European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of Allura Red AC (E 129) as a food additive [1]

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)[23]

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 Allura Red AC (E 129). Allura Red AC has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1980 and the EU Scientific Committee for Food (SCF) in 1984 and 1989. Both committees established an Acceptable Daily Intake (ADI) of 0-7 mg/kg body weight (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. New studies included a study by Tsuda *et al*. from 2001 reporting effects on nuclear DNA migration in the mouse *in vivo* Comet assay, and a study by McCann *et al*. from 2007 that concluded that exposure to a mixture including Allura Red AC, resulted in increased hyperactivity in 8- to 9-years old children. The Panel notes that Allura Red AC was negative in *in vitro* genotoxicity as well as in long-term carcinogenicity studies and that the effects on nuclear DNA migration observed in the mouse *in vivo* Comet assay are not expected to result in carcinogenicity. The Panel also concurrs with the conclusion from a previous EFSA opinion on the McCann *et al.* study that the findings of the study cannot be used as a basis for altering the ADI. The Panel concluded that the present database does not give reason to revise the ADI of 7 mg/kg bw/day. The Panel also concludes that at the maximum reported levels of use refined intake estimates are generally below the ADI, although in 1-10 years old children the high percentile of exposure (95th) can be slightly higher than the ADI at the upper end of the range.

KEY WORDS

Allura Red AC, E 129, CAS Registry Number 25956-17-6, Food Red No. 40, FD&C Red No. 40, disodium 2-hydroxy-1-(2-methoxy-5-methyl-4-sulphonatophenylazo)naphthalene-6-sulphonate, food colouring substance, EINECS number 247-368-0.

1 On request from the European Commission, Question No EFSA-Q-2008-230, adopted on 23 September 2009.

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SUMMARY

Following a request from the European Commission, the Panel on Food Additives and Nutrient Sources added to Food has been asked to provide a scientific opinion re-evaluating the safety of Allura Red AC (E 129) when used as a food colouring substance.

Allura Red AC (E 129) is an azo dye allowed as a food additive in the EU that has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1980 and the EU Scientific Committee for Food (SCF) in 1984 and 1989. Both committees have established an Acceptable Daily Intake (ADI) of 0-7 mg/kg body weight (bw)/day.

The SCF, JECFA and TemaNord evaluations concluded, based on *in vivo* and *in vitro* mutagenicity studies available at that time, that Allura Red AC did not show any genotoxic activity.

Recent results indicated that in an *in vivo* Comet assay, Allura Red AC induced significant increases in migration of nuclear DNA in both glandular stomach and colon tissue, in the absence of general cytotoxicity in these tissues. The Panel considered in the light of negative carcinogenicity studies, that the biological significance of the Comet assay results is uncertain.

In contrast to this, all *Salmonella* genotoxicity tests with Allura Red AC have been negative. Because the activation process of these azo dyes in animals is complex, *Salmonella* tests with S9 might not be suitable to detect mammalian genotoxicity.

The conversion of the parent compound by azo-reduction *in vivo* results in the formation of sulphonated naphtylamines that may not be formed in the standard *in vitro* genotoxicity tests. Previously, a range of sulphonated aromatic amines, including the ones formed from Allura Red AC upon azo-reduction, was shown to be in general not associated with genotoxicity *in vitro* and *in vivo*.

The Panel also noted that the specifications on the purity of Allura Red AC permit concentrations of unidentified unsulphonated aromatic amines to be present in concentrations of up to 100 mg/kg Allura Red AC. Although some aromatic amines may be associated with genotoxicity or even carcinogenicity, the Panel notes that Allura Red AC was negative in *in vitro* genotoxicity as well as in long-term carcinogenicity studies.

Long-term carcinogenicity studies on Allura Red AC were re-evaluated by the Panel. Several long-term carcinogenicity studies in rats at dose levels up to 2829 mg/kg bw/day in males and 3604 mg/kg bw/day in females respectively, and in mice at dose levels up to 7422 mg/kg bw/day in males and 8304 mg/kg bw/day in females, revealed no evidence of carcinogenicity. This includes the absence of neoplasms in the stomach and the large intestine, shown to be the most sensitive organs in the *in vivo* Comet assay in mice. Allura Red AC induced significant dose-related DNA damage in mice in the glandular stomach at doses of 100 mg/kg bw and higher, and in the colon at doses of 10 mg/kg bw and higher. However, carcinogenicity in these tissues was not observed at dose levels several times higher, up to 7422 and 8304 mg/kg bw/day for male and female mice, respectively. The Panel noted that Allura Red AC was negative in *in vitro* genotoxicity as well as in long term carcinogenicity studies, and that the effects on nuclear DNA migration observed in the mouse *in vivo* Comet assay are not expected to result in carcinogenicity.

Based on the same data set for long-term toxicity/carcinogenicity, previous evaluations by JECFA, the SCF and TemaNord also concluded that there was no evidence of carcinogenicity of Allura Red AC.

A study by McCann *et al*. has concluded that exposure to one of the two mixtures of four synthetic colours plus the preservative sodium benzoate in the diet, in particular Mix B (containing Allura Red AC), resulted in increased hyperactivity in 8- to 9-year old, but not in 3-year old children selected from the general population. In 2008, EFSA published an opinion on this McCann *et al.* study.

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC) concluded that:

- the McCann *et al.* study provides limited evidence that the two different mixtures of synthetic colours and sodium benzoate tested had a small and statistically significant effect on activity and attention in children selected from the general population, excluding children medicated for Attention Deficit Hypersensitivity Disorder, despite the effects not being statistically significant for the two mixtures in both age groups;

- since mixtures and not individual additives were tested in the study by McCann *et al.*, it is not possible to ascribe the observed effects to any of the individual compounds, and;

- in the context of the overall weight of evidence and in view of the considerable uncertainties, such as the lack of consistency and relative weakness of the effect, and the absence of information on the clinical significance of the behavioural changes observed, the findings of the study cannot be used as a basis for altering the ADI of the respective food colours or sodium benzoate.

The Scientific Panel on Food Additives and Nutrient Sources added to Food concurs with these conclusions.

Overall, the Panel concluded that the present dataset on genotoxicity, semi-chronic, reproductive, developmental and long-term toxicity, and carcinogenicity as well as the McCann *et al*. study, does not give reason for the revision of the ADI of 7 mg/kg bw/day.

The Panel concluded that while some sensitivity reactions after Allura Red AC intake (such as urticaria, rhinitis and asthma) have been reported, mostly when Allura Red AC is taken within mixtures of other synthetic colours, no conclusion on the induction of hypersensitivity by Allura Red AC could be drawn from the limited scientific evidence available. The Panel also notes that sensitive individuals may react at dose levels within the ADI.

The dietary exposure to Allura Red AC 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 Allura AC (Tier 3), as identified by the Panel from the data made available by the FSA, FSAI, AFSSA, UNESDA, CEPS, ELC, CIAA. For children population (1-10 years old), estimates have been calculated for nine European countries (Belgium, France, UK, the Netherlands, Spain, Czech Republic, Italy, Finland and Germany). For the adult population, the Panel has selected the UK population as representative of the EU consumers for Allura Red AC exposure estimates.

When considering MPLs (Tier 2), the mean dietary exposure to Allura Red AC for European children, (aged 1-10 years), ranged from 0.8 to 3.4 mg/kg bw/day, and from 1.8 to 9.4 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 2.1 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks.

When considering the maximum reported use levels (Tier 3), the mean dietary exposure to Allura Red AC for European children (aged 1-10 years) ranged from 0.5 to 3 mg/kg bw/day, and from 1.2 to 8.5 mg/kg bw/day at the 95th percentile. Estimates reported for the UK adult population give a mean

dietary exposure to Allura Red AC of 0.8 mg/kg bw/day, and of 1.9 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks.

The Panel concludes that at the maximum reported levels of use of Allura Red AC, refined (Tier 3) intake estimates are generally below the ADI of 7 mg/kg bw/day. However, in 1-10 years old children the high percentile of exposure (95th) can be 1.2-8.5 mg/kg bw/day and thus slightly higher than the ADI at the upper end of the range.

The Panel further notes that the specifications for Allura Red AC 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 notes that the JECFA specification for lead is $\leq$ 2 mg/kg whereas the EC specification is $\leq$ 10 mg/kg.

The Panel notes 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

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 Allura Red AC (E 129) when used as a food colouring substance.

Allura Red AC (E 129) is an azo dye allowed as a food additive in the EU and previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1980 and 1981, and the EU Scientific Committee for Food (SCF) in 1984 and 1989.

