laboratory, 2010; [(e)]Slovakia, 2009

For those categories where no reported use level was provided, the value presented in Table 1 (MPL) has been considered for the estimate calculation

[14] The matching FoodEx food codes reported under item "desserts including flavoured milk products" were amended to include codes 01.4 and 01.6. The matching FoodEx food codes reported under item "sauces, seasonings, pickles, relishes, chutney and piccalilli" were amended to include code 12.2.

2.8. Information on existing authorisations and evaluations

Patent Blue V (E 131) is authorised as a food additive in the EU under Commission Regulation (EU) No 1129/2011 on food additives for use in foodstuffs. Specific purity criteria on Patent Blue V have been defined in the EU Directive 2008/128/EC and new specifications according to Commission Regulation (EU) No 231/2012 will apply from 1 December 2012.

JECFA in 1970 established a temporary ADI of 0-1 mg/kg bw (JECFA, 1970). In 1975 JECFA withdrew the previously set temporary ADI (JECFA, 1975) due to the fact that information on the metabolism of the colour, a long-term study in a second species and a short-term study in a non-rodent species were lacking while JECFA required them in 1970. Up till now, JECFA has not allocated an ADI to Patent Blue V (E 131) (JECFA, 1982).

The SCF allocated an ADI of 0-15 mg/kg bw to Patent Blue V in 1983 based on the no-adverse level of 1500 mg/kg bw/day, the highest dose tested in a long-term mouse study (SCF, 1983).

The International Agency for Research on Cancer (IARC) evaluated Patent Blue V in 1987. It was concluded that Patent Blue V is carcinogenic in rats following its subcutaneous or intramuscular injection; it produced sarcomas at the site of repeated injections. No case reports or epidemiological studies were available. Patent Blue V has been considered in group 3, not classifiable as to carcinogenicity to humans (IARC, 1987).

2.9. Exposure assessment

2.9.1. Food consumption data used for exposure assessment

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been built from existing national information on food consumption at a detailed level. Competent authorities in the European countries provided EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b).

Overall, the food consumption data gathered at EFSA were collected by different methodologies and thus direct country-to-country comparison should be made with caution.

For calculation of chronic exposure, intake statistics have been calculated based on individual average consumption over the total survey period excluding surveys with only one day per subject. High level consumption was only calculated for those foods and population groups where the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011b). The Panel estimated chronic exposure for the following population groups: toddlers, children, adolescents, adults and the elderly. Calculations were performed using individual body weights.

Thus, for the present assessment, food consumption data were available from 26 different dietary surveys carried out in 17 different European countries as mentioned in the Table 4:

Table 4: Population groups considered for the exposure estimates of Patent Blue V

Population	Age range	Countries with food consumption surveys covering more than one day
Toddlers	from 12 up to and including 35 months of age	Belgium, Bulgaria, Finland, Germany, Italy, Netherlands, Spain
Children[15]	from 36 months up to and including 9 years of age	Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden
Adolescents	from 10 up to and including 17 years of age	Belgium, Cyprus, Czech Republic, Denmark, France, Germany, Italy, Latvia, Spain, Sweden
Adults	from 18 up to and including 64 years of age	Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Spain, Sweden, UK
The elderly[15]	Older than 65 years	Belgium, Denmark, Finland, France, Germany, Hungary, Italy

Consumption records were codified according to the FoodEx classification system (EFSA, 2011a). Nomenclature from FoodEx classification system has been linked to the Food Classification System as presented in the Commission Regulation (EU) No 1129/2011, part D, to perform exposure estimates.

2.9.2. Exposure to Patent Blue V from its use as a food additive

Exposure to Patent Blue V from its use as a food additive has been calculated by using (1) MPLs as listed in Table 2 and (2) data on reported use levels or data reported on analytical levels as listed in Table 3 including data following the rules for QS regulations (refined exposure assessment), both combined with national consumption data for the five population groups (Table 4).

High level exposure (typically 95th percentile of consumers only) was calculated by adding the 95th percentile of exposure from one food group (i.e. the one having the highest value) to the mean exposure resulting from the consumption of all other food groups.

This is based on the assumption that an individual might be a high level consumer of one food category and would be an average consumer of the others. This approach has been tested several times by the Panel in the re-evaluation of food colours and has shown reasonable correlation with high level total intakes when using the raw food individual consumption data. Therefore, this approach was preferred for the calculations based on the MPLs and maximum reported use levels in order to avoid excessively conservative estimates.

However, the Panel notes that its estimates should be considered as being conservative as it is assumed that all processed foods contain Patent Blue V added at the MPLs or the maximum reported use levels.

Table 5 summarises the estimated exposure to Patent Blue V from its use as a food additive of all five population groups.

[15] The terms "children" and "the elderly" correspond respectively to "other children" and the merge of "elderly" and "very elderly" age groups in the EFSA Guidance on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b).

Table 5: Summary of anticipated exposure to Patent Blue V from its use as a food additive using MPLs and reported use levels or analytical data on use levels in five population groups (mg/kg bw/day)

	Toddlers (12-35 months)	Children (3-9 years)	Adolescents (10-17 years)	Adults (18-64 years)	The elderly (>65 years)
Estimated exposure using MPLs					
• Mean	1-4.5	1.1-3.6	0.5-1.8	0.3-1.4	0.2-0.6
• High level[16]	2.9-7.5	2.4-7	1.3-3.7	0.9-2.9	0.6-1.5
Estimated exposure using reported use levels or analytical data					
• Mean	0.2-1.5	0.4-1.2	0.3-0.7	0.1-0.5	0.04-0.3
• High level[16]	0.6-2.7	1-2.4	0.7-1.7	0.4-1	0.1-0.8

2.9.3. Main food categories contributing to exposure to Patent Blue V using MPLs

Table 6: Main food categories contributing to exposure to Patent Blue V using MPLs and number of surveys (between brackets) in which each food category is contributing

	Toddlers	Children	Adolescents	Adults	The elderly
Food Categories	**% contribution to total exposure (Number of Surveys)**				
1.4. Flavoured fermented milk products including heat treated products	22-68 (6)	13-32 (9)	12-13 (3)	14-29 (2)	16-27 (2)
1.6. Cream					12-14 (3)
1.7.1. Unripened cheese (excluding category 16)		14 (1)	15 (1)	22 (1)	22 (19)
3. Edible ices	14 (1)	11 (2)			
5.2.1. Other confectionery with added sugar		10 (1)			
7.2. Fine bakery wares	13-50 (5)	11-45 (13)	12-37 (11)	11-34 (12)	10-31 (6)
9.2. Processed fish and fishery products including mollusks and crustaceans		15 (1)			
12.2. Herbs, spices, seasonings			14 (1)		
12.5. Soups and broths		13 (1)	12 (1)	16 (1)	16 (1)
12.6. Sauces	11 (1)	11-23 (8)	14-28 (7)	12-29 (10)	11-26 (5)
14.1.4.1. Flavoured drinks with sugar	13-21 (4)		15-55 (7)	11-48 (12)	12-39 (3)
14.1.4.2. Flavoured drinks with sweeteners	15 (1)		20-27 (2)	11-37 (3)	
14.2. Alcoholic beverages, including alcohol-free and low-alcohol counterparts				11-13 (2)	
16. Desserts excluding products covered in category 1, 3 and 4	12-15 (2)				

[16] Typically 95th percentile of consumers only.

