SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of Azorubine/Carmoisine (E 122) as a food additive[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion re-evaluating the safety of Azorubine/Carmoisine (E 122). Azorubine/Carmoisine has previously been evaluated by JECFA in 1983 and the SCF in 1984. Both committees established an Acceptable Daily Intake (ADI) of 0-4 mg/kg bw/day. The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. New studies included a study reporting alterations in the morphology of somatic chromosomes in *Secale cereale* (rye), and a study by McCann *et al*. that concluded that exposure to mixtures including Azorubine/Carmoisine, resulted in increased hyperactivity in 3-years old and 8- to 9-years old children. The Panel notes that the study in rye was not a standard genotoxicity assay, and concluded, given that all other genotoxicity tests were negative and that Azorubine/Carmoisine does not contain a structural alert, that there is no concern with respect to genotoxicity. The Panel also concurs 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 concludes that the present database does not give reason to revise the ADI of 4 mg/kg bw/day. The Panel also concludes that at the maximum reported levels of use, refined intake estimates are below the ADI, although in 1- to 10-year old children the high percentile of exposure (95th) can be slightly higher than the ADI at the upper end of the range.

KEY WORDS

Azorubine, Carmoisine, E 122, CAS 3567-69-9, CI Acid Red 14, CI Food Red 3, food colouring substance, EINECS number 222-657-4.

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

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SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion on Azorubine/Carmoisine (E 122) when used as a food colouring substance.

Azorubine/Carmoisine (E 122) is an azo dye allowed as a food additive in the EU and has previously been evaluated by the Joint FAO/WHO Joint Expert Committee on Food Additives (JECFA) in 1983 and the EU Scientific Committee for Food (SCF) in 1984. Both committees established an Acceptable Daily Intake (ADI) of 0-4 mg/kg bw/day.

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

The Panel concurs with the view expressed in previous evaluations by JECFA and TemaNord that the absorption of Azorubine/Carmoisine is limited, but that after reduction in the gastrointestinal tract, free sulphonated aromatic amines may reach the systemic circulation.

The SCF and also the JECFA and TemaNord evaluations concluded, based on *in vivo* and *in vitro* studies available at that time, that Azorubine/Carmoisine does not show any genotoxic activity.

In a more recent study, Zaharia and Pavel have tested four colourings, Tartrazine (E 102), Azorubine/Carmoisine (E 122), Patent Blue (E 131) and Acid Green 50 (E 142), on the frequency of divisional cells, mitotic index, mutagenic process, and the potential of these synthetic colourings to induce chromosomal modifications. Using cytogenetic analysis, the researchers claim to have proven that important alterations in the morphology of somatic chromosomes occur in *Secale cereale* (= rye) in the presence of Azorubine/Carmoisine. The Panel notes that this was not a standard genotoxicity assay, and concluded, given that all other genotoxicity tests were negative and that Azorubine/Carmoisine does not contain a structural alert, that there is no concern with respect to genotoxicity of Azorubine/Carmoisine.

There are no indications that Azorubine/Carmoisine induces tumour formation. Various non-neoplastic lesions have been observed after feeding of Azorubine/Carmoisine to mice and rats, but these findings have in the past been disregarded mainly based on the fact that high incidences were also seen in historical controls. The 1982 National Toxicology Program studies on carcinogenicity in rats and mice indicated that Azorubine/Carmoisine was not associated with the incidence of any type of tumour.

Based on the same dataset for long-term toxicity/carcinogenicity, previous evaluations by JECFA, the SCF and TemaNord also concluded that based on the data available there is no concern with respect to carcinogenicity or genotoxicity.

A study by McCann *et al*. has concluded that exposure to two mixtures of four synthetic colours plus the preservative sodium benzoate in the diet, both of them, Mix A and Mix B, containing Azorubine/Carmoisine, were reported to result in increased hyperactivity in 3- and 8- to 9-year old children in the general population. Recently, the European Food Safety Authority (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 the activity and attention in children selected from the general population, excluding children medicated for the Attention Deficit Hypersensitivity Disorder (ADHD), although the effects were not 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. Altogether, the Panel concludes that the present database does not give reason for revision of the ADI of 0-4 mg/kg bw/day.

The Panel concluded that while some sensitivity reactions after Azorubine/Carmoisine intake have been reported, mostly when Azorubine/Carmoisine is taken within mixtures of other synthetic colours, no conclusion on the induction of sensitivity by Azorubine/Carmoisine 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 Azorubine/Carmoisine 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 Scientific Cooperation (SCOOP) Task 4.2. The Panel calculated a theoretical maximum daily exposure of 8.1 mg/kg bw/day both for adults and for a typical 3 year-old child.

Refined exposure estimates have been performed for both 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 Azorubine/Carmoisine listed in Table 3, as identified by the Panel from the data by the UK Food Standards Agency, the Food Safety Authority of Ireland, the Agence Française de Sécurité Sanitaire des Aliments, the Union of European Beverage Associations, the European Spirits Organisation, the Federation of European Food Additives, Food Enzymes and Food Culture Industries and the Confederation of the Food and Drink Industries of the EU (Tier 3). For children (1-10 years old), estimates have been performed for nine European countries (Belgium, France, the Netherlands, Spain, UK, Czech Republic, Italy, Finland and Germany). For the adult population, the Panel has selected the UK population as representative of the EU consumers for Azorubine/Carmoisine intake estimates.

When considering MPLs (Tier 2), the mean dietary exposure to Azorubine/Carmoisine for European children (aged 1-10 years), ranged from 0.3 mg/kg bw/day to 2.5 mg/kg bw/day, and from 0.7 mg/kg bw/day to 6.7 mg/kg bw/day at the 95th percentile. Estimates reported for the UK adult population give a mean dietary exposure to Azorubine/Carmoisine of 0.5 mg/kg bw/day and of 1.1 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks.

When considering the maximum reported use levels, the mean dietary exposure to Azorubine/Carmoisine for European children (aged 1-10 years), ranged from 0.25 mg/kg bw/day to 2.4 mg/kg bw/day and from 0.6 mg/kg bw/day to 6.5 mg/kg bw/day at the 95th percentile. Estimates reported for the UK adult population give a mean dietary exposure of 0.4 mg/kg bw/day and of 1.0 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 Azorubine/Carmoisine, refined intake estimates are below the ADI, although in 1- to 10-year old children the high percentile of exposure (95th) can be slightly higher than the ADI at the upper end of the range.

The Panel further notes that the specifications of Azorubine/Carmoisine 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 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 for 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 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 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 Azorubine/Carmoisine (E 122) when used as a food colouring substance.