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

Allura Red AC (E 129) is an azo dye with the molecular formula $C_{18}H_{14}N_2Na_2O_8S_2$. It has a molecular mass of 496.43 and CAS Registry Number 25956-17-6. Its full chemical name is disodium 2-hydroxy-1-(2-methoxy-5-methyl-4-sulphonatophenylazo) naphthalene-6-sulphonate. Its structural formula is:

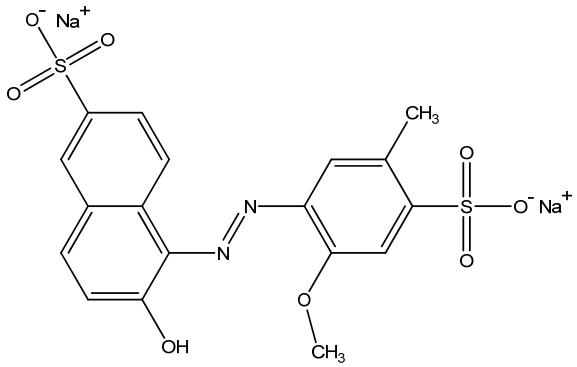

Figure 1. The structural formula of Allura Red AC

At least 17 synonyms are in use (ChemIDplus advanced, via internet, 2006). The most commonly used synonyms in published literature are Allura Red AC, Food Red No. 40 and FD&C Red No. 40. In the past, the synonym Red Z-4576 was frequently used in unpublished literature.

Allura Red AC is soluble in water and slightly soluble in 50 % ethanol (Merck Index, 2006).

2.2. Specifications

Specifications have been defined in the EU legislation (Directive 2008/128/EC) and by JECFA (JECFA, 2006) (Table 1).

Allura Red AC consists essentially of disodium 2-hydroxy-1-(2-methoxy-5-methyl-4-sulphonato-phenylazo)naphthalene-6-sulphonate and subsidiary colouring matters, together with sodium chloride and/or sodium sulphate as the principal uncoloured components. Allura Red AC is described as the sodium salt; the calcium and the potassium salt are also permitted (EC, 2008).

The purity is specified as not less than 85 % of 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), ≤ 3 % subsidiary colouring matters and ≤ 0.3 % 6-hydroxy-2-naphthalene sulphonic acid, sodium salt, ≤ 0.2 % 4-amino-5-methoxy-2-methylbenzene sulphonic acid, and ≤ 1 % 6,6-oxybis(2-naphthalene sulphonic acid) disodium salt, originating from the manufacturing process.

Thus, if the existing specifications could be extended to include ≤ 15 % sodium chloride and/or sodium sulphate as the principal uncoloured components, 99.9 % of the material would be accounted for.

Table 1. Specifications for Allura Red AC 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	≤ 3.0 %	≤ 3.0 %
- 6-hydroxy-2-naphthalene sulphonic acid, sodium salt	≤ 0.3 %	≤ 0.3 %
- 4-amino-5-methoxy-2-methylbenzene sulphonic acid	≤ 0.2 %	≤ 0.2 %
- 6,6-oxybis(2-naphthalene sulphonic acid) disodium salt	≤ 1.0 %	≤ 1.0 %
Unsulphonated primary aromatic amines	≤ 0.01 % (calculated as aniline)	≤ 0.01 % (calculated as aniline)
Ether extractable matter	≤ 0.2 % (from a solution of PH 7)	≤ 0.2 %
Arsenic	≤ 3 mg/kg	-
Lead	≤ 10 mg/kg	≤ 2 mg/kg
Mercury	≤ 1 mg/kg	-
Cadmium	≤ 1 mg/kg	-
Heavy metals (as Pb)	≤ 40 mg/kg	-

The Panel noted that the specifications on the purity of Allura Red AC would permit concentrations of unsulphonated aromatic amines to be present in concentrations of up to 100 mg/kg Allura Red AC. Given the maximal allowed concentration of Allura Red AC 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.

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 (EC, 2008).

JECFA does not give specifications for aluminium lakes of Allura Red AC, other than reference to the General Specifications for Aluminium Lakes of Colouring Matters (JECFA, 2006). The Allura Red AC 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 notes 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, 2008b) and that therefore specifications for the maximum level of aluminium in the lakes may be required.

2.3. Manufacturing process

Allura Red AC is manufactured by coupling diazotized 5-amino-4-methoxy-2-toluenesulphonic acid with 6-hydroxy-2-naphthalene sulphonic acid (HSDB, 2006). Allura Red AC 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 foods

Allura Red AC can be quantified in soft drinks by differential pulse polarography (Combeau *et al.,* 2002), and by a High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD) method described for water-soluble foods like fruit flavoured drinks, alcoholic drinks, jams, sugar confectionery and sweets upon dilution or water extraction (Minioti *et al.,* 2007). Allura Red AC in soft drink powder can be detected by double divisor-ratio spectra derivative, inverse least squares and principal component regression methods (Dinç *et al.,* 2002).

2.5. Reaction and fate in foods

No data were available in the published literature specifically on Allura Red AC. However, 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).

2.6. Case of need and proposed uses

Permitted use levels have been defined in the EU legislation (Directive 94/36/EC).

Currently, Allura Red AC is an allowed synthetic food colouring substance in the EU with a maximal allowed use level of 25 to 500 mg/kg food for various foodstuffs. Allura Red AC is also allowed in alcoholic beverages at levels up to 200 mg/L and non-alcoholic beverages up to 100 mg/L. Table 2 summarizes those beverages and foodstuffs that are permitted to contain Allura Red AC up to specified maximum permitted levels (MPL) set by EC legislation (EC, 1994).

Table 2. Maximum Permitted Levels of use of Allura Red AC in beverages and foodstuffs according to European Parliament and Council Directive 94/36/EC

Beverages	**Maximum permitted level** (mg/L)
Non-alcoholic flavoured drinks Bitter soda, bitter vino Liquid food supplements/dietary integrators	100
Spirituous beverages Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails Fruit wines, cider and perry	200
Foodstuffs	**Maximum permitted level** (mg/kg)
Luncheon meat Breakfast sausages with a minimum cereal content of 6 %	25
Complete formulae for weight control intended to replace total daily food intake or an individual meal Complete formulae and nutritional supplements for use under medical supervision Soups	50
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
Edible ices Desserts including flavoured milk products	150
Fine bakery wares Candied fruit and vegetables, Mostarda di frutta Preserves of red fruits Extruded or expanded savoury snack products	200
Pre-cooked crustaceans	250
Confectionery Mustard Fish roe Solid food supplements/dietary integrators	300
Decorations and coatings Sauces, seasonings, pickles, relishes, chutney and piccalilli Salmon substitutes Surimi	500
Edible cheese rind and edible casings	*Quantum satis*

2.7. Information on existing authorisations and evaluations

Allura Red AC is permitted as a food additive in the EU under Directive 94/36/EC. Allura Red AC has been evaluated previously by JECFA in 1974, 1980 and 1981 and the SCF in 1975, 1984 and 1989. Both committees have established an Acceptable Daily Intake (ADI) of 0-7 mg/kg bw.

2.8. Dietary exposure

2.8.1. Actual levels of use of Allura Red AC

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

2.8.1.1. Beverages

For non-alcoholic flavoured drinks, the UK Food Standards Agency (FSA) conducted an *ad hoc* survey in which artificial colours were analytically determined in 201 retail ready-to-drink soft drinks selected for being distinctly coloured (FSA, 2003). Allura Red AC was found to be present at a level higher than 0.1 mg/L (Limit Of Detection - LOD) in six products, with levels varying from 9 to 42 mg/L. In another survey, conducted in 2005 by the Food Safety Authority of Ireland (FSAI), Allura Red AC was found to be present at a level higher than 1.0 mg/L (Limit Of Quantification -LOQ) in three out of 54 soft drinks; the concentration in these products ranged from 1 to 75.6 mg/L (FSAI, 2009). A usage survey, conducted by the Union of European Beverage Associations (UNESDA) in 2005, suggests that the highest current use level of Allura Red AC in beverages is 21 mg/L (Tennant, 2006). A more recent report from UNESDA in 2009 gives a range of use levels from 1 to 70 mg/L (UNESDA, 2009). French companies reported use levels ranging from 0.5 to 60 mg/L (unpublished data provided by AFSSA). The Confederation of the Food and Drink Industries of the EU (CIAA) also reported other current use levels of Allura Red AC ranging from 1 to 50 mg/L (CIAA, 2009). The Federation of European Food Additives, Food Enzymes and Food Culture Industries (ELC) has provided from its UK member association, Food Additives and Ingredients Association (FAIA), further data which give a range of typical low - maximum use levels for Allura Red AC from 5 to 100 mg/L (ELC, 2009).

For spirituous beverages, including products with less than 15 % alcohol, in the survey conducted by the FSAI (2009), Allura Red AC was found to be present in two out of 14 retail samples (below 1 mg/L). The European Spirits Organisation reported a range of use levels of Allura Red AC from 0 to 200 mg/L (CEPS, 2009).

For fruit wines (still or sparkling), cider and perry, the CIAA reported no uses at the date of the survey.

2.8.1.2. Foodstuffs

For confectionery products, the Panel was also provided with data from an *ad hoc* survey conducted by the FSA, in which artificial colours were analytically determined in 176 retail samples of brightly coloured packaged sweets, selected for being distinctly coloured (FSA, 2002). Allura Red AC was found to be present in 47 products with levels varying from 3 to 208 mg/kg. According to the FSAI data, Allura Red AC was present at a level higher than 1.0 mg/kg in 83 out 183 confectionery products, with levels varying from 1 to 171 mg/kg (FSAI, 2009). Data provided by French industries on Allura Red AC in sweets showed use levels varying from 14 to 250 mg/kg (unpublished data provided by AFSSA). Data provided by the ELC (2009), give a range of typical low and maximum use levels from 0 to 240 mg/kg. A range of current use levels from 0.1 to 200 mg/L has also been reported by the CIAA.