2.9.4. Main food categories contributing to exposure to Patent Blue V using reported use levels or reported data on analytical levels

Table 7: Main food categories contributing to exposure to Patent Blue V using reported use levels or reported data on analytical levels and number of surveys (between brackets) in which each food category is contributing

	Toddlers	Children	Adolescents	Adults	The elderly
Food Categories	**% contribution to total exposure (Number of surveys)**				
1.4. Flavoured fermented milk products including heat treated products	12-28 (2)				
1.7.1. Unripened cheese (excluding category 16)	11-18 (2)	14 (1)	19 (1)	14-28 (2)	11-27 (3)
5.2.1. Other confectionery with added sugar	11 (2)	12-32 (6)	16-28 (2)	26-28 (2)	15-17 (2)
7.2. Fine bakery wares	15-71 (7)	10-70 (15)	19-62 (11)	15-64 (14)	31-59 (6)
9.2. Processed fish and fishery products including mollusks and crustaceans	16-19 (2)	13-22 (5)	12-15 (3)	19 (1)	
12.5. Soups and broths	17 (1)	13-26 (2)	24 (1)	14-31 (2)	
14.1.4.1. Flavoured drinks with sugar	11-16 (2)	10-31 (11)	11-40 (7)	11-27 (2)	15-25 (2)
14.1.4.2. Flavoured drinks with sweeteners	12 (1)		12-18 (2)	12-38 (8)	15-25 (2)
14.2. Alcoholic beverages, including alcohol-free and low-alcohol counterparts				11-12 (2)	
15.1. Potato-, cereal-, flour- or starch-based snacks	18 (1)		12-14 (2)		
17. Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children					17 (1)

3. Biological and toxicological data

Patent Blue V (E 131) has been evaluated previously by JECFA in 1970 and 1975, and the SCF in 1983. It was also evaluated by TemaNord (2002). The present opinion briefly reports the major studies included in these evaluations and describes the additionally, reported new literature in more detail.

3.1. Absorption, distribution, metabolism and excretion

JECFA (1975) describes one study regarding the toxicokinetics of Patent Blue V (E 131). In this study, after intravenous injection in rats (0.5 ml, 50 mg/ml aqueous solution) and subcutaneous injection in man (no details), the colour was excreted in the urine during a 12-hour observation period following injection (Truhaut, 1962).

The SCF (1983) states that Patent Blue V (E 131) is poorly absorbed by rats and dogs after single oral administration, and that the nature of the metabolites was not determined.

In vitro data

An in vitro study has been performed in 2011 (Guerbet/Eurofins, 2011) in order to measure the metabolism of Patent Blue V (E 131) in hepatic microsomes from rats and humans. Patent Blue V (1,

5, 10 and 100 µM), was added in the presence of 150 µg microsomal proteins (for the concentrations 10 and 100 µM) and 500 µg microsomal proteins (for the concentrations 1 and 5 µM). Patent Blue V used in this assay was in accordance with the EU specifications for this food colour (purity ≥ 85 %). Omeprazole, a known metabolic substrate for human and rat microsomal enzymes, was used as positive control. The Omeprazole concentrations tested were 0.58, 1, 5, and 100 µM in the same experimental conditions as those used for Patent Blue V (E 131). After incubation of 1 µM or 5 µM Patent Blue V, no metabolism was observed with either human or rat microsomes. After incubation of Patent Blue V at 10 µM, mean metabolism observed was 1 % when incubated with human microsomes and no metabolism was observed for rat microsomes. After incubation of Patent Blue V at 100 µM, mean metabolism observed was 3.4 % when incubated with human microsomes and 0.35 % for rat microsomes. After incubation of 0.58 µM Omeprazole, mean metabolism observed was 68 % for human microsomes and 96 % for rat microsomes, validating the enzyme activity of the microsomes at this concentration. Based upon these in vitro results, the authors conclude that Patent Blue V is not metabolised at concentrations of 1 to 100 µM in the presence of human or rat hepatic microsomes.

In vivo data

Rats

In preliminary studies in rats (LEMM, 1978a), only low levels of radioactivity (representing a maximum of less than 0.03 % of the dose) were detected in serial blood samples taken at intervals of up to 72 hours from males given a range of single oral doses of 0.34 and 1.21 (^{14}C) Patent Blue V mg/kg bw (position of labelling not specified). Although the peak concentration of radioactivity (which occurred within one hour of dosing) fell by 46-84 % in 24 hours, radioactivity was detectable in the blood at 72 hours. Following a single i.v. injection in rats of about 0.35 (^{14}C) Patent Blue V mg/kg bw (position of labelling not specified), elimination from the blood was rapid. Serial blood sampling revealed that the levels of radioactivity after one hour were only about 13-14 % of the initial value. At 4 hours these had fallen to 3-4 % and at 24 hours this was 1 %. The majority of radioactivity was excreted in the faeces (more than 90 % within 24 hours) but, in contrast to the mouse in which urinary excretion amounted to 24 % of the i.v. dose at 24 hours, only up to 2 % of the radioactivity injected appeared in the urine of the rat within this time-period. Less than 0.1 % of the administered dose of radioactivity was detected in expired air. Less than 0.5 % of the administered dose was excreted in the urine, whilst over 80 % appeared in the faeces; for both routes of administration, the majority was excreted within 24 hours. At 72 hours, less than 0.5 % of the dose was detected in the carcass. Analysis of the major organs of rats 72 hours or 96 hours after oral administration of radiolabelled Patent Blue V or 72 hours following i.v. dosing indicated a wide distribution. Higher levels of radioactivity were, as expected, associated with the gastrointestinal tract and organs involved in excretion. In addition, concentrations of radioactivity were several folds higher in the thyroid, compared to most other tissues in animals dosed by both the i.v. and oral routes (LEMM, 1978a; BIBRA, 1982).

More detailed studies, where groups of male and female Sprague-Dawley rats were given oral doses between 1.6 and 3.2 mg (^{14}C) Patent Blue V/kg bw, and then killed at intervals of up to 48 hours, were in agreement with the preliminary findings (LEMM, 1978b). A kinetic study showed that there were 2 phases of elimination from the blood with half-lives of 15 minutes and 39 hours, respectively. Low levels of activity remained in the carcass after 48 hours; again slightly elevated concentrations were noted in the thyroid compared to other tissues. In 6 male rats given oral doses of 1 to 1.5 mg (^{14}C) Patent Blue V/kg bw, a total of 93 % of the dose appeared in the faeces within 24 hours. Radioactivity extracted from the faeces in these studies was in the form of unchanged colour. Less than 0.5 % of the radioactivity was excreted in the urine (most within the first 4 hours), and of this, 20-50 % was as the unchanged colour. In addition, 2 substances representing 30-32 % and 12-14 % of the urinary radioactivity and 3 minor components were detected but not identified. The excretion in both urine and faeces appeared to be biphasic. The early rapid elimination of the majority of the radioactivity was followed by a much slower excretion of the remainder (possibly as a result of coprophagy) (LEMM, 1978b, c; BIBRA, 1982).