Azorubine/Carmoisine (E 122) is an azo dye allowed as a food additive in the EU and has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1983 and the EU Scientific Committee for Food (SCF) in 1984.

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

2. Technical data

2.1. Chemistry

Azorubine/Carmoisine (E 122) is an azo dye with the formula $C_{20}H_{12}N_2Na_2O_7S_2$. It has a molecular weight of 502.44 g/mol and CAS Registry Number 3567-69-9. Its full chemical name is disodium 4-hydroxy-3- (4-sulphonato-1-naphthylazo) naphthalene-1-sulphonate. Its structural formula is:

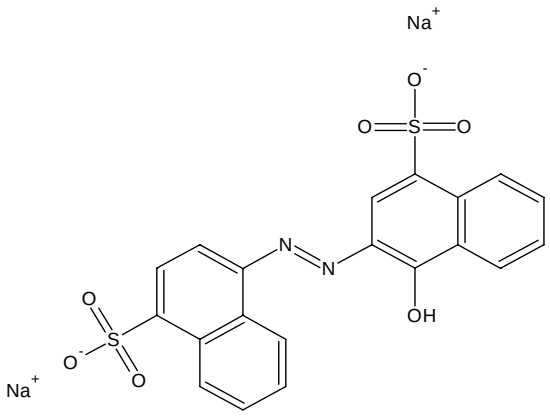

Figure 1. The structural formula of Azorubine/Carmoisine

At least 143 synonyms are in use (ChemIDplus advanced, via internet, 2007). The most commonly used synonyms in published literature are Carmoisine, Azorubine, CI Acid Red 14 and CI Food Red 3.

Azorubine/Carmoisine is soluble in water and slightly soluble in ethanol (Merck, 2006).

2.2. Specifications

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

Azorubine/Carmoisine consists essentially of disodium 4-hydroxy-3-(4-sulphonato-1-naphthylazo) naphthalene-1-sulphonate and subsidiary colouring matters, together with sodium chloride and/or sodium sulphate as the principal uncoloured components. Azorubine/Carmoisine is described as the sodium salt. The calcium and the potassium salts are also permitted (Directive 2008/128/EC).

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), ≤ 2% subsidiary colouring matters and ≤ 0.5% 4-aminonaphthalene-1-sulphonic acid and 4-hydroxynaphthalene-1-sulphonic acid, 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 Azorubine/Carmoisine 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	≤ 2%	≤ 1%
Organic compounds other than colouring matters: - 4-aminonaphthalene-1-sulphonic acid - 4-hydroxynaphthalene-1-sulphonic acid	} ≤ 0.5%	} ≤ 0.5%
Unsulphonated primary aromatic amines	≤ 0.01% (calculated as aniline)	≤ 0.01% (calculated as aniline)
Ether extractable matter	≤ 0.2% under neutral conditions	≤ 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 notes that the specifications on the purity of Azorubine/Carmoisine would permit concentrations of unsulphonated aromatic amines to be present in concentrations of up to 100 mg/kg Azorubine/Carmoisine. Given the maximal allowed concentration of Azorubine/Carmoisine 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 Directive 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 (Directive 2008/128/EC).

JECFA does not give specifications for aluminium lakes of Azorubine/Carmoisine other than reference to the General Specifications for Aluminium Lakes of Colouring Matters (JECFA, 2004). The Azorubine/Carmoisine 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, not more than 0.2%

ether-extractable matter, not more than 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

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

2.4. Methods of analysis in food

Several methods for the determination of Azorubine/Carmoisine in foods are described in the literature, of which variations of High Performance Liquid Chromatography (HPLC) appear to be most generally employed.

Azorubine/Carmoisine can be directly quantified by HPLC with diode-array detection (HPLC-DAD) in water-soluble foods like fruit flavoured drinks, alcoholic drinks, jams, sugar confectionery and sweets after dilution or water extraction (Minioti *et al.,* 2007).

2.5. Reaction and fate in food

Some data on the reaction and fate of Azorubine/Carmoisine in food can be obtained from a review by Scotter and Castle (2004). Azorubine/Carmoisine is reduced by sulphur(IV) oxo-anions with the transfer of two electrons, thus facilitating the conversion of sulphur(IV) to sulphate. Furthermore, it was suggested that Azorubine/Carmoisine interacts with sulphite species to form a complex, and that a hydrazo compound may then be formed via hydrolysis.

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, Azorubine/Carmoisine (E 122) is an allowed synthetic food colouring substance in the EU with a maximal allowed use level of 50-500 mg/kg food for various foodstuffs. Azorubine/Carmoisine is also allowed in alcoholic beverages at levels up to 200 mg/L and non-alcoholic flavoured drinks

(soft drinks) up to 50 mg/L. Table 2 summarises those beverages and foodstuffs that are permitted to contain Azorubine/Carmoisine up to specified Maximum Permitted Levels (MPLs) set by EC legislation (EC, 1994).

Table 2. Maximum Permitted Levels of use of Azorubine/Carmoisine in beverages and foodstuffs according to Council Directive 94/36/EC

Beverages	**Maximum permitted level** (mg/L)
Non-alcoholic flavoured drinks	50
Liquid food supplements/dietary integrators Americano Bitter soda, bitter vino	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)
Confectionery Fine bakery wares Edible ices Desserts including flavoured milk products 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
Candied fruit and vegetables, Mostarda di frutta Preserves of red fruits Extruded or expanded savoury snack products	200
Pre-cooked crustaceans	250
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

Azorubine/Carmoisine is permitted as a food additive in the EU under Directive 94/36/EC. Azorubine/Carmoisine has been evaluated previously by JECFA in 1983 (JECFA, 1983a; 1983b) and the SCF in 1984 (SCF, 1984). Both committees have established an Acceptable Daily Intake (ADI) of 0-4 mg/kg bw/day.

2.8. Dietary exposure

2.8.1. Actual levels of use of Azorubine/Carmoisine

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). Azorubine/Carmoisine was found to be present at a level higher than 0.1 mg/L (Limit of Detection - LOD) in 64 products, with levels varying from <0.5 to 59 mg/L. In another survey, conducted in 2005 by the Food Safety Authority of Ireland (FSAI), Azorubine/Carmoisine was found to be present at a level higher than 0.1 mg/L (Limit of Quantification - LOQ) in 16 out of 34 soft drink; the concentration in these products ranged from 1 to 59 mg/L (unpublished data provided by FSAI). A usage survey, conducted by the Union of European Beverage Associations (UNESDA) in 2005 suggests that the highest current use level of Azorubine/Carmoisine in beverages is 50 mg/L (Tennant, 2006). A more recent report from UNESDA in 2009 gives a range of use levels from 1 to 48 mg/L (UNESDA, 2009). The Confederation of the Food and Drink Industries of the EU (CIAA) also reported other current use levels of Azorubine/Carmoisine ranging from 1 to 48 mg/L (CIAA, 2009). French companies reported use levels ranging from 6 to 40 mg/L (unpublished data provided by AFSSA). 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 from 4 to 50 mg/L (ELC, 2009).