For candied fruit, vegetables, mostarda di frutta, a range of current use levels from 60 to 100 mg/kg has also been reported by the CIAA.

For preserved red fruits, the FSAI survey (2009) gave a range of analytical values of < 2 to 191 mg/kg for ten retail samples; the CIAA reported a range of typical use levels of Allura Red AC from 60 to 100 mg/kg.

For decorations and coatings, data from the FSAI survey gave a range of analytical values from < 5 to 82 mg/kg for four retail samples; the CIAA reported a range of typical use levels of Allura Red AC from 0.1 to 200 mg/kg.

For fine bakery wares, the CIAA (2009) reported a range of typical use levels of Allura Red AC from 25 to 110 mg/kg, whereas the ELC provided further data from the FAIA, which gave typical use levels ranging from 45 to 50 mg/kg.

For edible ices, the FSAI (2009) survey gave analytical values of Allura Red AC ranging from 1 to 6.1 mg/kg for 30 retail samples. The CIAA reported usages according to the current legislation.

For flavoured processed cheese and edible cheese rind and edible casing, the CIAA reported a typical maximum value for Allura Red AC of 0.01 mg/kg.

For desserts, including flavoured milk products, the FSAI survey (2009) gave a range of analytical values from < 2 to 245 mg/kg detected in 35 retail samples, and the CIAA reported a range of typical use levels of Allura Red AC from 7 to 100 mg/kg.

For sauces, seasonings, pickles, relishes, chutney and picalilli, the FSAI survey (2009) gave a range of analytical values from 2 to 102 mg/kg for five retail samples; the CIAA reported usages according to the current legislation.

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 either on maximum actual usage, maximum analytical data or *quantum satis* rules for Allura Red AC 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 3 summarises the maximum reported use levels of Allura Red AC 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 3. Maximum reported use levels of Allura Red AC in beverages and foodstuffs used for the refined exposure assessment

Beverages	**Maximum reported use level (mg/L)**
Non-alcoholic flavoured drinks Bitter soda, bitter vino Liquid food supplements/dietary integrators	100
Spirituous beverages Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails Fruit wines, cider and perry	200
Foodstuffs	**Maximum reported use level (mg/kg)**
Flavoured processed cheese Edible cheese rinds and edible casings*	0.01
Luncheon meat Breakfast sausages with a minimum cereal content of 6 %	25
Complete formulae for weight control intended to replace total daily food intake or an individual meal Complete formulae and nutritional supplements for use under	50

medical supervision Soups	
Fish paste and crustaceans paste Smoked fish Savoury snack products and savoury coated nuts Meat and fish analogues based on vegetable proteins Desserts including flavoured milk products Candied fruit and vegetables, Mostarda di frutta Preserves of red fruits	100
Fine bakery wares	110
Edible ices	150
Extruded or expanded savoury snack products Decorations and coatings	200
Pre-cooked crustaceans Confectionery	250
Mustard Fish roe Solid food supplements/dietary integrators	300
Sauces, seasonings, pickles, relishes, chutney and piccalilli Salmon substitutes Surimi	500

* For the Tier 2 approach, the Panel defined some rules in Annex A for identifying the maximum practical used levels to deal with *quantum satis* authorisation. A value of 100 mg/kg was proposed for edible cheese rinds and 25 mg/kg for edible casings.

2.8.2. Exposure assessment

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 intakes. The approach goes from the conservative estimates that form the first Tier (Tier 1) of screening, to progressively more realistic estimates that form the Second (Tier 2) and Third (Tier 3) Tier.

2.8.2.1. Crude estimates (Budget method)

The dietary exposure to Allura Red AC 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 Allura Red AC, the maximum permitted use level considered for beverages was 200 mg/L. The maximum permitted level considered for solid foods was 500 mg/kg (Table 2).

The default proportion (25 %) of beverages and solid food that could contain the additive was considered adequate. In effect, even though Allura Red AC 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 solid foods with the same colour added. In the case of beverages, uses are reported for a limited number of beverages; however, some of these may constitute a significant proportion of liquid intake (i.e., non-alcoholic flavoured drinks) with consumer loyalty to a single brand (and therefore to a specific colour) often being high for this category of product. The 25 % proportion was therefore considered adequate also for beverages (EC, 1998). This assumes that a typical adult, weighing 60 kg, consumes daily 1.5 litres of beverages and 375 g of solid foods, containing Allura Red AC. The 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.

For children, the level of Allura Red AC considered in beverages was 100 mg/L (after exclusion of alcoholic drinks), and in solid food 500 mg/kg. The proportion of 25% used, for beverages, was recognised to be inadequate for children, as the corresponding consumption rate of 375 mL/day could easily be exceeded by young children. This conclusion was derived from UK data on consumption of soft drinks by children aged less than 5 years, where the 97.5th percentile of consumption was between 70 and 80 mL/kg bw/day and a proportion factor of 100 % for beverages was recommended for children in the SCOOP Task 4.2 (EC, 1998). 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 Allura Red AC.

The overall theoretical maximum daily exposure 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 Allura Red AC may be used *quantum satis* in edible cheese rinds 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 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 maximum permitted use levels presented in Table 2 and maximum practical used levels presented in Table 3 to deal with the specific cases of *quantum satis* authorization for edible cheese rinds and edible casings, and for Tier 3 using the maximum reported use levels presented in Table 3, for children and adult populations.

Exposure estimates for children (1-10 years old) have been performed by the EXPOCHI consortium, based on detailed individual food consumption data from eight European countries (Belgium, France, the Netherlands, Spain, Czech Republic, Italy, Finland and Germany) for Tier 2 and Tier 3. 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 the detailed individual food consumption data (UK NDNS, 1992-1993) available from the UNESDA report (Tennant, 2006) and with the MPLs of use as specified in the Directive 94/36/EC on food colours from Table 2 (Tier 2 approach), and with the maximum reported use levels from Table 3 (Tier 3 approach).

Since the UK population is considered to be one of the highest consumers of soft drinks in Europe and as estimates were provided on more refined adult food consumption data, in comparison to those available to the Panel (e.g. EFSA Concise European Food Consumption Database, which gives access to aggregate food categories consumed in 15 European countries), the Panel decided to select the UK population as representative of the EU consumers for the Allure Red AC intake estimates for adults.

Estimates of Allura Red AC exposure from the UK adult population (>18 years old) have been made by the Panel with the use of the detailed individual food consumption data (UK NDNS, 2000-2001) available from the UNESDA report (Tennant, 2006) and with the MPLs of use as specified in the Directive 94/36/EC (EC, 1994) for Tier 2 approach (Table 2), and with the maximum reported use levels for Tier 3 approach (Table 3).

Table 4 summarises the anticipated exposure of children and adults to Allura Red AC.

In the case of Allura Red AC, when considering MPLs of use (Tier 2), the mean dietary exposure of European children (aged 1-10 years and weighing 25-30 kg) considered by the EXPOCHI consortium ranged from 0.8 to 3.4 mg/kg bw/day, and from 1.8 to 9.4 mg/kg bw/day at the 95th percentile. The

main contributors to the total anticipated exposure to Allura Red AC (>10 % in all countries), were soft drinks (13 to 41 %), fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (10 to 47 %), and desserts, including flavoured milk products (12 to 63 %). Sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli accounted for 10 to 50 % of exposure in four countries. Confectionery accounted for 11 % of exposure in one country.

For UK children aged 1.5 to 4.5 years and weighing 15 kg, the mean dietary exposure was 3.1 mg/kg bw/day and 7.3 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10 %) for UK pre-school children were soft drinks (55 %), confectionery (13 %) and desserts, including flavoured milk products (12 %).

Estimates reported for the UK adult population give a mean dietary exposure to Allura Red AC of 0.9 mg/kg bw/day, and of 2.1 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure to Allura Red AC (>10 %) were soft drinks ((50 %) for average consumers and 80% for high consumers).

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

When considering the maximum reported use levels from Table 3, the mean dietary exposure of European children (aged 1-10 years and weighing 25-30 kg), considered by the EXPOCHI consortium, ranged from 0.5 to 3 mg/kg bw/day, and from 1.2 mg/kg bw/day to 8.5 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure to Allura Red AC (>10% in all countries), were soft drinks (10 to 54 %), fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (12 to 39 %), and desserts, including flavoured milk products (14 to 58 %). Sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli accounted for 10 to 57 % in five countries.

For UK children, aged 1.5 to 4.5 years and weighing 15 kg, the mean dietary exposure was 2.7 mg/kg bw/day and 6.8 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10 %) for UK pre-school children were soft drinks (63 %), and confectionery (10 %).

Estimates reported for the UK adult population give a mean dietary exposure to Allura Red AC of 0.8 mg/kg bw/day and of 1.9 mg/kg bw/day for the high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10 %) were soft drinks (53 % for average consumers and 80% for high consumers).