Periodic collection of bile at intervals for up to 96 hours, from 2 cannulated male rats given a single oral dose of 0.81 or 0.89 mg/kg bw (^{14}C) Patent Blue V revealed biliary excretions of 6.1 % of the doses within 54 hours. The peak in the radioactivity occurred within the first three hours, with little excretion occurring after 12 hours. The radioactivity present was in the form of unchanged colour. The total radioactivity excreted in the bile and urine and remaining in the carcass indicated that around 6.7 % and 4.4 % of the dose was absorbed (LEMM, 1978b; BIBRA, 1982).

The relatively low absorption after oral administration was confirmed by analysis of the organs from rats given oral doses 1.8 - 3.2 mg/kg of (^{14}C)radiolabelled Patent Blue V, and killed at intervals of up to 48 hours, and from rats given 0.87 - 1.18 mg/kg and killed at 120 hours. Wide tissue distribution was again observed. In tissues other than the gastrointestinal tract, the findings suggest that about 10 % of the orally administered dose was present at 0.5 hour. This fell to 2 % by 24 hours and 0.2 % by 48 hours. Small amounts of the dose were present in the carcass at 72 hours and less than 0.1 % of the dose was present at 120 hours. There was some evidence for preferential accumulation of radioactivity in the thyroid; concentrations were several folds higher than in many other tissues throughout the study.

Autoradiographs of 3 rats treated orally with 0.48, 8.75 or 9.08 mg (^{14}C) Patent Blue V/kg bw and killed at 1, 24 and 48 hours after dosing, confirmed that the absorbed radioactivity was widely distributed (LEMM, 1978b; BIBRA, 1982).

Dogs

(^{14}C) Radiolabeled Patent Blue V (label position not specified) complying with JECFA specifications was used in studies in which 2 groups of 1 male and 1 female 8-month-old Beagle dogs received single oral doses of 0.23 and 0.25 mg/kg bw or single i.v. doses of 0.11 mg/kg, respectively (LEMM, 1978d; BIBRA, 1982). Excretion following i.v. injection was in 2 phases, with half-lives in the blood of 2.7 and 36.5 hours, respectively. Less than 1 % of the initial concentration of radioactivity remained in the blood at 24 hours. Totals of 3.3 and 15.6 % of the amount injected intravenously were excreted in the urine within 72 hours, the majority being excreted within 24 hours. 14.8 % and 89.3 % of the injected doses, were excreted in the faeces within 72 hours, thus it was likely that biliary excretion had occurred.

After oral dosing, low levels of radioactivity (which peaked after 1.25 hours at concentrations representing about 0.002 % of the dose/g blood) were detected in the blood. Examination of the blood kinetics revealed a slower elimination rate than when the dogs were dosed by the i.v. route, involving 3 compartments with half lives of 0.3 hour, 2.7 hours and 33.3 hours. Delayed clearance of residual levels of radioactivity was again noted. In total, 1.3 and 1.4 % of the dose were excreted in the urine in 24 hours, whilst 41.1 % and 77.6 % were recovered in the faeces. Up to 12 % of the dose of radioactivity was recovered from the cage.

Chromatographic study of the faeces and urine from animals dosed by both routes indicated that little of the colour was metabolised, but no attempt was made to identify the metabolites present. Radioactivity remained in the tissues after 72 hours (1 % of the injected dose and 0.1 % of the oral dose); no specific accumulation in the thyroid was noted, but concentrations of radioactivity in the testes were higher than the other tissues (excluding those involved in excretion) (LEMM, 1978d,c).

Overall, in vitro data showed that Patent Blue V was not metabolised by rat or human hepatic microsomal enzymes. Data available on the absorption, distribution, metabolism and excretion of Patent Blue V after oral administration in rats and dogs showed that Patent Blue V has low absorption and limited systemic availability. It was mainly excreted in faeces.

3.2. Toxicological data

3.2.1. Acute oral toxicity

JECFA described a study by Truhaut (1962) in which acute toxicity studies were conducted with Patent Blue V in mice and rats. The oral LD_{50} values in mice and rats were greater than 3000 and 5000 mg/kg bw respectively.

3.2.2. Short-term and subchronic toxicity

Cats

In a study in 3 cats, the animals received daily oral doses of 250, 500 and 750 mg (5 % aqueous solution) of the colour. Without presenting details on which parameters were examined, it is stated that no abnormalities were found (Truhaut, 1962).

Dogs

Patent Blue V (calcium salt) was incorporated into the diet of groups of 4 male and 4 female 6- to 8-month old Beagle dogs for 13 weeks at levels of 0, 0.3 and 1 %. These levels, which were shown to be of acceptable palatability in preliminary studies, provided daily intakes of about 0, 80 and 280 mg/kg bw/day (IFREB, 1978a; BIBRA, 1982). No mortality occurred during the study. Group body weights appeared to be slightly reduced in the treated animals, but differences were small and food and water consumption were not affected by treatment. At the end of the study, ocular examination, urine and blood biochemistry revealed no treatment-related effects, apart from a tendency to reduced creatinine clearance. In both groups of dosed females, at week 13 the reduction in creatinine clearance was significantly reduced (Student's t test, $P < 0.05$) compared to controls. However, the relationship was not strictly dose-related and the values were considered by the authors to remain within normal limits. According to the authors of the BIBRA (1982) report, the No-Observed-Adverse-Effect Level (NOAEL) of this study was 280 mg/kg bw/day, the highest dose tested. The Panel agreed with this conclusion.

3.2.3. Genotoxicity

JECFA did not address the genotoxicity of Patent Blue V (JECFA, 1970; 1975). The SCF mentioned that in vitro mutagenicity studies were available to the Committee (SCF, 1983).

In vitro studies

Patent Blue V did not demonstrate any evidence of mutagenic activity in strains of tryptophan-requiring *Escherichia coli* and histidine-requiring *Salmonella typhimurium* (4 strains) both with and without metabolic activation (Viola and Nosotti, 1978; Gubbini et al., 1975; Haveland-Smith and Combes, 1980).

An Ames test with Patent Blue V was performed in 2010 according to the OECD guideline for testing of chemicals No. 471 (Guerbet/Ricerca, 2010). The reported purity of Patent Blue V used in this assay was 86 %, in compliance with the EU specifications for this food colour (purity $\geq 85\%$).

Five histidine-dependent strains of *S. typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) were used to evaluate the mutagenic potential of Patent Blue V, both in the absence and presence of rat liver S9 metabolism. The study was carried out using both the plate incorporation and the pre-incubation methods. Patent Blue V was tested as a dark blue solution in water for injection.

The first experiment, using the plate incorporation method was carried out using the dose range of 52-5000 μg/plate, both in the absence and presence of rat S9 metabolism. Following treatment, no precipitation and cytotoxicity (reduction in the number of revertants and/or thinning of the background

lawn) were noted in the absence or in the presence of metabolic activation. Statistically and biologically significant increases in the number of revertants were observed only with the TA98 tester strain in the presence of metabolic activation.