For spirituous beverages, including products with less than 15% alcohol, the survey conducted by the FSAI shows no detected samples (LOD of 1mg/L) from the analysis of 14 retail samples. The European Spirits Organisation (CEPS), reported a range of use levels of Azorubine/Carmoisine from 0 to 100 mg/L (CEPS, 2009).

For fruit wines (still or sparkling), cider and perry, the CIAA reported a range of typical maximum use levels of below 1 mg/L.

2.8.1.2. Foodstuffs

For confectionery products, the Panel was provided with data from an *ad hoc* survey conducted by the FSA, in which artificial colours were analytically determined in 195 retail samples of brightly coloured packaged sweets selected for being distinctly coloured (FSA, 2002). Azorubine/Carmoisine was found to be present at a level higher than 0.3 mg/kg (LOD) in 56 products, with levels varying from 0.7 to 61 mg/kg. According to the FSAI data, Azorubine was present at a level higher than 1.0 mg/kg in 25 out of 183 confectionery products, with levels varying from 1 to 106 mg/kg (unpublished data provided by FSAI). Data provided by French industries on Azorubine/Carmoisine in sweets showed use levels varying from 0 to 43 mg/kg (unpublished data provided by AFSSA). Data provided by the ELC (2009), give a range of typical low and maximum use levels from 3 to 50 mg/L. A range of current use levels from 10 to 50 mg/kg has also been reported by the CIAA (2009).

For candied fruit, vegetables, mostarda di frutta and preserves of red fruits, to date no uses were reported by CIAA members.

For decorations and coatings, data from the FSAI survey gave a range of analytical values below 5 mg/kg for four retail samples; the CIAA (2009) reported a range of typical low and maximum use levels of Azorubine/Carmoisine from 10 to 100 mg/kg.

For fine bakery wares, the CIAA (2009) reported a range of typical low and maximum use levels of Azorubine/Carmoisine from 5 to 50 mg/kg.

For edible ices, the FSAI survey gave a range of analytical values of Azorubine/Carmoisine from 1 to 76 mg/kg for 5 detected samples out of 30 retail samples analysed, whereas the ELC provided further data from the FAIA, which gave a range of typical low and maximum use levels from 1 to 5 mg/kg.

For flavoured processed cheese, and edible cheese rind and edible casing, the CIAA (2009) reported a typical maximum value of 0.004 mg/kg.

For desserts, including flavoured milk products, the FSAI survey (2009) gave a range of analytical values from 1 to 85 mg/kg for 11 detected samples out of 35 retail samples analysed, and the CIAA (2009) reported a range of typical low and maximum use levels of Azorubine/Carmoisine of 3 to 30 mg/kg.

For sauces, seasonings, pickles, relishes, chutney, the FSAI survey (2009) gave a range of analytical values from 2 to 10 mg/kg for 5 retail samples; the CIAA to date reported no uses from its members.

For fish paste and crustacean pastes and mustard, no uses were reported from the CIAA's members.

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 Azorubine/Carmoisine. 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 Azorubine/Carmoisine 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 Azorubine/Carmoisine in beverages and foodstuffs used for the refined exposure assessment

Beverages	**Maximum reported use level** (mg/L)
Fruit wines, cider and perry	1
Non-alcoholic flavoured drinks	50
Liquid food supplements/dietary integrators Americano Bitter soda, bitter vino Spirituous beverages	100
Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails	200
Foodstuffs	**Maximum reported use level** (mg/kg)
Flavoured processed cheese Edible cheese rind and edible casings*	0.004
Desserts including flavoured milk products	30
Confectionery Fine bakery wares	50

Edible ices 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	
Fish paste and crustaceans paste Smoked fish Savoury snack products and savoury coated nuts Meat and fish analogues based on vegetable proteins Decorations and coatings	100
Candied fruit and vegetables, Mostarda di frutta Preserves of red fruits Extruded or expanded savoury snack products	200
Pre-cooked crustaceans	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 use 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 intake estimates. 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 Azorubine/Carmoisine 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 Azorubine/Carmoisine, the maximum permitted use level considered in beverages was 200 mg/L. The maximum permitted use level considered for solid foods was 500 mg/kg.

The default proportion (25%) of beverages and solid food that could contain the additive was considered adequate. In fact, even though Azorubine/Carmoisine 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 Azorubine/Carmoisine. 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 Azorubine/Carmoisine considered in beverages was 50 mg/L (after exclusion of alcoholic drinks) and in solid food 500 mg/kg. The proportion of 25% used, for beverages, was changed to 100% for children, in order to compensate the fact that 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 under 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 Azorubine/Carmoisine. The overall theoretical maximum daily exposure in children would therefore be:

(50 x 0.1 x 1) + (500 x 0.025 x 0.25) = 5 + 3.12 = 8.1 mg/kg bw/day.

It was noted that Azorubine/Carmoisine may be used *quantum satis* in edible cheese rind and edible casings. As this is a very specific food category, which is unlikely to be consumed in high amounts on a daily basis, if at all, this category was excluded from the Budget calculation, as 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 the Tier 2 using maximum permitted use levels presented in Table 2 and maximum practical use levels presented in Table 3 to deal with the specific cases of *quantum satis* authorisation for edible cheese rinds and edible casings, and for the 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 from UK children (aged 1.5 to 4.5 years) were made by the Panel with the use of detailed individual food consumption data (UK NDNS, 1992-1993) available from the UNESDA report (Tennant., 2006) and with the MPL of use as specified in 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 calculated from more refined adult food consumption data, than 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 Azorubine/Carmoisine intake estimates for adults.

Estimates of Azorubine/Carmoisine 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 Directive 94/36/EC (EC, 1994) for the Tier 2 approach (Table 2) and with the maximum reported use levels for the Tier 3 approach (Table 3).

Table 4 summarises the anticipated exposure of children and adults to Azorubine/Carmoisine.

In the case of Azorubine/Carmoisine, 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 0.3 mg/kg bw/day to 2.5 mg/kg bw/day, and from 0.7 mg/kg bw/day to 6.7 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 61%), desserts, including flavoured milk products (14 to 56%), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (16 to 68%). Fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) accounted for 11 to 29% of exposure in 5 countries, and surimi 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 to Azorubine/Carmoisine was 1.4 mg/kg bw/day and 3.5 mg/kg bw/day for the high level (97.5th percentile) consumers of beverages. The main contributors to the total anticipated exposure (>10%) for UK pre-school children were soft drinks (60%).