Table 4. Summary of anticipated exposure to Allura Red AC using tiered approach (EC, 2001) in children and adult populations

	Adult UK population (>18 years old)	**Pre-school UK children** (1.5 - 4.5 years old, 15 kg body weight)	**Children EXPOCHI population** (1-10 years old, 25-30 kg body weight)
	mg/kg bw/day		
Tier 1. Budget method	8.1	13.1	
Tier 2. Maximum Permitted Level			
• Mean exposure	0.9	3.1	0.8 - 3.4
• Exposure 95th* or 97.5th percentile **	2.1	7.3	1.8 – 9.4
Tier 3. Maximum reported use levels			
• Mean exposure	0.8	2.7	0.5 - 3.0
• Exposure 95th*or 97.5th percentile**	1.9	6.8	1.2 – 8.5

* 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

Allura Red AC has been evaluated previously by the JECFA in 1980 and 1981, and the SCF in 1984 and 1989. It was also evaluated by TemaNord (2002). The present opinion briefly reports the major studies evaluated in these opinions and describes the additionally reported new literature data in some more detail.

3.1. Absorption, distribution, metabolism and excretion

Previous evaluations reported studies on the following: excretion in rats fed a diet containing 5.19 % of the colour (White, 1970), absorption, distribution, retention and excretion in rats and dogs fed radio-labelled Allura Red AC through their diet (dose not specified) (Guyton and Reno, 1975), and metabolism in rats and dogs (no details on route of administration or dose) (Guyton and Stanovick, 1975).

The main conclusions of the JECFA (1980) and TemaNord (2002) evaluations were that the parent compound is absorbed to only a limited extent and that the major route of excretion is through the faeces (29 % parent compound). Excretion of the parent compound in urine is negligible. Several metabolites, possibly resulting from azo-reduction in the gastrointestinal tract (two identified as aromatic amines, *p*-cresidine sulphonic acid being the major one), were also found in the faeces and urine. Finally, significant retention in the washed intestines of rat was observed, probably due to adhesion to the intestinal wall.

The SCF indicated that there were some uncertainties with respect to the metabolic data, particularly in relation to the fact that a number of metabolites was not identified, and the number of test animals being too small. The SCF accepted that the data were less than totally adequate, but considered that in the context of an overall assessment of all data, there was no need to repeat the studies. Furthermore, the SCF considered that *p*-cresidine sulphonic acid was unlikely to break down into the carcinogenic *p*-cresidine and this was supported by the absence of any carcinogenic effect in long-term studies. The Panel concurs with the conclusion of the SCF.

Recently, Kuno and Mizutani (2005) have investigated the influence of Allura Red AC on the activities of human phase I and phase II drug-metabolizing enzymes (CYP2A6, UGT1A6, and UGT2B7). Their findings indicate that Allura Red AC is neither a substrate, nor an inhibitor of the enzymes studied.

3.2. Toxicological data

3.2.1. Acute oral toxicity

The JECFA evaluation contains information on acute toxicity. After administration of Allura Red AC to rats by gavage at doses varying from 215 to 10000 mg/kg, no compound-related adverse effects were observed (Weir, 1965a). Based on these studies, JECFA set the LD_{50} for rats at 10000 mg/kg bw. Based on two other studies with rabbits and dogs, the LD_{50} values have been set at 10000 and 5000 mg/kg bw, respectively (Weir, 1967, 1965b).

The SCF states that acute toxicity studies indicate no colour-induced toxic responses.

In a study by Sasaki *et al.* (2002), LD_{50} values were determined for several food additives, including Allura Red AC. In these limited acute toxicity experiments on four to five mice, no death was observed at 2000 mg/kg Allura Red AC, and the LD_{50} was defined as > 2000 mg/kg.

Overall, it can be concluded that the acute oral toxicity of Allura Red AC is low.

3.2.2. Short-term and subchronic toxicity

In previous evaluations, four studies on short-term toxicity were reported (JECFA, 1980; TemaNord, 2002).

Groups of 20 rats (10/sex) were fed diets containing 0, 0.37, 0.72, 1.39, 2.69 and 5.19 % (equivalent to 0, 185, 360, 695, and 2595 mg/kg bw/day) of Allura Red AC for six weeks. No compound-related effects were observed on body weight, food consumption, survival, organ weights, gross and microscopic pathology, haematology or urinalysis (Weir and Crews, 1966a). This reveals a No-Observed-Adverse-Effect Level (NOAEL) of at least 2595 mg/kg bw/day.

In a dog study (1/sex/group), animals received Allura Red AC in capsule form at doses of 0, 125, 250 and 500 mg/kg bw/day (duration not specified). At the highest level, slight ill-defined hepatic parenchymal changes were observed in both sexes. No effects were observed in terms of body weight, food consumption, survival, organ weights, gross and histopathology, haematology or clinical chemistry (Weir and Crews, 1966b). The Panel concluded that this study is too limited to derive a NOAEL.

In another dog study, groups of 8 dogs (4/sex) were fed 0, 0.37, 0.72, 1.39 and 5.19 % (equivalent to 0, 92.5, 180, 347.5 and 1297.5 mg/kg bw/day) of the colourant in their diet, daily for 104 weeks. The only effect observed was a transient vacuolization of the adrenal cortical cells at the highest dose levels. No anomalies were found in appearance, behaviour, haematology, gross and histopathology or clinical chemistry (Olson *et al.*, 1970). The Panel concluded that this study is too limited to derive a NOAEL.

Finally, pigs (2 males and 2 females/group) were observed after gavage administration of a dose of 1000 mg/kg bw/day for 21 days followed by an increased dose of 1500 mg/kg/day for an additional 54 days. No compound-related effects with regard to clinical and haematological parameters, and observable pathological changes were noted (Sondergaard *et al.*, 1977).

No new short-term studies have been published since these previous evaluations.

3.2.3. Genotoxicity

The JECFA describes a total of seven studies in its evaluation on mutagenicity of Allura Red AC.

Jorgenson *et al.* (1978) conducted a mouse heritable translocation test, which detects structural and numerical chromosome changes in mammalian germ cells, as recovered in first generation progeny. No induction of inheritable translocations were observed in 8- to 10-week old male mice which were fed diets containing dose levels of 4000 and 20000 mg/kg feed for eight weeks.

In different genotoxicity studies, a wide range of strains of *Saccharomyces cerevisiae* and *Salmonella typhimurium* were tested. None of these test systems demonstrated mutagenic activity (Anonymous, 1977a; Brown, 1978; Brusick, 1976; Muzzall and Cook, 1979; Viola and Nosotti, 1978).

The Panel noted that Prival and Mitchell (1982) demonstrated that the metabolic conditions of the standard Ames test protocol were not appropriate for testing azo dyes for mutagenic activity in *Salmonella typhimurium* and developed a specific protocol including use of flavin mononucleotide (FMN) rather than riboflavin to reduce the azo compounds to free amines, and hamster liver S9 rather than rat liver S9 for metabolic activation. The Panel therefore noted that a final conclusion from negative Ames test results obtained under standard conditions cannot be drawn.

Furthermore, additional *Salmonella* genotoxicity tests of Allura Red AC (Fujita and Sasaki, 1993; Fujita *et al.,* 1995; NTP, 2000), including tests with the modified metabolic conditions (Prival *et al.,* 1988) have been negative.

Another study examined the influence of Allura Red AC in a recessive lethality test in *Drosophila melanogaster*. Genotoxic events and parameters were scored in various categories. There was no significant increase in the proportion of mutations in any category compared to controls (Anonymous, 1977b, 1978).

The SCF, and also the JECFA and Tema Nord evaluation, conclude that based on *in vivo* and *in vitro* mutagenicity studies, Allura Red AC does not show any genotoxic activity.

More recently, Tsuda *et al.* (2001) have used an *in vivo* Comet assay to measure DNA damage after gavage feeding Allura Red AC to groups of male mice at doses of 0, 1, 10, 100, 1000 and 2000 mg/kg bw. Three hours after administration, Allura Red AC induced significant increases in migration of nuclear DNA in the glandular stomach at doses of 100 mg/kg bw and higher. In the colon, significant differences between the treatment group and controls were observed at doses from 10 mg/kg bw and higher; the effect was slightly dose-related and plateaued at doses higher than 100 mg/kg bw. In the lung, a slight increase in nuclear DNA migration was observed only at a dose of 1000 mg/kg bw. In the highest dose group of 2000 mg/kg bw, nuclear DNA migration was also determined at 6 and 24 hours after administration. Both in the glandular stomach and the colon, the magnitude of migration of nuclear DNA decreased with time being significantly increased in the stomach at 6 hours and in the colon at both 6 and 24 hours. A modest but significant effect was observed also in pregnant mice three hours after treatment with a dose of 2000 mg/kg, only in the colon. Necropsy and histopathological examination revealed no treatment-related effect on the colon and glandular stomach. The authors therefore concluded that the effect observed was not likely to be due to general cytotoxicity. The Panel considered that the indications provided by the study of Tsuda *et al*. (2001) should not be disregarded.

The data from the study by Tsuda *et al.* (2001) were also used in a more comprehensive study on the genotoxicity (Comet assay) of a broad range of food additives (Sasaki *et al.*, 2002). This study is not further discussed as it does not present any new data.

In another experiment, Zeiger and Margolin (2000) tested a random selection of 100 chemicals on mutagenic activity using a pre-incubation modification of a *Salmonella* microsome mutagenicity (Ames) test. Allura Red AC was found to be non-mutagenic in four different strains of *Salmonella*.