On the basis of the results obtained a second experiment using the pre-incubation method was carried out in the dose range of 492-5000 µg/plate, both in the absence and presence of rat liver S9 metabolism. Following treatment, no precipitation or cytotoxicity were noted in the absence or in the presence of metabolic activation. Statistically and biologically significant increases in the number of revertants were confirmed with the TA98 tester strain in the presence of metabolic activation.

The Panel noted that the positive outcome obtained in the presence of metabolic activation and at higher dose levels only, strongly indicates that mutagenicity could have been caused by the presence of impurities given the low purity of the test item (86 %) and the absence of reported metabolic conversion of Patent Blue V.

In the study by Masannat et al. (2009), which aimed to investigate potential genotoxicity of Methylene Blue, Patent Blue V and Indigo Carmine commonly used in sentinel node biopsies, Patent Blue V was assessed for potential DNA damaging properties in an in vitro alkaline Comet assay in breast epithelial MCF-7 and HB-2 cell lines. The enzyme Fapy-DNA glycosylase (FpG) was also incorporated in the Comet assay to enable detection of additional oxidative DNA damage. Treatments were performed for 5 minutes at the highest dose level of 25 mg/ml; the same dose level used in clinical practice. Three lower dose levels (0.1,1 and 10 mg/ml) were also employed. Results obtained showed dose-related and statistically significant increases of "tail DNA". However, the Panel observed that the 2 higher dose levels employed largely exceeded the highest recommended dose-levels (5 mg/ml) to be used in mammalian cells in vitro (Seeberg et al., 1988; Galloway et al., 1994; OECD guidelines for testing of chemicals No. 473 and 487). The results observed at the two lower levels, although significant, were within the range of variability of untreated controls. On this basis, the study was judged to be unreliable.

In an unpublished study report, the mutagenic potential of Patent Blue V was assessed in the in vitro mammalian cell gene mutation assay using L5178Y ($Tk^{+/-}$) mouse lymphoma cells (Guerbet/Ricerca, 2011a). The study was conducted in compliance with the OECD guideline No. 476 and the ICH guidelines S2A and S2B. The reported purity of Patent Blue V was 86 % which is in compliance with the EU specifications for this food colour (purity≥ 85 %). Long treatment (approximately 24 hours) without metabolic activation and short treatment (approximately 4 hours) both in the absence and presence of rat liver S9 metabolism were performed. Replicate cultures were set up at each experimental point.

Treatments were performed at dose levels ranging from 0.86 to 2500 µg/ml for approximately 24 hours in the absence of S9 metabolism and at dose levels ranging from 246 to 2500 µg/ml and 43 to 2500 µg/ml for approximately 4 hours in the absence and presence of S9 metabolism respectively. No presence of detectable precipitation was observed at any experimental point.

No cytotoxicity (decrease in the relative survival and/or in the relative total growth) was noted at any tested dose level, in the absence or in the presence of metabolic activation. No statistically or biologically significant increases in the mutant frequency were noted up to the maximum tested dose level of 2500 µg/ml at any treatment time in the absence or presence of metabolic activation.

Overall, from the previous study the Panel considered that Patent Blue V did not show mutagenic potential in mammalian cells at doses up to 2500 µg/ml. The Panel noted that according to the report it was not possible to test higher doses of Patent Blue V in this assay due to the reported limit of solubility of 100 mg/ml in aqueous solvent. However, the Panel noted that the use of culture medium as direct solvent for Patent Blue V could have allowed to reach the upper limit of concentration (5000 µg/ml) indicated by the relevant OECD guideline (OECD 476) and possibly the detection of impurities

showing mutagenic effect in bacteria. Therefore, the Panel considered the results of this assay inconclusive.

In vivo studies

In the study by Misra et al. (1986), which aimed to investigate potential in vivo genotoxicity of 3 commonly used dyes, Patent Blue V was assessed for potential clastogenicity in an in vivo bone marrow micronucleus assay in Swiss male mice. The test item used was identified as Blue VRS (purity not reported), Acid Blue 1 or Food Blue 3 with CAS no. 129-17-9 and a molecular weight of 566.7 g/mol. Purity was not reported. The test compound was administered to groups of 3 mice by 2 intraperitoneal injections 24 hours apart at dose-levels of 100, 200, 400 and 800 mg/kg bw. Animals administered 800 mg/kg of the test item died shortly after treatment. Surviving animals were sacrificed 6 hours after the second injection and a total of 30 slides from each animal group (i.e. 10 slides for each animal) were prepared and subsequently stained and scored at microscope magnification x 1000. A minimum number of 750 erythrocytes (both polychromatic and normochromatic erythrocytes) were scored, when possible, from each slide. Results obtained indicated that the test item induced marked increases in the percentage of cells with micronuclei at the higher dose level both in the polychromatic and normochromatic erythrocytes. However, the Panel noted that marked increases in micronucleated normochromatic erythrocytes cannot be attributable to test compound genotoxicity based on the experimental protocol applied (30 hour sampling time) due to the physiology of erythrocytes maturation following expulsion of nuclei. Their appearance is not expected not earlier than 30 hours and therefore the presence of micronucleated normochromatic erythrocytes cannot be attributed to treatment with the test substance but to a previous exposure to genotoxic compounds or an unhealthy status of animals. Therefore the outcome of the study generates strong doubts about the reliability of scoring of micronucleated cells. On these bases the study has been judged unreliable.

In the study by Durnev et al. (1995) which aimed to investigate potential in vivo genotoxicity of 6 food colours, Patent Blue V (purity unknown) was assessed for potential clastogenicity in an in vivo bone marrow chromosome aberration assay in C57Bl6 mice. The test item was administered by oral gavage at daily dose levels of 0.08 and 0.8 mg/kg bw for 5 days. Animals were sacrificed 6 hours after the administration of the last dose level. In the last 2 hours mice also received colchicine at 2.5 mg/kg bw to accumulate cells in metaphases. A minimum number of 100 well spread metaphases were scored for each animal in the control and treated groups. Results obtained indicated that Patent Blue V did not induce statistically significant increases of chromosomal aberrations in the mouse bone marrow cells erythrocytes under the reported experimental conditions. However, the Panel noted that mitotic indices, important to detect bone marrow cytotoxicity, were not evaluated and that scoring of aberrations did not follow internationally recognised methods as described in the current literature.