Estimates reported for the UK adult population give a mean dietary exposure to Azorubine/Carmoisine of 0.5 mg/kg bw/day and of 1.1 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure to Azorubine/Carmoisine (>10%) were soft drinks (40%), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (14%) and fruit wines, cider and perry (13%).

Further data suggest that current use levels of Azorubine/Carmoisine 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 Azorubine/Carmoisine (Tier 3).

When considering the maximum reported use levels from Table 3, the mean dietary exposure to Azorubine/Carmoisine of European children (aged 1-10 years and weighing 25-30 kg) considered by the EXPOCHI consortium, ranged from 0.25 mg/kg bw/day to 2.4 mg/kg bw/day, and from 0.6 mg/kg bw/day to 6.5 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure (>10% in all countries) were soft drinks (17 to 58%), fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (10 to 34%), desserts, including flavoured milk products, (15-42%) and sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (15-70%). Surimi 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 to Azorubine/Carmoisine was 1.3 mg/kg bw/day and 3.4 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 (65%).

Estimates reported for the UK adult population give a mean dietary exposure to Azorubine/Carmoisine of 0.4 mg/kg bw/day and of 1.0 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%), sauces and seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (17%).

Table 4. Summary of anticipated exposure to Azorubine/Carmoisine using tiered approach (EC, 2001) in children and adult populations

	Adult UK population (>18 years old)	**Pre-school UK children** (1.5 to 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	8.1	
Tier 2. Maximum Permitted Level			
• Mean exposure	0.5	1.4	0.3-2.5
• Exposure 95th* or 97.5th percentile**	1.1	3.5	0.7-6.7

Tier 3. Maximum reported use levels			
• Mean exposure	0.4	1.3	0.25-2.4
• Exposure 95th* or 97.5th percentile**	1.0	3.4	0.6-6.5

* 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

Azorubine/Carmoisine has been evaluated previously by JECFA in 1983 (JECFA, 1983a; 1983b) and the SCF in 1984 (SCF, 1984). 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 (JECFA, 1983a,b; SCF, 1984; TemaNord, 2002) reported that research with ^{14}C-Azorubine/Carmoisine revealed that absorption is limited. Blood ^{14}C-radioactivity decay curves after i.v. injection in mice and rats indicate that distribution to the tissues is rapid and that elimination occurs according to a two-compartment model. In mice given a single oral dose of ^{14}C-Azorubine/Carmoisine, peak levels of radioactivity were seen in plasma, liver, lung, testes and spleen (after 8 hours). In rats however, after administration by gavage, no radioactivity was detected in the lung, testes and spleen, but no time specification was given. Furthermore, blood-radioactivity curves after oral and intravenous administration, revealed that bioavailability of ^{14}C-Azorubine/Carmoisine in rats was less than 10%.

Some of the ingested Azorubine/Carmoisine undergoes azo reduction in the intestine. Besides unmodified Azorubine/Carmoisine, 5 unidentified metabolites were found in the faeces. In urine, naphthionic acid, 2-amino-1-naphthol-4-sulphonic acid (2-ANS), 1,2-naphthoquinone-4-sulphonate (1,2-NQS), and a fourth unidentified metabolite were determined. The toxicokinetic profile of Azorubine/Carmoisine was not affected by pregnancy. Contradictory results were found on trans-placental transfer, as *in utero* exposure of rats to ^{14}C-Azorubine/Carmoisine revealed no trans-placental transfer of the dye or its metabolites, whereas, in another rat study, radioactivity in fetuses was similar to that in maternal tissues. The majority of radioactivity was excreted in faeces and urine in the first 24 hours. Substantially, the entire dose was recovered in the excreta within 72 hours, the majority being accounted for in the faeces (60-75%).

No new data on the toxicokinetic aspects of Azorubine/Carmoisine have been published since the TemaNord evaluation.

3.2. Toxicological data

3.2.1. Acute oral toxicity

JECFA mentions acute toxicity studies in rats and mice. In these studies, LD_{50} values were found to be > 8000 and > 10000 mg/kg/bw, respectively. In addition, acute toxicity tests were conducted with intraperitoneal and intravenous administration, but these studies are considered to be of little relevance for the toxicological evaluation of food additives.

No new literature on the acute toxicity of Azorubine/Carmoisine was found.

3.2.2. Short-term and subchronic toxicity

JECFA describes five short-term studies.

Weanling rats (3) were given a 0.1% solution of Azorubine/Carmoisine to drink for 28 days (daily consumption approximately 15 mg). No toxic effects were noted (Goldblatt and Frodsham, 1952).

Groups of rats (16/sex/group) were fed 0, 0.05, 0.1, 0.5 or 1% Azorubine/Carmoisine (equivalent to 0, 25, 50, 250 or 500 mg/kg bw/day, respectively) for 90 days. No deleterious effects on body weight, food consumption, haematology, renal or hepatic function parameters were noted. Females at the 1% dietary level were found to have elevated renal weight, but without untoward pathology. Gross pathology was normal and no non-spontaneous, compound-induced tumours were found. The authors established a no-effect level of 0.5% (250 mg/kg bw/day) based on the elevated renal weights observed at 500 mg/kg bw/day (Gaunt *et al.*, 1967). As however, no untoward pathology was noted, and in none of the other studies conducted with Azorubine/Carmoisine any effects on kidneys or renal function were observed, this No-Observed-Adverse-Effect Level (NOAEL) has not been taken into consideration by JECFA and the SCF. The Panel agrees that the increased renal weights can be considered toxicologically insignificant.

In another study in rats, animals (10/sex/group) were fed 0, 2, 4, 6 or 8% Azorubine/Carmoisine in the basal diet (equivalent to 0, 1000, 2000, 3000, or 4000 mg/kg bw/day) for 9 weeks. At the two highest dose levels a reduction in body weight gain was noted, based on which a no-effect level of 2000 mg/kg bw/day was reported by the study authors. No other toxic effects were observed (Holmes *et al.*, 1978a).

Groups of miniature pigs (3/sex/group) received Azorubine/Carmoisine at levels of 0, 250, 500 and 1000 mg/kg bw/day admixed with their basal diets for 90 days. No untoward toxicology or pathology was noted, and no significant differences between control and treated animals were detected. A no-effect level of 1000 mg/kg/day was assigned based upon the results of this study (Gaunt *et al.*, 1969).