Azo-reduction of Allura Red AC may produce sulphonated aromatic amines. Jung *et al.* (1992) have reviewed the genotoxicity data of a range of sulphonated aromatic amines. To provide insight in the effect of sulphonation on the genotoxic potential of phenyl- and naphtylamines, the genotoxicity of sulphonated aromatic amines was compared with their unsulphonated analogues. It was found that in general sulphonated phenyl- and naphtylamines, including the azo-reduction products of Allura Red AC, are non-mutagenic to *Salmonella* in Ames tests. For some other sulphonated aromatic amines no genotoxicity was also demonstrated with a variety of other test systems *in vitro* and *in vivo* (no details given). Based on the available data, the authors concluded that sulphonated aromatic amines, in contrast with their unsulphonated analogues, have no or very low genotoxic potential. Hence, the authors concluded that exposure to sulphonated aromatic amines, derived from metabolic cleavage or present as contaminants in colourings are unlikely to induce any significant genotoxic risk.

3.2.4. Chronic toxicity and carcinogenicity

The JECFA, in its evaluation of Allura Red AC, refers to five long-term studies. The Panel noted that these studies were all performed before OECD guidelines and Good Laboratory Practice (GLP) were established.

Mice (50/sex) were treated dermally with a 5 % Allura Red AC test solution twice weekly for 20 months. No dye-related anomalies were noted in terms of survival, or gross and histopathology of major organs and the skin (Voelker, 1970).

In a 92-week study, 30 rats/sex received 0, 0.37, 1.39 and 5.19 % Allura Red AC in their daily diet (equivalent to 0, 185, 695 and 2595 mg/kg bw/day). A moderate growth depression was observed at the highest dose level in both sexes. Furthermore, there is report of a slight tendency to anaemia, however no details on dosage or significance are given. No compound-related effects were observed regarding appearance, behaviour, survival, organ weights, clinical laboratory studies, or gross and histopathology (Olson and Voelker, 1970).

Three studies also described in the section on reproductive and developmental toxicity also report on the carcinogenic potential of Allura Red AC in mice (Serota *et al.*, 1977a; Reno *et al.*, 1978, later published in the open literature as Borzelleca *et al.,* 1991), and rats (Serota *et al.*, 1977b, later published in the open literature as Borzelleca *et al.*, 1989). These studies are briefly re-addressed below.

In the first study by Serota *et al.* (1977a), later published in the open literature (Borzelleca *et al.,* 1991), 50 mice/sex were fed Allura Red AC daily throughout life at doses of 0.37, 1.39 and 5.19 % in the diet (equivalent to 0, 507, 1877 and 7422 mg/kg bw/day for males and 0, 577, 2043 and 8304 mg/kg bw/day for females) (Borzelleca *et al.,* 1991). Early onset of neoplasms was found and histologically diagnosed as lymphomas. Lymphomas were observed in one low-dose male, one mid-dose female and two males and females from the high-dose group between weeks 31 and 37. Lymphomas were not observed among controls until weeks 85 and 70 for males and females, respectively. To further investigate this possible early onset of tumours, an interim sacrifice was performed at 42 weeks. Statistical analysis however, did not reveal any increase in tumour incidence due to compound administration.

A second lifetime dietary mouse study was conducted using a protocol identical to the study performed by Serota *et al.* (1977a) (dose levels equivalent to 0, 507, 1877 and 7422 mg/kg bw/day for males, and 0, 577, 2043 and 8304 mg/kg bw/day for females) (Borzelleca *et al*., 1991), with the exception that 100 animals/sex were used and instead of one negative control group there were two control groups (Reno *et al.*, 1978). Also this study was subsequently reported in the open literature (Borzelleca *et al.,* 1991). In this study, in contrast to the first study in mice, increased incidences of lymphocytic lymphomas or acceleration of the appearance of lymphomas were not observed. Statistical evaluation of neoplasms of the reticuloendothelial system did not reveal a significant dose-related effect on tumour response at dose levels up to 7318 mg/kg bw/day for males and 8356 mg/kg bw/day for females, the highest dose levels tested. There was also no evidence for a dose-related increase in the incidence of other spontaneously-occurring tumours up to the highest dose levels tested.

In addition, a lifetime toxicity/carcinogenicity study in rats was reported using 50 rats/sex in three test groups and a negative control group, and administered diets containing 0, 0.37, 1.39 and 5.19 % Allura Red AC (Serota *et al.*, 1977b; later published as Borzelleca *et al*., 1989) (equivalent to 180, 701 and 2829 mg/kg bw/day for males and 0, 228, 901 and 3604 mg/kg bw/day for female rats, according to Borzelleca *et al.,* 1989). Histological evaluation revealed a variety of lesions, including neoplasms, among the control and treated rats. The lesions were present at similar incidences in control and

treated rats. The authors concluded that the lesions appeared to be spontaneous and that none of them were compound-related.

All previous evaluations concluded that there was no evidence of carcinogenicity of Allura Red AC (SCF, 1984 and 1989; JECFA, 1980; ThemaNord, 2002).

No new literature on Allura Red AC-induced long-term toxicity was published since these previous evaluations.

3.2.5. Reproductive and developmental toxicity

The JECFA evaluated only one study under the heading of reproduction. In this study, groups of 10 male and 20 female rats were fed 0, 0.37, 0.72, 1.39 or 5.19 % (equivalent to 0, 185, 360, 695 and 2595 mg/kg bw/day) of Allura Red AC in their diet through two parental (P1, P2) and two filial (F1A/B and F2A/B) generations (F1A was P2). Mating occurred after 27 weeks of exposure, for both P1 and P2 generations. Fertility indices were found to be low in the two filial generations, but it is unclear from the text whether they were significantly lower than the controls, as the animals in control groups also showed low fertility indices. Furthermore, a slight growth suppression was observed, mainly at the high test levels in F1 and F2 pups.

No anomalies were detected regarding growth, litter size, pup weight, gross pathology, implantation sites, resorptions sites, live fetuses indices, appearance and anatomy and structure (Blackmore *et al.,* 1969). The JECFA concluded that the NOAEL is 695 mg/kg bw/day, considering the slight growth suppression observed mainly at the high test level in F1 and F2 pups. The JECFA has used this NOAEL, together with the NOAEL from the rat study described below (Serota *et al.,* 1977b) to establish the ADI of 0-7 mg/kg bw/day.

Two studies are categorized as teratogenicity studies by the JECFA.

In the first study, groups of 24, 19, 20, 21 and 16 pregnant rats received 0, 15, 30, 100 and 200 mg/kg bw/day of the dye, respectively, by gavage during gestational days 0-19. No dye-induced effects were observed in terms of early or late deaths, resorptions, pre-implantation loss, litter size and average fetus weight (Collins, 1974). The Panel concluded that the NOAEL of this study amounts to 200 mg/kg bw/day, the highest dose tested.

A second teratogenicity study was conducted in rabbits. In this study groups, 14 rabbits were fed 0, 200 or 700 mg/kg bw/day by gavage from gestational day 6 to 18. No compound-related effects were observed regarding appearance and behaviour, body weight, gross necropsy findings, implantation, litter data, or other fetal abnormalities (Reno, 1974). The Panel concluded that the NOAEL in this study would be the highest dose tested being 700 mg/kg bw/day, and thus in line with the NOAELs taken from the study of Blackmore *et al*. (1969) from which JECFA derived an ADI of 0-7 mg/kg bw/day (JECFA, 1980).

In addition, the JECFA described three long-term studies by Serota *et al.* (1977a, 1977b) and Reno *et al.* (1978), in which the reproductive and developmental toxicity were concurrently monitored.

In a mouse study, 50 mice/sex were fed 0, 0.37, 1.39 and 5.19 % Allura Red AC in their diet for a lifetime (estimated in the initial study report to be equivalent to 0, 529, 1986 and 7414 mg/kg bw/day) (Serota *et al.*, 1977a; JECFA 1980) (calculated in a subsequent peer reviewed publication of the study to be equivalent to 0, 507, 1877 and 7422 mg/kg bw/day in male mice and 0, 577, 2043 and 8304 mg/kg bw/day in female mice)(Borzelleca *et al.,* 1991). Animals were also exposed throughout gestation and lactation. At the highest dose level, lower body weights and effects on organ weight and organ/body weight ratios were observed. However, these effects were considered to be

inconsequential. No significant treatment-related effects were noted in terms of clinical signs, clinical laboratory data, and gross and microscopic examination (Serota *et al.,* 1977a, later published in Borzelleca *et al.*, 1991).

In addition, a lifetime dietary mouse study was conducted using a protocol identical to the study performed by Serota *et al.* (1977a) (dose levels equivalent to 0, 492, 1821, 7318 mg/kg bw/day in male, and 0, 526, 2057 and 8356 mg/kg bw/day in female mice, according to Borzelleca *et al*., 1991) with the difference that 100 animals/sex were used and instead of one negative control group there were two control groups (Reno *et al.*, 1978, later published in Borzelleca *et al.,* 1991). In this study, sporadic changes in the clinical signs, body weight, food consumption and survival rates were observed, but these were not statistically significant and considered not to be related to the administration of the colouring substance. The absolute and relative weights of the adrenals and thyroids were altered in several treatment groups, but the significance of these findings could not be established, because histological examination of these tissues in the high-dose group animals did not show any abnormalities. No distinct treatment-related effects were found after gross pathological and histopathological examination.