In an unpublished study report, the mutagenic potential of Patent Blue V was assessed in the in vivo bone marrow micronucleus assay in Sprague-Dawley rats (Guerbet/Ricerca, 2011b). The study was conducted in compliance with the OECD guideline 474. The reported purity of Patent Blue V used in this assay was 86 %, which is in compliance with the EU specifications for this food colour (purity≥ 85 %). On the basis of a preliminary experiment in which mortality was observed at 600 mg/kg bw, the study was carried out using groups of 5 male and 5 female rats administered dose levels of 75, 150 and 300 mg/kg, by a single intravenous injection. Saline (0.9 % NaCl) was used as vehicle. Animals were sacrificed 24 and 48 hours after administration. Clinical signs were noted only at the highest dose level of 300 mg/kg bw and consisted of irregular breathing and/or lowered activity in some animals. When compared to the negative control group (0.9 % NaCl), no biologically significant decreases in the polychromatic/normochromatic erythrocytes ratio (PCEs/NCEs) were noted in any animal treatment groups. For either sex or both sexes combined, when compared to the negative control group (0.9 % NaCl), no biologically significant increase in the mean frequencies of micronucleated polychromatic erythrocytes (MNPCEs) was noted in the animal groups treated with Patent Blue V up to the highest dose level of 300 mg/kg. The authors concluded that under the reported experimental conditions Patent Blue V did not prove to induce micronuclei in rat bone marrow erythrocytes. The Panel agreed with this conclusion.

In an unpublished study report, the DNA damaging capabilities of Patent Blue V (E 131) were assessed in the in vivo single cell gel/Comet assay in rats (Fiorio Colori, 2012a). The study was conducted in compliance with the internationally agreed protocols, since no OECD test guidelines are yet available and in compliance with the OECD principles of Good Laboratory Practice (GLP) as revised in 1997. The reported purity of Patent Blue V used in this assay was 90 % and therefore in compliance with the EU specifications for this food colour (purity ≥ 85 %). Patent Blue V (E 131) was examined for genotoxic properties by evaluating the induction of DNA damage in cell suspensions isolated from liver, jejunum/ileum and peripheral blood of rats after in vivo treatment using the alkaline (pH>13) version of the Comet Assay. In the main experiment, only male animals were treated since no substantial inter-sex differences in toxicity were observed. Groups of 5 Sprague-Dawley male rats were treated twice at 24 hour intervals with the vehicle only (sterile distilled water of injectable grade), or Patent Blue V (E 131) at the dose levels of 500, 1000 and 2000 mg/kg/day. The highest dose level represents the maximum dose level to be used for non-toxic compounds. Ethyl methanesulphonate (EMS), at 200 mg/kg/day served as positive control. Animals were sacrificed approximately 3-4 hours after the second dosing. Peripheral blood and cell suspensions isolated from liver and jejunum/ileum were embedded in agarose gel on microscope slides. No statistically significant increases in tail moment and tail intensity values compared with the vehicle control values were observed at any dose-level in the treated groups. The authors concluded that Patent Blue V (E 131) does not induce any effect on DNA migration in rat liver, jejunum/ileum and peripheral blood after in vivo treatment under the reported experimental conditions. The Panel agreed with this conclusion.

According to new studies provided by industry on request of EFSA, the Panel considered that Patent Blue V (E 131) (at purity level ≥ 90 %) is not of concern with respect to genotoxicity.

3.2.4. Chronic toxicity and carcinogenicity

Mice

In 1983, the SCF allocated an ADI of 15 mg/kg bw/day based on the no-adverse level of 1500 mg/kg bw/day in the following mouse study (SCF, 1983).

A combined reproductive toxicity and long-term toxicity study has been performed in mice. Groups of 65 male and female mice were given diets containing 0.1, 0.3 or 1 % Patent Blue V (equivalent to 150, 500 and 1500 mg/kg bw/day) for 9 weeks prior to mating and throughout mating, gestation and rearing of their offspring (F_1 generation) (IFREB, 1981, as referred to by BIBRA, 1982).

According to BIBRA (1982), groups of 50 (treatment groups) and 100 (control) mice of each sex were selected from the offspring (1 male plus 1 female from each litter) and given the same treatment as their parents for 21 months (males) or 23 months (females). The colour used in this study complied with the requirements of the JECFA specification.

There were no treatment-related behavioural or clinical findings. Growth rates and food consumption were roughly comparable in all groups, but the body weights of the males in the top dose group were frequently significantly reduced (Student's t-test). The high-dose males demonstrated a slightly increased mortality; by the end of the study, 60 % of controls compared to 76 % of the high-dose males, had died.

Haematological analyses carried out on 20 animals of each sex from the high-dose and control groups at 3, 6, 12 and 18 months, and on all surviving animals at the end of the experiment, did not identify any consistent effects, apart from significantly reduced values for haemoglobin, haematocrit and red blood cell counts detected in the males ($P < 0.01$ % in each case) and females ($P < 0.5$) from the high-dose group at 3 months and at the end of the study in the males. These values were not significantly reduced at the other examinations. A significant ($P < 0.01$) reduction in reticulocyte count and an increase in the total number of white blood cells in the high-dose males ($P < 0.01$) and females ($P <$

0.5) were observed at 12 months only. After 21 months, there was a highly significant reduction in the lymphocyte and total leucocyte counts in males and the polymorph count was significantly elevated ($P < 0.01$), but their distribution was random, and appeared unrelated to treatment (more details were not available).

Comparison of the absolute and relative weights of the organs revealed a highly significant reduction ($P < 0.01$) in the relative and absolute liver weights of the males in the intermediate dose group, but not at the top dose. The absolute weight of this organ was also significantly reduced ($P < 0.01$) in the females of the intermediate dose group.

Increases in the absolute ($P < 0.01$) and relative ($P < 0.05$) kidney weights of the females were reported at the intermediate dose, but at the high-dose level only the absolute weight of the kidneys in females was statistically significant ($P < 0.05$). Females in the 2 higher dose groups exhibited increased relative heart weights ($P < 0.01$), and females in the highest dose group had increased caecal weights (full, $P < 0.05$; and empty, $P < 0.01$).

Full histopathological examination of a comprehensive selection of organs and tissues from the high-dose and control groups and gross lesions seen at autopsy in the intermediate and low-dose groups revealed a range of non-neoplastic and neoplastic pathology. The observed non-neoplastic lesions which frequently occurred in both control and treatment groups (mainly involving the lungs, kidneys or liver) or were isolated occurrences, were not considered to be related to the administration of Patent Blue V.

A number of neoplastic lesions occurred in the treated but not in the control animals, such as squamous cell carcinoma (1 animal in each dose), osteosarcoma (1 animal at each dose), nephroblastoma (1 male at intermediate dose), adrenocortical adenoma (1 male at lowest and intermediate doses, 2 males at highest dose), adrenocortical carcinoma (1 male at highest dose), adenocarcinoma of the thymus (1 female at intermediate dose), adenoma of mammary gland (1 female at intermediate and highest doses). 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. In the absence of the full study report, the Panel considered that the haematological effects observed at the highest dose tested are biologically significant. Therefore, the Panel considered that a NOAEL of 500 mg/kg bw/day (intermediate dose tested) can be derived from this study, based on growth reduction and alterations of haematological parameters reported at the highest dose tested, mainly in males.

Rats

In 1970, JECFA based its temporary ADI of 1 mg/kg bw/day on the following study.