Cats were given Azorubine/Carmoisine (5% solution) at doses of 1000 mg (day 1) and 100 mg (days 9 and 18). No Heinz bodies were observed (DFG, 1957).

No new data have become available.

3.2.3. Genotoxicity

JECFA describes five studies on genotoxicity.

Azorubine/Carmoisine tested in cultures of *Escherichia coli* at a concentration of 0.5 g/100 mL revealed no mutagenic potential (Lück and Rickerl, 1960).

Azorubine/Carmoisine was not mutagenic in an assay with *Salmonella typhimurium* (TA1538) at 50 µg/plate, with or without metabolic activation (Garner and Nutman, 1977).

No mutagenic effect was found when Azorubine/Carmoisine was tested for cytotoxicity and mutagenicity in *Salmonella typhimurium* (TA1535, TA1538, TA100 and TA98) at concentrations of 0, 1, 2, 20, 500 and 1000 µg/plate/10^8 bacteria, either with or without metabolic activation (Viola and Nosotti, 1978).

No evidence of mutagenic potential was obtained after exposure of *Escherichia coli* (WPZ) and *Salmonella typhimurium* (TA1538) to 5 mg/mL Azorubine/Carmoisine, either with or without metabolic activation (Haveland-Smith and Combes, 1980).

Azorubine/Carmoisine did not induce mitotic gene conversion in *Saccharomyces cerevisiae* (BZ 34) at 5 mg/mL, when treated either in stationary-phase or log-phase culture without microsomal activation. Neither significant cell killing nor inhibition of cell division was observed (Sankaranarayanan and Murthy, 1979).

In addition, TemaNord (2002) mentions some *in vitro* and *in vivo* studies in which the genotoxic potency of Azorubine/Carmoisine has been investigated. According to TemaNord, no evidence for genotoxicity was found in a wide range of *in vitro* studies, such as *Salmonella*/microsome test, mouse lymphoma TK +/- assay, chromosome aberration/sister chromatid exchange test in Chinese hamster ovary cells, and rat hepatocyte/DNA repair assay (no further details on any of these studies were given) (Ashby *et al.*, 1988; Benigni, 1989; Cameron *et al.*, 1987; Gulati *et al.*, 1989; Kornbrust and Barfknecht, 1985; McGregor *et al.*, 1988; Mortelmans *et al.*, 1986; Shelby and Stasiewics, 1984; Sweeney *et al.*, 1994; Tennant *et al.*, 1986, 1987; Zeiger, 1987).

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.

In a single *in vivo* study, mice received Azorubine/Carmoisine at oral doses of 1 or 10 mg/kg bw/day for 5 days. Metaphase slides did not reveal any increase in the level of cells with chromosomal damages (Durnev *et al.*, 1995).

The SCF provides no additional data on this subject.

In a more recent study, Zaharia and Pavel (2003) tested four colours (Tartrazine (E 102), Azorubine/Carmoisine (E 122), Patent Blue (E 131) and Acid Green 50 (E 142)) on the frequency of divisional cells, mitotic index, mutagenic process and the probability of these synthetic colourings to induce chromosomal modifications. Using cytogenetic analysis, the researchers claim to have proven that important alterations in the morphology of somatic chromosomes occur in *Secale cereale* (= rye) in the presence of Azorubine/Carmoisine. The Panel noted that this is not a standard genotoxicity assay.

As described above, metabolism of Azorubine/Carmoisine produces sulphonated aromatic amines. Jung *et al.* (1992) have reviewed the genotoxicity of a range of sulphonated aromatic amines. Although the paper was published over a decade ago, it is discussed in this section as it has not been referred to in any previous evaluations. To provide insight in the effect of sulphonation on the genotoxic potential of phenylamines and naphthylamines, the genotoxicity of sulphonated aromatic amines was compared with their unsulphonated analogues. It was found that in general sulphonated phenylamines and naphthylamines (including naphthionic acid) are non-mutagenic to *Salmonella* in Ames tests. For some sulphonated aromatic amines the absence of genotoxicity was also demonstrated with a variety of other test systems *in vitro* and *in vivo* (no details provided). Based on the available data, the authors concluded that sulphonated aromatic amines, in contrast to their unsulphonated analogues, have no or very low genotoxic potential. Furthermore, the authors conclude that exposure to sulphonated aromatic amines, derived from metabolic cleavage or present as micro-contaminants in colourings is unlikely to induce any significant genotoxic risk.

3.2.4. Chronic toxicity and carcinogenicity

The Panel noted that the studies on chronic toxicity and carcinogenicity were all performed before OECD guidelines and Good Laboratory Practice (GLP) were established.

In a study in mice, Azorubine/Carmoisine was administered to animals (30/sex/group; 60/sex/controls) at levels of 0, 0.01, 0.05, 0.25 or 1.25% of the diet (equivalent to approximately 0, 14, 71, 375 or 1786 mg/kg bw/day) for 80 weeks. High-dosed females displayed significantly lowered haemoglobin levels 12 and 52 weeks after exposure commenced. A decreased packed cell volume was observed in males fed 0.25% and 1.25% Azorubine/Carmoisine after 52 weeks. However, as at the 0.25% level the observed changes were of doubtful significance, the Lowest-Observed-Adverse-Effect Level (LOAEL) was considered to be 1.25%. Consequently, a NOAEL of 375 mg/kg bw/day (0.25%) was ascribed to Azorubine/Carmoisine. Further, no effects were noted on behaviour, body weight or organ weight, and no (compound-related) change in tumour distribution was detected (Mason *et al.*, 1974). It is concluded that in this long-term mouse study by Mason *et al.* (1974), a dose of 1786 mg/kg bw/day Azorubine/Carmoisine induced a significant decrease of haemoglobin levels and a decreased packed cell volume, based on which a NOAEL of 375 mg/kg bw/day was determined. Conceivably, the SCF and JECFA used this study, in addition to the rat developmental study, for calculation of the current ADI.

In another study in mice, animals (15/sex/group) received two subcutaneous injections weekly with 6 mg Azorubine/Carmoisine in arachis oil for 6 months, followed by two subcutaneous injections weekly with 3 mg Azorubine/Carmoisine in arachis oil for another 6 months (resulting in a total dose of 468 mg). Controls received arachis oil alone. After the 12-month exposure period, the animals were allowed to survive until natural death.

After 89 weeks from the start of exposure, 7 females had been found to develop lymphomas; no subcutaneous sarcomas or hepatomas were observed. As the observed lymphomas also developed spontaneously in control animals, these findings were considered to be of no toxicological significance and the authors concluded that Azorubine/Carmoisine was non-carcinogenic in mice (Bonser *et al.*, 1956).