Overall, in these two lifetime toxicity studies in mice, no compound-related adverse effects were observed. Borzelleca *et al.* (1991) concluded that the NOAELs in these studies were 5.19 % in the diet, amounting to approximately 7300 and 8300 mg/kg bw/day for male and female mice, respectively.

Another long-term study subdivided 50 rats/sex in three test groups and a negative control group, and administered diets containing 0, 0.37, 1.39 and 5.19 % Allura Red AC (Serota *et al.*, 1977b; later published as Borzelleca *et al*., 1989) (estimated in the initial study report to be equivalent to 0, 185, 695 and 2595 mg/kg bw/day) (Serota *et al.,* 1977b) (calculated in a subsequent peer reviewed publication of the study to be equivalent to 180, 701 and 2829 mg/kg bw/day for male and 0, 228, 901 and 3604 mg/kg bw/day for female rats) (Borzelleca *et al*., 1989). Administration started throughout parental breeding, gestation and lactation, and lasted for 118 and 121 weeks for male and female rats, respectively. For female rats, the mean body weights and growth rates were significantly lower (-12.5 %) in the high-dose group compared to controls. Although some abnormalities in gross pathological and histopathological appearance of the kidney were observed in the colour-treated groups, statistical analysis of the dose-response relationship indicated that these could not be attributed to administration of the compound.

Overall, in these lifetime toxicity studies, no compound-related adverse effects were observed, except for a reduction in body weight in high-dosed females at the end of the study. Borzelleca *et al.* (1989) concluded that the NOAELs in this study were 5.19 % (2829 mg/kg bw/day) for male rats and 1.39 % (901 mg/kg bw/day) for female rats.

The JECFA has used the NOAEL of 1.39 % in the diet, estimated to be equivalent to 695 mg/kg bw/day derived from this study (Serota *et al.,* 1977b), together with the NOAEL from the rat study described above (Blackmore *et al.*, 1969) to establish the ADI of 0-7 mg/kg bw/day.

The TemaNord report (2002) refers to four additional studies on reproductive and developmental toxicity.

In one study, rats (no detail on group numbers) received a daily diet containing 0, 2.5, 5 or 10 % of the colour in the diet (equivalent to 0, 1250, 2500 and 5000 mg/kg bw/day) over two generations.

Reproductive success (dams producing litters) was reduced at all dose levels, although not dose-related. Litter mortality at 22-24 days of age was increased at a concentration of 10 % in the diet. Offspring displayed reduced cerebellar weight from a dose level of 2.5 % and reduced brain stem weight at the 5 % concentration level. Two significant and dose-dependent behavioural effects,

decreased running wheel activity (2.5, 5 and 10 % groups), and increased open-field rearing activity (5 and 10 % groups) were also noted (Vorhees *et al.*, 1983). The Panel concluded that this study reveals a LOAEL of 1250 mg/kg bw/day, the lowest dose tested, and that a NOAEL cannot be derived.

A study in mice (10/sex/group) fed 0, 0.42, 0.84 or 1.68 % Allura Red AC in the diet (equivalent to 0, 600, 1200 and 2400 mg/kg bw/day) over two generations revealed few adverse effects on litter size, weight and behaviour, including a reduction in the ratio of male to female offspring from 1.33 in the control to 0.71 in the 0.42 % dose level group (Tanaka, 1994). The reduction in the ratio of male to female offspring was found at the low-dose level, but the authors concluded that this change of sex ratio at birth might not be caused by the treatment with Allura Red AC, since there was no consistent effect in the other two treatment groups at the higher dose levels. The average body weight of offspring during the lactation period was significantly increased in the lower-dosed groups of each sex. The survival index at post-natal day (PND) 21 showed no consistent adverse effects in treatment groups, but was significantly reduced for male offspring in the low-dose group only, and significantly increased for female offspring in the high-dose group only. No adverse effects were observed in the behavioural development during the lactation period. There were few adverse effects of Allura Red AC on either movement activity or maze learning in F1 generation mice, compared with controls in each sex. The author concluded that the dose levels of Allura Red AC tested in the study produced few adverse effects on the reproductive and neurobehavioural parameters in mice. The author of the study also concluded that the results suggest that Allura Red AC will not produce adverse effects on reproduction and behaviour at typical human intake.

In a rat study (no details on group numbers) providing Allura Red AC in the drinking water at levels of 0, 0.2, 0.4 or 0.7 % (corresponding to 274, 546 and 939 mg/kg bw/day) from gestational days 0-20, no fetal malformations were observed. TemaNord does not give further details, but based on the abstract of the original study there were no dose-related changes in maternal findings, number of fetuses, fetal viability, or external or visceral variations. The only effect observed was a significant increase in the incidence of fetuses with reduced ossification of the hyoid at the 0.7 % dose level (Collins *et al.*, 1989a). Thus, the Panel concluded that the NOAEL in this study was 546 mg/kg bw/day.

Gavage administration of the compound to rats (no details on group numbers) at concentrations of 0, 30, 75, 150, 300, 600 and 1000 mg/kg bw/day from day 0 up to gestational day 19 induced no teratological effects or effects on reproduction. There were no dose-related changes in terms of maternal daily observations, food consumption, body weight gain, implantations, or in fetal viability, body weight, body length, sex distribution or external variations. Although incidental increases in the number of male fetuses, number of females with two or more resorptions, number of litters with three or more sternebral variations, and incidence of 14th rib bud occurred, these were not considered dose-related (Collins *et al.*, 1989b). The Panel concluded that the NOAEL in this study was 1000 mg/kg bw/day, the highest dose tested.

3.2.6. Hypersensitivity

The JECFA describes a human study in which hypersensitivity to Allura Red AC was examined. Fifty-two patients suffering urticaria or angioedema were placed on a diet free of urticaria-causing ingredients. When most of the patients were free of symptoms, they were challenged with Allura Red AC orally in either a dose of 1 or 10 mg. A positive reaction in 15 % of the subjects was observed (Mikkelsen *et al.*, 1978).

Furthermore, several skin sensitization studies are presented by the JECFA, but these are considered of little importance in the context of food consumption and therefore not discussed in this evaluation.

Reactions to food colourings, including those triggered by immune (immediate and delayed type hypersensitivity) and non-immune (intolerance) mechanisms are assumed to be infrequent in the population, and prevalence of 0.14 to around 2 % has been reported (Young *et al.,* 1987; Hannuksela and Haahtela, 1987; Fuglsang, 1993, 1994). Adverse reactions after Allura Red AC intake, mostly taken within mixtures of other synthetic colours, have been reported for urticarial and vasculitic reactions (Mikkelsen *et al.,* 1978; Lowry *et al.,* 1994). Reports are often characterized by poorly controlled challenge procedures. Recent studies performed under properly controlled conditions imply that sensitivity to food additives in patients with chronic urticaria/angioedema or asthma is uncommon (Simon, 2003; Supramaniam and Warner, 1986).

3.2.7. Other studies

The study by McCann *et al*. (2007) has concluded that exposure to two mixtures of four synthetic colours plus the preservative sodium benzoate in the diet result in increased hyperactivity in 3-year old and 8- to 9-year old children in the general population. In an earlier study by the same research team, there was some evidence for adverse behavioural effects of a mixture of four synthetic colours and sodium benzoate in 3-year old children on the Isle of Wight (Bateman *et al.,* 2004). In this recent study, the effects of two combinations of Tartrazine (E 102), Quinoline Yellow (E 104), Sunset Yellow FCF (E 110), Ponceau 4R (E 124), Allura Red AC (E 129), Carmoisine (E 122) and sodium benzoate (E 211) on children's behaviour were studied.

The study involved 153 3-year old and 144 8- to 9-year old children. A global hyperactivity aggregate (GHA) score was the main outcome of the study, and this parameter was based on aggregated z-scores of observed behaviours and ratings by teachers, class room observers and parents, plus, for 8- to 9-year old children, a computerised test of attention.

Mix A in this study contained Ponceau 4R, Tartrazine, Sunset Yellow FCF, Carmoisine and sodium benzoate. Mix B in this study contained Allura Red AC, and in addition Sunset Yellow FCF, Carmoisine, Quinoline Yellow and sodium benzoate.

Mix A significantly increased the GHA scores for all 3-year old children compared to the placebo control GHA scores (effect size 0.20 [CI 0.01 to 0.39], $p < 0.05$). This result persisted when analysis was restricted to 3-year old children who consumed more than 85 % of juice and had no missing data (complete case group); in this analysis the effect of Mix A in the 3-year old children was still significantly increased compared to the placebo control (effect size 0.32 [CI 0.05 to 0.60, $p < 0.05$).

For the 8- to 9- year old children a significant effect of Mix A (effect size 0.12 [CI 0.02 to 0.23], $p < 0.05$) and Mix B (effect size 0.17 [0.07 – 0.28], $p < 0.001$) was seen when analysis was restricted to those children consuming at least 85 % of drinks with no missing data (complete case group). When all 8- to 9- year old children that completed the study were taken into account, Mix A had no effect on the GHA scores compared to the placebo control (effect size 0.08 [CI -0.02 to 0.17]). The clinical significance of the observed effects for normal functioning of the exposed children remains unclear.