Rats (30 animals/sex) were given in their diet 10 000 mg of Patent Blue V/kg diet, equivalent to 500 mg/kg bw/day of the colour for their life-span. The colour used was pure (no impurities were found by paper chromatography studies) (Truhaut, 1962). The average life-span of treated animals was 24 months and the last animal died at an age of 37.5 months. Forty animals were used as controls. The average life-span of these animals was 22.5 months. For the second experiment, 30 rats (15 males and 15 females) were given the same diet with 10 000 mg of Patent Blue V/kg diet, and also, a subcutaneous injection of 1 ml of 1 % Patent Blue V aqueous solution, once a week, for 15-19 months. The average life-span of the animals was 18 months. The last animal died at an age of 30 months. Twenty-six rats were used as controls and were given 1 ml of distilled water subcutaneously for the same period as the treated animals. The average life-span of these animals was 19 months. No abnormal histopathological findings or sarcomas at the site of injection were observed.

3.2.5. Reproductive and developmental toxicity

Groups of 65 male and female mice were given diets containing 0.1, 0.3 or 1 % Patent Blue V (equivalent to 150, 500 and 1500 mg/kg bw/day) for 9 weeks prior to mating and throughout mating,

gestation and rearing of their offspring (F_1 generation) (IFREB, 1981, as referred to by BIBRA, 1982). No adverse effects were reported. No further details on the study were available to the Panel. The authors concluded that the NOAEL was 1500 mg/kg bw/day.

In a developmental study, groups of 25 mated OFA (Sprague-Dawley derived) rats were fed diets containing 0, 0.3 or 1 % Patent Blue V (calcium salt), meeting JECFA specifications, equivalent to 0, 150 and 500 mg/kg bw/day, from day 6 to 15 of gestation, and were killed 21 days after mating. No mortality was observed during the study and growth and food consumption was similar in all 3 groups. One female from the low-dose group with a single implantation miscarried during the study, and in one female from the high-dose group, total resorption occurred (12 implantations). The average number of resorptions, implantations and living foetuses and their weights did not appear to differ markedly between control and treatment groups (statistical comparisons were not reported). Skeletal staining of two-thirds of the offspring and organ examination and sectioning of the remaining offspring did not reveal any major or minor abnormality which might be related to treatment, but individual data were not reported (IFREB, 1978b, as referred to by BIBRA, 1982).

The Panel agreed with the authors that the NOAEL of this study was 500 mg/kg bw/day, the highest dose tested.

3.2.6. Hypersensitivity, allergenicity, intolerance

They are no reported cases of allergy or anaphylactic reactions after ingestion of Patent Blue V in humans.

Allergic reactions to Patent Blue V have been reported from its use in surgery for the identification of the primary draining lymph nodes (sentinel nodes biopsy) in breast cancer and malignant melanoma. The incidence of reactions ranged from 0.6 % up to 2.7 %, and severe anaphylaxis is described in 0.06 % of the cases (Scherer et al., 2006; Barthelmes et al., 2010; Bézu et al., 2010). Most patients experiencing a severe allergic reaction had no particular medical history of allergy (Barthelmes et al., 2010).

An allergic reaction (erythematous urticarial rash) was also described after topical use of Patent Blue V in a healthy five year old girl with no history of atopic conditions, given a disclosing tablet containing Patent Blue V to demonstrate the presence of plaque on teeth (Chadwick et al., 1990).

Anaphylactic reactions to Patent Blue V observed during surgery are likely to be mediated by IgE (Johansson et al., 2010) and are often characterized by an increased expression of CD63, a marker of activation in basophils (Eberlein-König et al., 2004; Johansson et al., 2010).

Given its relatively low molecular weight and its capacity to (weakly) bind proteins, Patent Blue V may likely act as a hapten, with the carrier protein(s), sugar or lipid being so far unknown (Tsopelas et al., 2002; Johansson et al., 2010).

The Panel noted that anaphylactic reactions to Patent Blue V occur during surgery. However, the application of Patent Blue V in various fields (cosmetics, textiles, paints, inks) could potentially cause sensitization to this colour.

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.

Patent Blue V is a triarylmethane dye allowed as a food additive in the EU that has been previously evaluated by JECFA in 1970 and 1975, and the EU SCF in 1983.

JECFA established a temporary ADI of 0-1 mg/kg bw/day in 1970, but withdrew it in 1975. Until now JECFA has not allocated an ADI to Patent Blue V. In 1983, the SCF established an ADI of 0-15 mg/kg bw/day on the basis of a long-term toxicological study in mice, which had not been available for the JECFA evaluation in 1975.

Furthermore, the Panel noted that the specifications on the purity of Patent Blue V would allow concentrations of unidentified unsulphonated aromatic amines to be present in concentrations of up to 100 mg/kg Patent Blue V. Given the maximum allowed concentration of Patent Blue V that can be added to food (500 mg/kg food), the concentration of these amines in food could be up to 50 μg/kg food. Intake estimates of Patent Blue V at levels up to the ADI of 15 mg/kg bw/day could amount to intakes of these aromatic amines at levels up to 1.5 μg/kg bw/day. As some aromatic amines may be associated with genotoxicity or even carcinogenicity, this may give reason for concern. The Panel concluded that it would be prudent to modify the specifications to indicate that the level of unsulphonated aromatic amines should be as low as reasonably achievable.

In vitro data on metabolism in humans and rats showed that Patent Blue V is not metabolised by rat or human hepatic microsomal enzymes. Data available on the absorption, distribution, metabolism and excretion of Patent Blue V after oral administration in rats and dogs show that Patent Blue V has low absorption, limited systemic availability and is mainly excreted unchanged in faeces.

Concerning subacute and subchronic toxicity data, only one subacute toxicity study in dogs was available. The effects reported in this study were considered by the Panel to be without any toxicological relevance, and the NOAEL was 280 mg/kg bw/day, the highest dose tested.

Patent Blue V did not demonstrate any evidence of mutagenic activity in strains of tryptophan-requiring *Escherichia coli* and histidine-requiring *Salmonella typhimurium* (4 strains), both with, and without metabolic activation in old studies. In contrast, Patent Blue V induced in the Ames test, performed (in 2010) according to OECD Guidelines, biologically significant increases in the number of revertants in the TA98 strain in the presence of metabolic activation at high concentrations of colour. In the presence of low concentrations, there were no effects. This observation could suggest that an impurity present in the food colour could be responsible for this mutagenic effect.

A mouse lymphoma assay performed in 2011 according to OECD Guidelines with Patent Blue V in accordance with EU specifications, was negative.

Two in vivo micronucleus assays are available for Patent Blue V. The first one (in 1986), performed in mouse after intraperitonal injection, was evaluated as not reliable..The second one (in 2011), performed in rat after intravenous injection did not show any clastogenic effect.

In an unpublished study report, the DNA damaging capabilities of Patent Blue V (E 131) were assessed in the in vivo single cell gel/Comet assay in rats. The Panel concluded that Patent Blue V (E 131) does not induce any effect in DNA migration in rat liver, jejunum/ileum and peripheral blood after in vivo treatment under the reported experimental conditions.

According to new studies provided by industry on request of EFSA, the Panel considered that Patent Blue V (E 131) (at purity level $\geq$ than 90 %) is not of concern with respect to genotoxicity.