Rats (10) were given Azorubine/Carmoisine in the drinking-water in a concentration of 1% for 209 days and were observed for 919 days. No tumours were found (DFG, 1957).

Rats (10) were given Azorubine/Carmoisine in the drinking-water in a concentration of 1% for 250 days and were observed for 545 days. No tumours were found (DFG, 1957).

Rats (10) were given a diet containing 0.2% of the colour for 417 days and were observed for 838 days. No tumours were found (DFG, 1957).

Rats (10) were given twice per week subcutaneous injections containing 5 mg Azorubine/Carmoisine for 1 year, and were observed for over 938 days. In one animal, an axillary tumour was observed. In a repeat experiment, another group of 10 rats was treated identically and was observed for 521 days. No tumours were observed (DFG, 1957).

In addition, JECFA (JECFA, 1983a) describes three long-term studies, which due to their nature, have been described in this opinion under the section on reproductive and developmental toxicity. Furthermore, JECFA describes two studies investigating carcinogenicity.

Groups of B6C3F1 mice (50/sex/group) were fed diets containing textile-grade Azorubine/Carmoisine at levels of 0, 0.3 or 0.6% (equivalent to approximately 0, 430 or 860 mg/kg bw/day) for 103-104 weeks (dye composition in the first 11 months: 71.4% dye, 7.39% water, 11.7% NaC1, 5.7% Na_2SO_4, 3.72% $NaHCO_3$; in the final 13 months: 67.3% dye, 7.48% water, 7.85% NaC1, 12.2% Na_2SO_4, 5.16% $NaHCO_3$).

No compound-related clinical signs were observed except for a slightly lower mean body weight in high-dose male mice. There was no significant decrease in survival.

Histopathological examination indicated that Azorubine/Carmoisine was not associated with the incidence of any tumour type. However, although not dose-related, non-neoplastic lesions such as lymphoid hyperplasia of the spleen, haematopoiesis in the liver and lymphoid hyperplasia of the sub-mucosa of the urinary bladder were observed in female mice (NTP, 1982). The National Toxicology Programme (NTP) study reports states that the various non-neoplastic lesions represented among both control and dosed animals have been encountered previously as spontaneous occurrences in aging 2-year old laboratory mice.

In a similar study in F344 rats, groups of animals (50/sex/group; 90/sex/controls) were fed diets containing textile-grade Azorubine/Carmoisine for 103-104 weeks. Males received 0, 0.6 or 1.25% (equivalent to 0, 300 or 625 mg/kg bw/day); females received 0, 1.25 or 2.5% (equivalent to 0, 625 or 1250 mg/kg bw/day) (dye composition in the first 11 months: 71.4% dye, 7.39% water, 11.7% NaC1, 5.7% Na_2SO_4, 3.72% $NaHCO_3$; in the final 13 months: 67.3% dye, 7.48% water, 7.85% NaC1, 12.2% Na_2SO_4, 5.16% $NaHCO_3$).

Although a significantly higher incidence of endometrial stromal polyps of the uterus was observed at the highest dose, this increase was similar to the historical rate in untreated female rats. Various non-neoplastic lesions found in both control and dosed animals were also encountered previously as spontaneous occurrences in ageing laboratory rats. An increased incidence of adrenal cortical focal hyperplasia (characterised by focal collections of basophilic, eosinophilic or vacuolated cells) was seen in high-dose rats of both males (controls: 5/89 (6%); low-dose: 6/49 (12%); high-dose: 8/50 (16%)) and females (controls: 7/86 (8%); low-dose: 7/50 (14%); high-dose: 18/50 (36%)). Furthermore, no compound-related clinical signs were observed, and survival was not affected in both male and female rats. Histopathological examination revealed no carcinogenic potential of Azorubine/Carmoisine in both sexes (NTP, 1982).

The Panel notes that these adrenal histopathological changes are rather common in ageing laboratory rats and moreover, these lesions generally vary considerably between different studies. Therefore, the Panel concluded that these adrenal histopathological changes are not toxicologically relevant, which is in accordance with the previous evaluation of these data by JECFA and the SCF.

No new chronic toxicity studies have been published since the TemaNord evaluation.

3.2.5. Reproductive and developmental toxicity

JECFA describes several studies on reproductive and developmental toxicity.

In a study on reproduction, groups of rats (25/sex/group) received 0 or 1% Azorubine/Carmoisine in their drinking-water (equivalent to 0 or 500 mg/kg bw/day) for 6 months. Weight gain, mortality and general condition were similar in both groups. After 7 months the animals were mated to produce an F1 generation. After weaning, the F1 pups also received 1% Azorubine/Carmoisine and after 4 months mated to produce an F2 generation. No abnormalities regarding litter number or fertility were noted. The F2 generation was also given 1% Azorubine/Carmoisine for 200 days and subsequently kept on normal diet and water for 2 years. No adverse effects were seen on mortality or tumour incidence (Hecht, 1966).

Female rats were given Azorubine by oral intubation at levels of 0 (60 animals), 100 (22 animals), 300 (24 animals) or 1000 mg/kg bw/day (22 animals) on Gestational Days (GD) 6-15. Twenty two animals were dosed with 30 mg trypan blue/kg bw/day, as a positive control. No embryotoxic or teratogenic

effects were observed in any of the Azorubine/Carmoisine-dosed groups of animals (Smith *et al.*, 1972b).

In another rat study, groups of pregnant animals (3 or 6/group) received 200 mg/kg ^{14}C-Azorubine (25 μCi) by gavage on GD 11. Animals were killed on GD 19. No significant differences in maternal body weight, food intake of dams, number of fetuses, litter size and fetal weight were observed (Galli *et al.*, 1982).

In a study in female rabbits, animals were administered Azorubine/Carmoisine by oral intubation at levels of 0 (47 animals), 40 (15 animals), 120 (15 animals), or 400 mg/kg bw/day (20 animals) on GD 6-18. Thalidomide (150 mg/kg bw/day) was administered to 15 rabbits as a positive control. No effect of Azorubine/Carmoisine was seen on body weight gain. An increase in the number of spontaneous deaths among dams of the high-dose group was noted, but was statistically non-significant. All females to which Azorubine/Carmoisine had been administered displayed a decrease in the implantation efficiencies, which was however not deemed compound-related, as implantation was assumed to have occurred prior to the initiation of the dye administration. Furthermore, no signs of toxicity or fetal abnormalities were found (Smith *et al.*, 1972a).