Another study reported that Allura Red AC was able to inhibit aromatase activity in an *in vitro* model system using rat ovarian microsomes (Satoh *et al*., 2008) with an IC_{50} of 24 µM. Aromatase catalyses the conversion of androgens to estrogens and presents a target for endocrine disrupting chemicals. The Panel noted that Allura Red AC may thus mimic the effect of some endocrine disrupting chemicals. However, given the limited systemic bioavailability of Allura Red AC, the IC_{50} for this *in vitro* effect of 24 µM, and the fact that the reproduction studies did not show related adverse effects, the Panel concluded that this *in vitro* effect on aromatase does not give reason for concern.

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 notes that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

Allura Red AC (E 129) is an azo dye allowed as a food additive in the EU and previously evaluated by JECFA in 1980 and the SCF in 1984 and 1989. Both committees have established an ADI of 0-7 mg/kg bw.

Specifications have been defined in the EU legislation directives 2008/128/EC and by JECFA (JECFA 2005). The purity is specified as 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), ≤ 3 % subsidiary colouring matters, ≤ 0.3 % 6-hydroxy-2-naphthalene sulphonic acid, sodium salt, ≤ 0.2 % 4-amino-5-methoxy-2-methylbenzene sulphonic acid, and ≤ 1 % 6,6-oxybis(2-naphthalene sulphonic acid) disodium salt. Thus, if the existing specifications would be extended to include the percentage of sodium chloride and/or sodium sulphate as the principal uncoloured components, most of the material would be accounted for.

The ADI as defined by JECFA was based on a NOAEL of 695 mg/kg bw/day derived from a reproductive toxicity study in rats which revealed slight growth suppression observed mainly at the high test levels of 2595 mg/kg bw/day in F1 and F2 pups (Blackmore *et al.,* 1969), and from a teratogenicity study in rats which revealed lower body weights and growth rates at the highest dose level of 2595 mg/kg bw/day but not at 695 mg/kg bw/day (Serota *et al.*, 1977b) (1.39 % in the diet, later defined to be equivalent to 701 mg/kg bw/day for male and 901 mg/kg bw/day for female rats, when the study was published in the peer reviewed literature) (Borzelleca *et al*., 1989).

A subchronic toxicity study in rats, which was not yet available at the time of the JECFA evaluation, revealed some adverse behavioural effects including a decrease in running wheel activity and an adverse effect on reproductive success at relatively high-dose levels with a LOAEL of 1250 mg/kg bw/day, being the lowest dose tested in this study (Vorhees *et al.*, 1983). There was no clear dose-response relation for the reported effects in this study. Furthermore, another study performed in mice at dose levels up to 1200 and 2400 mg/kg bw/day over two generations (Tanaka, 1994), revealed no systematic significant adverse effects on reproductive and behavioural parameters.

Several other subchronic, reproductive, developmental and long-term studies revealed NOAEL values of, respectively, 2595 mg/kg bw/day (highest dose tested) in rats (Weir and Crews, 1966a), 200 mg/kg bw/day (highest dose tested) in rats (Collins, 1974), 700 mg/kg bw/day (highest dose tested) in rabbits (Reno, 1974), 7300 mg/kg bw/day for male and 8300 mg/kg bw/day for female mice (Serota *et al.,* 1977a; Borzelleca *et al*., 1991), 546 mg/kg bw/day in rats (Collins *et al.*, 1989a) and 1000 mg/kg bw/day (highest dose tested) in rats (Collins *et al.*, 1989b).

The only significant long-term effect observed is limited to the moderate growth depression in rats at the 5.19 % dietary level (Olson and Voelker, 1970). A reduction in growth rates (and mean body weights) was also observed at the same concentration in the reproductive and developmental study in rats (Serota *et al.,* 1977b; Borzelleca *et al.,* 1989). The NOAEL for these effects was 1.39 % in the diet (estimated to be equivalent to 695 mg/kg bw/day (Serota *et al.,* 1977b), (later defined to be equivalent to 701 mg/kg bw/day for male and 901 mg/kg bw/day for female rats when the study was published in the peer reviewed literature) (Borzelleca *et al.*, 1989).

The Panel concurs with the view expressed in previous evaluations (JECFA, 1980; TemaNord 2002) that the absorption of Allura Red AC is limited, but that after reduction in the gastrointestinal tract, its metabolites in the form of free sulphonated aromatic amines may reach the systemic circulation.

The SCF indicated that there were some uncertainties with respect to the metabolic data, particularly in relation to the fact that a number of metabolites was not identified, and the number of test animals being too small. The SCF accepted that the data were less than totally adequate, but considered that in the context of an overall assessment of all data, there was no need to repeat the studies. Furthermore, the SCF considered that *p*-cresidine sulphonic acid was unlikely to break down into the carcinogenic *p*-cresidine and this was supported by the absence of any carcinogenic effect in long-term studies. The Panel concurs with the conclusion of the SCF.

The SCF, the JECFA and the TemaNord evaluation concluded, based on *in vivo* and *in vitro* studies available at that time, that Allura Red AC did not show any genotoxic activity.

Recent results obtained by Tsuda *et al.* (2001) suggest that in an *in vivo* Comet assay, Allura Red AC induced significant increases in migration of nuclear DNA in both glandular stomach and colon. Necropsy and histopathological examination revealed no treatment-related effects in the colon and glandular stomach, and the authors therefore concluded that the effect observed was not likely to be due to general cytotoxicity. The Panel considered in the light of negative carcinogenicity studies, that the biological significance of the Comet assay results is uncertain.

The conversion of Allura Red AC by azo-reduction *in vivo* results in the formation of sulphonated naphtylamines that may not be formed in the standard *in vitro* genotoxicity tests (Prival and Mitchell 1998). In a review by Jung *et al.* (1992), a range of sulphonated aromatic amines was shown, in general, not to be associated with genotoxicity *in vitro* and *in vivo*. Since all the sulphonated aromatic amine metabolites that could in theory be formed by azo-reduction of Allura Red AC were included in the study, the Panel concludes that the data reviewed by Jung *et al.* (1992) are sufficiently re-assuring to support the conclusion that the sulphonated aromatic amines formed from Allura Red AC by azo-reduction do not give reason for concern with respect to genotoxicity.

Furthermore, the Panel notes that the specifications on the purity of Allura Red AC would allow concentrations of unidentified unsulphonated aromatic amines to be present in concentrations of up to 100 mg/kg Allura Red AC. Given the maximal allowed concentration of Allura Red AC that can be added to food (500 mg/kg food), the concentration of these amines in food could be 50 µg/kg food. Although some aromatic amines may be associated with genotoxicity or even carcinogenicity, the Panel notes that Allura Red AC was negative in *in vitro* genotoxicity as well as in long term carcinogenicity studies.

Long-term carcinogenicity studies on Allura Red AC were re-evaluated by the Panel. Several long-term carcinogenicity studies at dose levels up to 2595 mg/kg bw/day in male rats (Olson and Voelker, 1970) and 2829 mg/kg bw/day in female rats (Serota *et al.,* 1977b; Borzelleca *et al.,* 1989), and in mice at dose levels up to 7422 mg/kg bw/day in males and 8304 mg/kg bw/day in females (Serota *et al.,* 1977a; Borzelleca *et al.*, 1991; Reno *et al.,* 1978), revealed no evidence of carcinogenicity. This included the absence of neoplasms in the stomach and the large intestine, shown to be the most sensitive organs in the *in vivo* Comet assay in mice. Allura Red AC induced significant dose-related DNA damage in mice in the glandular stomach at doses of 100 mg/kg bw and higher, and in the colon at doses of 10 mg/kg bw and higher. However, carcinogenicity in these tissues was not observed at dose levels several times higher up to 7422 and 8304 mg/kg bw/day for male and female mice, respectively (Serota *et al.,* 1977a; Borzelleca *et al.,* 1991; Reno *et al.,* 1978). Therefore the Panel concluded that the effects on nuclear DNA migration observed in the mouse *in vivo* Comet assay are not expected to result in carcinogenicity.

Based on the same dataset for long-term toxicity/carcinogenicity from previous evaluations, it was also concluded by SCF, JECFA and the authors of the TemaNord report that there was no evidence for carcinogenicity of Allura Red AC (SCF, 1984, 1989; JECFA, 1980; TemaNord, 2002).

The study by McCann *et al*. (2007) has concluded that after exposure to two mixtures of four synthetic colours plus the preservative sodium benzoate in the diet, one of them, Mix B, containing Allura Red AC, resulted in increased hyperactivity in 8 to 9-year old, but not in 3-year old children in the general population. In an earlier study by the same research team, there was some evidence for adverse behavioural effects of a mixture of four synthetic colours (not including Allura Red AC) and sodium benzoate in 3-years old children on the Isle of Wight (Bateman *et al.,* 2004).

Recently EFSA published an opinion on this McCann *et al*. study (EFSA, 2008a). In this opinion the AFC Panel also presented an overview of earlier studies that reported effects of food colours in general on child behaviour, the majority of these studies being conducted on children described as hyperactive or with a clinical diagnosis of ADHD.