There is one chronic toxicity study performed in mice. In the absence of the full study report, the Panel considered that the haematological effects observed at the highest dose tested are biologically significant. Therefore, the Panel considered that a NOAEL of 500 mg/kg bw/day (intermediate dose tested) can be derived from this study, based on growth reduction and alterations of haematological parameters reported at the highest dose tested mainly in males.

A reproductive toxicity study in mice did not reveal adverse effects. The NOAEL is the highest dose tested, 1500 mg/kg bw/day. A developmental study in rats is available. No adverse effects have been identified. The NOAEL derived by the Panel was 500 mg/kg bw/day, the highest dose tested.

The Panel concluded that the present dataset provided a rationale for a re-definition of the ADI. Using the NOAEL of 500 mg/kg bw/day from a chronic toxicity study in mice and applying an uncertainty factor of 100 to this NOAEL, the Panel established an ADI of 5 mg/kg bw/day for Patent Blue V of purity at least 90 %.

Exposure to Patent Blue V from its use as a food additive has been calculated by using MPLs as indicated in the Commission Regulation No 1129/2011 and by using data on reported use levels provided by food industry or data reported on analytical levels provided by national authorities or found in the literature. These data were combined with national consumption data for the population groups of toddlers, children, adolescents, adults and the elderly from the EFSA Comprehensive Food Consumption Database.

When considering MPLs, estimates calculated for toddlers, children, adolescents, adults and the elderly give dietary exposures at the mean in the range of 1-4.5, 1.1-3.6, 0.5-1.8, 0.3-1.4, and 0.2-0.6 mg/kg bw/day, respectively. High level exposures for these population groups were calculated to be in the range of 2.9-7.5, 2.4-7, 1.3-3.7, 0.9-2.9 and 0.6-1.5 mg/kg bw/day, respectively. The main contributors to the total anticipated exposure to Patent Blue V (>10 %) for adults were flavoured drinks (with sugar or with sweeteners) (11-48 %), flavoured fermented milk products (14-29 %), fine bakery wares (11-34 %), and sauces (12-29 %). For children, main contributors were flavoured fermented milk products (13-32 %), fine bakery wares (11-45 %), and sauces (11-23 %).

When considering maximum reported use levels or maximum analytical levels, estimates calculated for toddlers, children, adolescents, adults and the elderly give dietary exposures at the mean in the range of 0.2-1.5, 0.4-1.2, 0.3-0.7, 0.1-0.5 and 0.04-0.3 mg/kg bw/day, respectively. High level exposures for these population groups were calculated to be in the range of 0.6-2.7, 1-2.4, 0.7-1.7, 0.4-1 and 0.1-0.8 mg/kg bw/day, respectively. The main contributors to the total anticipated exposure to Patent Blue V (>10 %) for adults were flavoured drinks with sweeteners (12-38 %), and fine bakery wares (15-64 %). For children, main contributors were fine bakery wares (10-70 %), and flavoured drinks with sugar (10-31 %).

The Panel noted that at the maximum permitted levels of use of Patent Blue V, exposure estimates for high consumers are above the ADI of 5 mg/kg bw/day in toddlers and children population. At the maximum reported use levels of Patent Blue V, exposure estimates are below the ADI of 5 mg/kg bw/day for all groups of the population.

The Panel further noted that the specifications of Patent Blue V 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 chromium is < 50 mg/kg, whereas no specification for chromium is required in EC specifications.

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.

CONCLUSIONS

Patent Blue V (E 131) is a triarylmethane dye permitted for use as a food additive in the EU, that has been previously evaluated by JECFA in 1970 and 1975 and the EU SCF in 1983. JECFA established a

temporary ADI of 0-1 mg/kg bw/day in 1970 but withdrew it in 1975. In 1983, the SCF established an ADI of 0-15 mg/kg bw/day.

The Panel concluded that the present dataset provided a rationale for re-definition of the ADI. Using the NOAEL of 500 mg/kg bw/day from a chronic toxicity study in mice and applying an uncertainty factor of 100 to this NOAEL, the Panel established an ADI of 5 mg/kg bw/day for Patent Blue V. The Panel considered that this ADI will only apply to Patent Blue V with a purity of at least 90 %.

The Panel noted that at the maximum permitted levels of use of Patent Blue V, exposure estimates for high consumers are above the ADI of 5 mg/kg bw/day in toddlers and children. At the maximum reported use levels of Patent Blue V, exposure estimates are below the ADI of 5 mg/kg bw/day for all groups of the population.

The Panel further noted that the specifications for Patent Blue V 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 chromium is < 50 mg/kg, whereas no specification for chromium is required in EC specifications.

The Panel noted that the aluminium lake of the colour could add to the daily intake of aluminium and that therefore specifications for the maximum level of aluminium in the lakes are required.

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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

Figure 1: Rules defined by the Panel to deal with usage data or observed analytical data for all regulated food additives to be re-evaluated and procedures for estimating intakes using these rules.