Finally, JECFA describes some multi-generation reproduction studies under the heading of long-term toxicity. As the investigated animals were also exposed during gestation and lactation, these studies are discussed here.

A study was carried out by Holmes *et al.* (1978a) in which rats (10/sex/group; 20/sex/controls) were fed Azorubine/Carmoisine for 9 weeks at dietary concentrations of 0, 2, 4, 6 and 8% (equal to 0, 1320, 2760, 4500 or 6920 mg/kg bw/day for males, and 0, 1760, 3400, 5200 or 7600 mg/kg bw/day for females). At concentrations of 6 and 8%, a significant effect on body weight was noted in both sexes, which according to the researchers reflects a maximum no-effect level of 4%. Furthermore, no overall toxicity (not specified) was noted.

In the same publication by Holmes and co-workers, three generations of rats (30/sex/group; 50/sex/controls) were fed Azorubine/Carmoisine at dietary concentrations of 0, 0.35, 0.8 or 2% of the diet (approximately equal to 0, 160, 370 or 970 for males, and 0, 160, 440, or 1050 mg/kg bw/day for females.

An unexplained drop in food consumption was observed for the P0 females at week 10. Further, no significant Azorubine/Carmoisine-related effects were seen with regard to pup body weight (gain), litter size, number of stillborn, sex distribution, offspring viability, survival, or copulation and fertility indices. These results indicate a maximum no-effect level of 2% in the diet (roughly equal to an average of 1270 mg/kg bw/day for F1b and F2b males taken together, and 2150 mg/kg bw/day for F1b and F2b females taken together (at week 10)) (Holmes *et al.*, 1978a).

Another study, also by Holmes and colleagues (1978b), is an extension of the above described three-generation rat study. In this study, the F2-generation animals (30/sex/group; 50/sex/controls) were continued on the diets containing 0, 0.35, 0.8 and 2% Azorubine/Carmoisine up to 1 year of age (approximately equal to 0, 160, 370 or 970 for males, and 0, 160, 440, or 1050 mg/kg bw/day for females). A concentration-dependent decrease in monocyte levels was noted in females, but this was considered insignificant as no pathological situations could be linked with decreased monocyte counts. In addition, an increase of relative thyroid weights was observed in both sexes at a dietary concentration of 2%. As no gross or microscopic evidence of thyroid dysfunction was found, this effect was considered to be toxicologically insignificant. Finally, in 2% male rats, an increased incidence of several mild sub-clinical conditions was noted without overt clinical signs (statistically significant increase in bronchitis and tracheal irritation (histological)). Furthermore, no significant Azorubine/Carmoisine-related effects were noted on body weight gain, urinalysis, haematological values, relative organs weights, or at gross pathological and histopathological examination. Tumour incidence was not significantly increased. Although the deleterious effects seen at the 2% level were

considered to be minimal, a no-effect level of 0.8% (400 mg/kg/day) was assigned to Azorubine/Carmoisine (Holmes *et al.*, 1978b).

Lastly, F0 rats (66/sex/group; 114/sex/controls) were given diets to provide 0, 100, 400 or 1200 mg Azorubine/Carmoisine/kg bw/day and were allowed to mate (Stevenson *et al.*, 1982; published in the peer reviewed literature as Ford *et al*., 1987). Treatment continued throughout gestation and lactation, and ceased when the F1 generation was randomly selected (54/sex/group; 90/sex/controls). F1 rats were treated similarly to F0 for up to 110-115 weeks (females and males, respectively) (dye composition: dye content 89.5%; volatile matter 4.7%; NaCl 4.7%; Na_2SO_4 1.7%). The overall appearance of exposed animals was normal, apart from coloration of the fur, urine and faeces. High-dose animals of both generations had slightly decreased body weights, despite a small increase in food intake. Water intake was also increased in high-dose animals, accompanied by a tendency to excrete larger volumes of urine (the ability of the kidney to concentrate urine under condition of dehydration was not impaired). Increases in caecum weights were also noted in high-dose animals. Haematological analysis throughout (20/sex/group), and at the end of the study (survivors) revealed no consistent differences. Periodical examination of kidney function (20/sex/group) did not reveal any treatment-related changes. At the end of the study, serum-glucose concentrations were lowered in the high-dose groups of both sexes and mid-dose females (not firmly related to treatment due to absence of any associated findings).

Histological examination revealed some changes that showed a positive dose-related trend together with a significantly higher incidence when compared to controls. These changes consisted of papillary hyperplasia of the bladder in high-dose males, which was considered treatment-related and increased occurrence of blood/fibrin cysts and intimal hyperplasia/medial hypertrophy of the pancreatic blood vessels among high-dose females. With regard to the occurrence of blood/fibrin cysts, the researchers commented that the normal occurrence is approximately 25% in examined females. Furthermore, it was concluded that although the observed effects are undeniably an effect of treatment, the absence of parallel findings in the opposite sexes reduces the strength of this association.

The incidence of a range of individual tumour types and the summary of total malignant and benign tumours among F1 animals was given. The incidence of adrenal phaeochromocytoma (7.5%) was significantly increased in high-dose males. The researchers however remark that this is a relatively common and variable tumour, and the incidence is comparable or even lower than control incidences observed in three parallel studies (Stevenson *et al.*, 1982; published in the peer reviewed literature as Ford *et al*., 1987).

Overall, it is concluded that only in this study by Stevenson *et al.* (1982; published in the peer reviewed literature as Ford *et al*., 1987), were some effects noted that are attributable to Azorubine/Carmoisine. In rats, the compound was found to alter body weights and caecum weights, and to lower serum-glucose concentrations. Although unclear from the study description, it appears that these effects were seen in both generations. Histopathological examination revealed that in high dose male rats Azorubine/Carmoisine induced bladder hyperplasia. In females, blood/fibrin cysts and increased intimal hyperplasia/medial hypertrophy of the pancreatic blood vessels were noted. The researchers commented that although the observed changes were not severe, treatment-relatedness was undeniable, and consequently, a non-untoward-effect level of 400 mg/kg bw/day was established. Conceivably, this study was used by the SCF and JECFA for determination of the current ADI.

No new data on reproductive and developmental toxicity were available.

3.2.6. Hypersensitivity

In experiments on guinea-pigs, it was found that Azorubine/Carmoisine had no sensitising activity (no further details) (Bär and Griepentrog, 1960).

In the TemaNord evaluation (2002), an additional study is mentioned, but this appears to be a study on hyperactivity rather than hypersensitivity and therefore this study is discussed in the next section.

Worm *et al.* (2000) conducted a study in which a small sub-group responded to oral provocation with a mixture of food additives including Azorubine/Carmoisine. However, as the mixture contained 23 different food additives, no discrimination between the different pseudo-allergens can be made.