In its opinion, the AFC Panel concluded that the McCann *et al.* study provides limited evidence that the two different mixtures of synthetic colours and sodium benzoate tested had a small and statistically significant effect on activity and attention in some children selected from the general population, although the effects were not observed for all children in all age groups and were not consistent for the two mixtures. The AFC Panel also concluded that the findings may thus be relevant for specific individuals within the population, showing sensitivity to food additives in general or to food colours in particular.

However, the AFC Panel, assisted by experts in human behavioural studies in the *ad hoc* Working Group preparing the opinion, also concluded that the clinical significance of the observed effects remains unclear, since it is not known whether the small alterations in attention and activity would interfere with schoolwork and other intellectual functioning.

The AFC Panel also concluded that:

- since mixtures and not individual additives were tested in the study of McCann *et al.*, it is not possible to ascribe the observed effects to any of the individual compounds, and;

- in the context of the overall weight of evidence and in view of the considerable uncertainties, such as the lack of consistency and relative weakness of the effect, as well as the absence of information on the clinical significance of the behavioural changes observed, the findings of the study cannot be used as a basis for altering the ADI of the respective food colours or sodium benzoate.

The ANS Panel concurs with these conclusions.

Overall, the Panel concludes that the present database on genotoxicity, semi-chronic, reproductive, developmental and long-term toxicity, and carcinogenicity as well as the McCann *et al.* study (McCann *et al*., 2007) does not give reason to revise the ADI of 7 mg/kg bw/day.

Adverse reactions after Allura Red AC intake, mostly taken within mixtures of other synthetic colours, have been reported for urticarial and vasculitic reactions.Reports are often characterized by poorly controlled challenge procedures. Recent studies performed under properly controlled conditions imply that sensitivity to food additives in patients with chronic urticaria/angioedema or asthma is uncommon.

Therefore, the Panel concludes that while some sensitivity reactions after Allura Red AC intake (urticaria, rhinitis and asthma) have been reported, mostly when Allura red AC is taken within mixtures of other synthetic colours, no conclusion on the induction of sensitivity by Allura Red AC could be drawn from the limited scientific evidence available. The Panel also notes that sensitive individuals may react at dose levels within the ADI.

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 Allura Red AC 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, 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 Allura Red AC, as identified by the Panel from the data by the FSA, FSAI, AFSSA, UNESDA, CEPS, ELC, CIAA (Tier 3).

For children population (1-10 years old), estimates have been calculated for nine European countries (Belgium, France, UK, the Netherlands, Spain, Czech Republic, Italy, Finland and Germany). For the adult population, the Panel has selected the UK population as representative of the EU consumers for Allura Red AC intake estimates.

When considering MPLs (Tier 2), the mean dietary exposure to Allura Red AC for European children, (aged 1-10 years), ranged from 0.8 to 3.4 mg/kg bw/day, and from 1.8 to 9.4 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure (>10 % in all countries), were soft drinks (13 to 55 %), fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (10 to 47 %), and desserts, including flavoured milk products (12 to 63 %). Sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli accounted for 10-50 % of exposure in four countries. Confectionery accounted for 11 – 13 % of exposure in two countries.

Estimates reported for the UK adult population give a mean dietary exposure of 0.9 mg/kg bw/day and of 2.1 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10 %) were soft drinks (50 % for average consumers and 80 % for high consumers).

When considering the maximum reported use levels (Tier 3), the mean dietary exposure to Allura Red AC for European children (aged 1-10 years), ranged from 0.5 to 3 mg/kg bw/day, and from 1.2 to 8.5 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure (>10 % in all countries) were soft drinks (10 to 63 %), fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (12 to 39 %) and desserts, including flavoured milk products (14 to 58 %). Sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli accounted for 10 to 57 % of exposure in five countries.

Estimates reported for the UK adult population give a mean dietary exposure to Allura Red AC of 0.8 mg/kg bw/day and of 1.9 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10 %) were soft drinks (53 % for average consumers and 80 % for high consumers).

The Panel further notes that the specifications of Allura Red AC 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 notes that the JECFA specification for lead is $\leq$ 2 mg/kg whereas the EC specification is $\leq$ 10 mg/kg.

The Panel notes 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 (EFSA 2008b) and that therefore specifications for the maximum level of aluminium in the lakes may be required.

CONCLUSIONS

Allura Red AC (E 129) is an azo dye allowed to be used as a food additive in the EU and has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1980 and the EU Scientific Committee for Food (SCF) in 1984 and 1989. Both committees have established an ADI of 0-7 mg/kg bw/day.

The Panel concludes that the present dataset does not give reason to revise the ADI of 7 mg/kg bw/day.

The Panel concludes that at the maximum reported levels of use of Allura Red AC, refined (Tier 3) intake estimates are generally below the ADI of 7 mg/kg bw/day. However, in 1-10 years old children the high percentile of exposure (95^{th}) can be 1.2-8.5 mg/kg bw/day and thus slightly higher than the ADI at the upper end of the range.

The Panel concludes that while some sensitivity reactions after Allura Red AC intake (urticaria, rhinitis and asthma) have been reported, mostly when Allura red AC is taken within mixtures of other synthetic colours, no conclusion on the induction of sensitivity by Allura Red AC could be drawn from the limited scientific evidence available. The Panel also notes that sensitive individuals may react at dose levels within the ADI.

The Panel further notes that the specifications for Allura Red AC 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 notes that the JECFA specification for lead is $\leq$ 2 mg/kg whereas the EC specification is $\leq$ 10 mg/kg.

The Panel notes 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.
2. CEPS (European Spirits Organisation), 2009. Letter sent to DG SANCO, dated 17 September 2009/GP.TS-006-2009.
3. CIAA (Confederation of the Food and Drink Industries of the EU), 2009. CIAA data in response to the Commission request for data: "EFSA re-evaluation of food colours" - Southampton study colours) (SANCO/E3/OS/km D 53007, May 22, 2009).
4. ELC (Federation of European Food Additives, Food Enzymes and Food Culture Industries), 2009. ELC comments to EFSA in response to a written request from DG Sanco: "EFSA re-evaluation of food colours" – DG Sanco's additional call for data dated 8 April 2009, letter to EFSA on 20 May 2009).

5. UNESDA (Union of European Beverage Associations), 2009. Comments to the CIAA/DG Sanco in response to a written request from DG Sanco to the CIAA, dated April 8 2009: 'Use of certain colour additives in non-alcoholic beverages' (May 26, 2009).

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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 colours to be re-evaluated (30 July 09) and intake estimates

1. Decision rules taken to deal with QS authorisations:

a. In the category 'All other foodstuffs, the value of 500 mg/kg (the highest MPL) is used

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 (except if this value is available only for annatto-cf point c)

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

c. At the colour level: if there is no available value or if there is just a single value for annatto, 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**: if available use the pork-based products use level; if not available, the highest MPL of 500 mg/kg is used.
- **Edible cheese rinds:** 100 mg/kg (as the flavoured processed cheese category) is used, except for the E 120 (Cochineal) colour whose level is 125 mg/kg for red marbled cheese.

2. Rules defined to identify maximum reported use levels from maximum current usages or maximum observed analytical values:

a. If the identified maximum reported use level, adjusted for the highest current usage data or the highest analytical value, is lower than or equal to the actual MPL, then the actual MPL is used by default.

b. If analytical and current use level data are available, priority is given to the use level data, even if analytical values are higher; the figure is rounded up to the nearest integer.

c. If no use level data are available because no uses were reported (use level = 0) or industry was not asked, the choice is made between the highest analytical value or the MPL:

   i. if more than 10 analytical data are available, the highest value is used;

   ii. if less than 10 analytical data are available, the MPL is used.

d. If no data were reported by the industry, the MPL is used by default.

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

3. Tiered approach to intake estimation.

The basic principles of the stepwise approach for estimates of additives' intakes involve, for each successive Tier, further refinement of intakes from the conservative estimates that form the First Tier of screening until more realistic estimates that form the Second and Third Tiers (EC, 2001).

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

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

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

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

GLOSSARY /ABBREVIATIONS

ADHD	Attention-Deficit Hyperactivity Disorder
ADI	Acceptable Daily Intake
AFC	Scientific Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food
AFSSA	Agence Française de Sécurité Sanitaire des Aliments
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	Scientific Panel on Food Additives and Nutrient Sources added to Food
CAS	Chemical Abstracts Service
CEPS	The European Spirits Organisation
CIAA	Confederation of the Food and Drink Industries of the EU
DG Sanco	The Directorate General for Health and Consumers
EC	European Commission
EFSA	European Food Safety Authority
ELC	The Federation of European Food Additives, Food Enzymes and Food Culture Industries
EXPOCHI	Refers to EFSA Article 36 2008 call for Proposals Focused on Children and Food Consumption
FSA	UK Food Standard Agency
FSAI	Food Safety Authority of Ireland
GHA	Global Hyperactivity Aggregate
GLP	Good Laboratory Practice
HPLC-DAD	High-performance liquid chromatography
IC_{50}	Inhibitory Concentration
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
LOAEL	Lowest-Observed-Adverse-Effect Level
LOD	Limit Of Detection
LOQ	Limit of Quantification
MPL	Maximum Permitted Levels
NOAEL	No-Observed-Adverse-Effect Level
OECD	Organisation for Economic Co-operation and Development
PND	Post-Natal Day

SCF	EU Scientific Committee on 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
WHO/FAO	World Health Organization/Food and Agriculture Organization