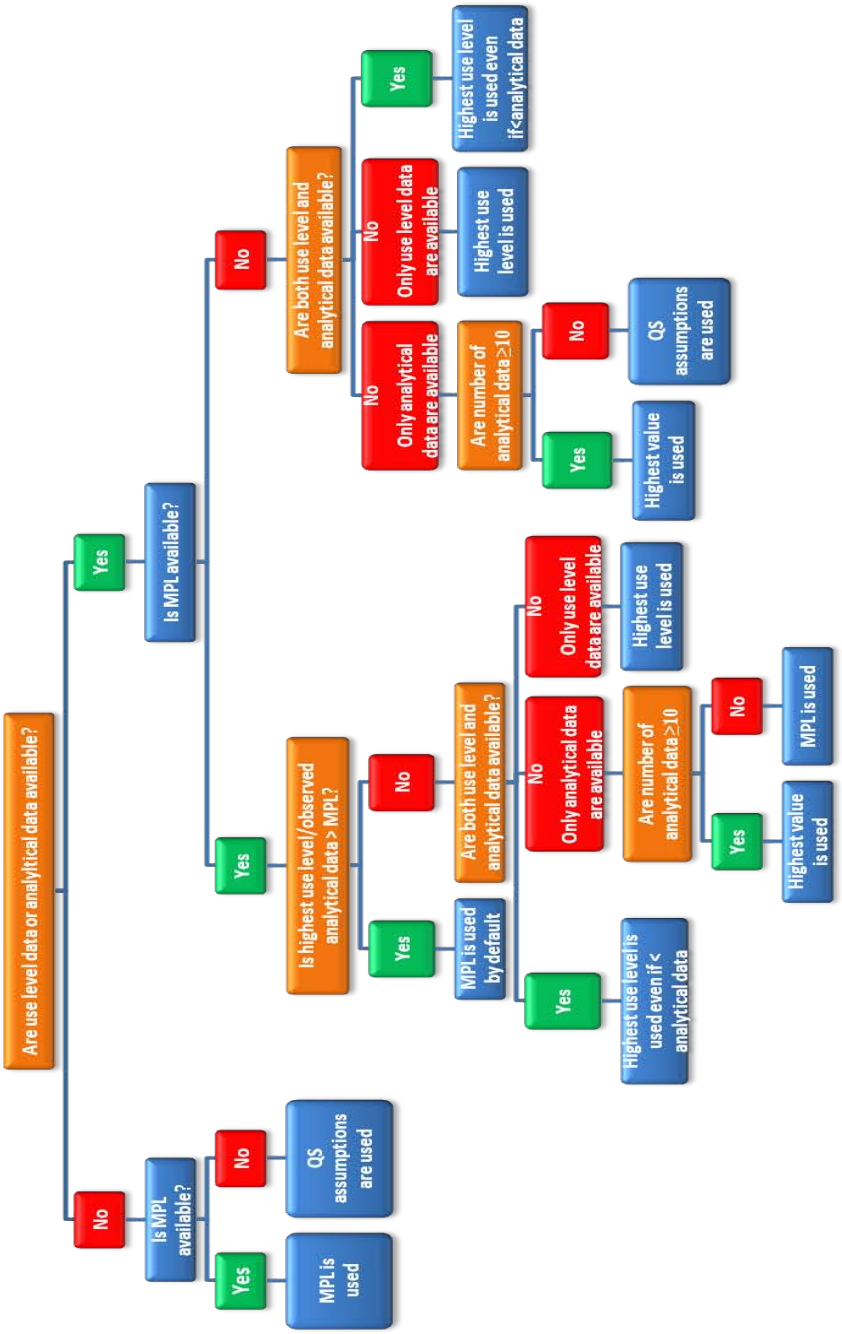
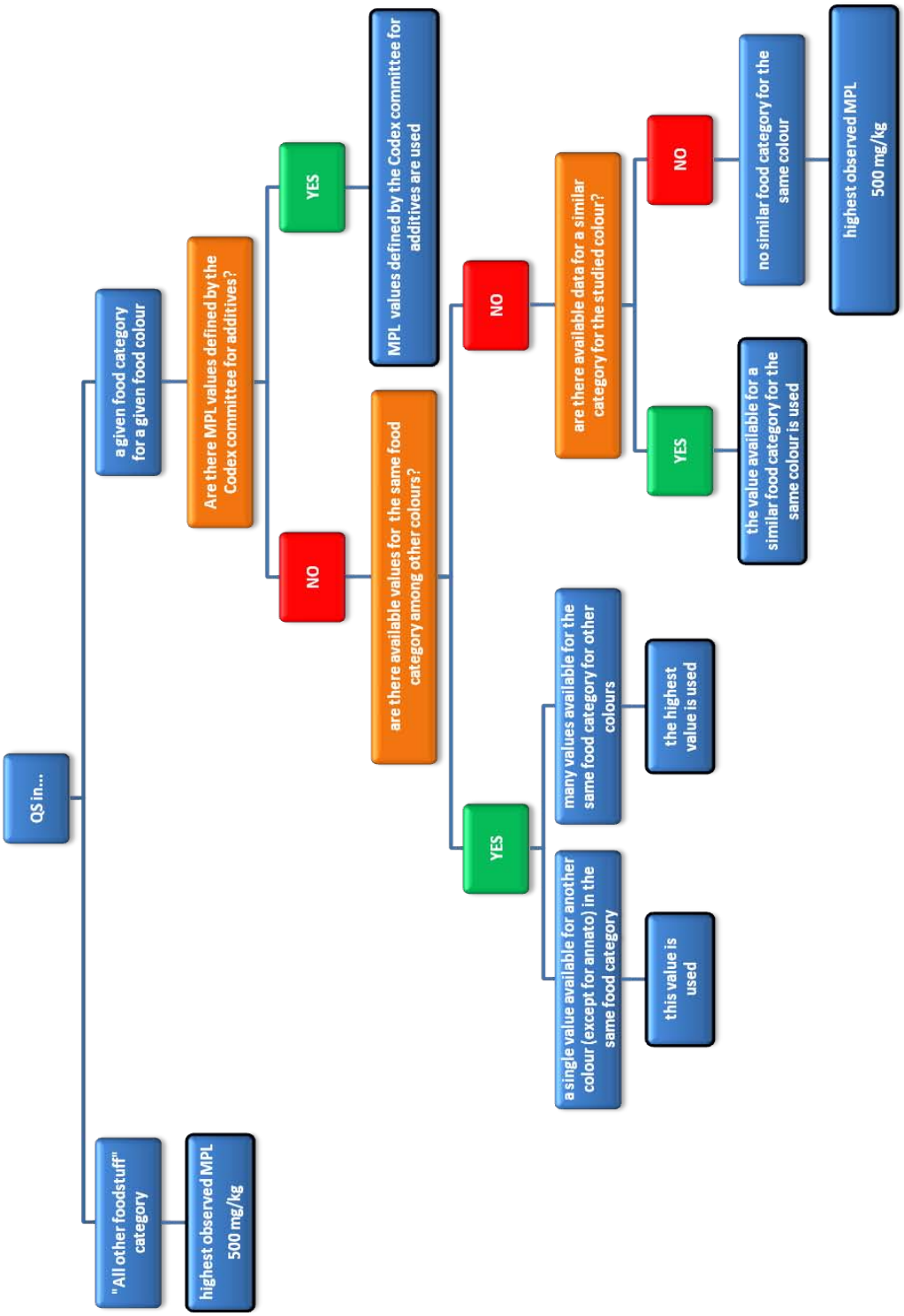


Figure 2: Rules defined by the Panel to deal with quantum satis (QS) authorisation.



GLOSSARY/ABBREVIATIONS

ADI	Acceptable Daily Intake
Aluminium lakes	Aluminium lakes are produced by the absorption of water soluble dyes ont substrate rendering the colour insoluble in water. The end product is coloure 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
bw	Body weight
CAS	Chemical Abstract Service
CIAA	Confederation of the Food and Drink Industries of the EU
EC	European Commission
EFSA	European Food Safety Authority
EMS	Ethyl methanesulphonate
EU	European Union
FSA	UK Food Standards Agency,
FAO/WHO	Food and Agriculture Organization/World Health Organization
FSAI	Food Safety Authority of Ireland
FpG	Fapy-DNA glycosylase
GLP	Good Laboratory Practice
HPLC	High-Performance Liquid Chromatography
IARC	The International Agency for Research on Cancer
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 if Detection
LOQ	Limit of Quantification
MNPCEs	Micronucleated Polychromatic erythrocytes
MPL	Maximum Permitted Level
NATCOL	Natural Food Colours Association
NDNS	UK National Diet and Nutrition Survey
NOAEL	No-Observed-Adverse-Effect Level
NCEs	Normochromatic Erythrocytes
OECD	Organisation for Economic Co-operation and Development
PCEs	Polychromatic Erythrocytes
QS	Quantum Satis
SCF	Scientific Committee for Food
SCOOP	A scientific cooperation (SCOOP) task involves coordination amongst Me pooled data from across the EU on particular issues of concern regarding fooc
TWI	Tolerable Weekly Intake

UNESDA	Union of European Beverage Associations
UV/VIS	Ultra-Violet/Visible Spectrum