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% have been reported (Young *et al.,* 1987; Hannuksela and Haahtela, 1987; Fuglsang, 1993, 1994) Adverse reactions after Azorubine/Carmoisine intake, mostly taken within mixtures of other synthetic colours, have been reported for vasculitic and urticarial reactions (Lowry *et al.,* 1994; Mikkelsen *et al.,* 1978). Reports are often characterised 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

TemaNord mentions two studies that have focussed on the effect of synthetic food colourings on childhood behaviour.

In a study by Rowe (1988), eight children whose parents claimed that a particular cluster of behaviours was associated with the ingestion of foods containing synthetic colourings, were included in a double-blind crossover study, employing a single-subject repeated measures design. Subjects were maintained on a diet free from synthetic additives, and were challenged daily for 18 weeks with either placebo or 50 mg of either Tartrazine or Azorubine/Carmoisine, each for two separate weeks. Two significant reactors were identified, whose behavioural pattern featured extreme irritability, restlessness and sleep disturbance.

Pollock and Warner (1990) conducted a study on, among others, behavioural effects in children exposed to Azorubine/Carmoisine and other food colours.

They performed an objective evaluation of 39 children whose behaviour was observed by their parents to improve on an artificial food additive-free diet and to deteriorate with dietary lapses. Nineteen children completed a double-blind placebo controlled challenge study with a combination of synthetic food colours: Azorubine/Carmoisine (25 mg), Tartrazine (50 mg), Sunset Yellow FCF (25 mg), and Amaranth (25 mg). These colours were shown to have an adverse effect on a daily Conners' rating of behaviour, although most parents could not detect these changes.

Carter *et al.* (1993) conducted a double-blind placebo controlled food challenge (DBPCFC) study in 19 children with hyperactive behaviour who were identified in an open-challenge study before being committed to the DBPCFC study protocol. Artificial colours were given in capsules rendered opaque with iron oxide. Each capsule contained 6-5 mg mixed colours (1 mg Tartrazine, 1 mg Sunset Yellow FCF, 1 mg Quinoline Yellow, 0.5 mg Azorubine/Carmoisine, 0 5 mg Brilliant Blue, 0 5 mg Erythrosine, 0 5 mg Green S, 0-5 mg Indigo Carmine, 0-5 mg Amaranth). The results of a crossover trial showed a significant effect for the provoking colours to worsen ratings of behaviour and to impair psychological test performance.

A study by McCann *et al*. (2007) concluded that exposure to two mixtures of four synthetic colours plus the preservative sodium benzoate in the diet, results 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 study, the effects of two combinations of Tartrazine, Quinoline Yellow, Sunset Yellow FCF, Ponceau 4R, Allura Red AC, Azorubine/Carmoisine and sodium benzoate 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, classroom 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, Azorubine/Carmoisine and sodium benzoate. Mix B contained Azorubine/Carmoisine, Sunset Yellow, Quinoline Yellow, Allura Red AC 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 the group of 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 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).

The effect of Azorubine/Carmoisine and naphthionic acid (a metabolite of Azorubine/Carmoisine) on both true and pseudo-cholinesterases (ChEs) was investigated *in vitro* (human plasma and erythrocytes) and *in vivo* (rat feeding study) (Osman *et al.,* 2004). The results of the *in vitro* study indicate that Azorubine/Carmoisine and naphthionic acid inhibit both human true and pseudo-ChE activities. Based on IC_{50} and Ki values, naphthionic acid produces greater inhibition of pseudo-ChE than Azorubine/Carmoisine. Inhibition by Azorubine/Carmoisine is non-competitive, whereas naphthionic acid produces competitive inhibition kinetic with plasma-ChE only. The inhibition is abolished by dialysis, indicating that the effects are reversible. The *in vivo* effect on rats fed on a diet supplemented with Azorubine/Carmoisine (4 mg/kg bw/day) resulted in a significant decrease in both true and pseudo-ChE activity. Naphthionic acid only inhibited pseudo-ChE activity (Osman *et al.*, 2004). However, it appears that the role of acetylcholine (ACh) in hyperactivity has not yet been elucidated (on the contrary, ChE inhibitors have been studied clinically to alleviate ADHD in children), and therefore the contribution of altered plasma-ChE levels to the behavioural changes noted cannot be decisively concluded upon. Furthermore, inhibition of plasma-ChE is considered of limited toxicological relevance.

4. Discussion

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

Azorubine/Carmoisine (E 122) is an azo dye allowed as a food additive in the EU and has been previously evaluated by JECFA in 1983 and the SCF in 1984. Both committees established an ADI of 0-4 mg/kg bw/day.

Specifications have been defined in Directive 2008/128/EC and by JECFA (2004). 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), ≤ 2% subsidiary colouring matters and ≤ 0.5% 4-aminonaphthalene-1-sulphonic acid and 4-hydroxynaphthalene-1-sulphonic acid. 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.

The ADI as defined by JECFA and the SCF was probably based on a NOAEL of 400 mg/kg bw/day derived from a rat developmental study by Stevenson *et al.* (1982) (published in the peer reviewed literature as Ford *et al*. 1987) and a mouse study by Mason *et al.* (1974). Stevenson *et al*. (1982) described a study in which body and caecum weights were altered, serum-glucose concentrations were lowered, bladder hyperplasia was seen in males, and blood/fibrin cysts and increased intimal hyperplasia/medial hypertrophy of the pancreatic blood vessels was noted in females. A mouse study by Mason *et al.* (1974), in which at the highest administered dose Azorubine/Carmoisine induced a significant decrease of haemoglobin levels and a decreased packed cell volume, and in which a NOAEL of 375 mg/kg bw/day was determined, may have been used by the SCF and JECFA, in addition to the rat developmental study, for calculation of the current ADI.

In the rat study by Gaunt *et al.* (1967), a NOAEL of 250 mg/kg bw/day was determined based on elevated renal weight at 500 mg/kg bw/day (highest dose level). Use of this NOAEL for the calculation of the ADI would lead to a lowering of the current ADI. As however, no untoward pathology was noted, and in none of the other studies conducted with Azorubine/Carmoisine any effects on kidneys or renal function were observed, this NOAEL has not been taken into consideration by JECFA and the SCF. The ANS Panel agrees that the increased renal weights can be considered toxicologically insignificant.

The Panel concurs with the view expressed in previous evaluations (JECFA, 1983a,b; TemaNord 2002) that the absorption of Azorubine/Carmoisine is limited, but that after reduction in the gastrointestinal tract, free sulphonated aromatic amines may reach the systemic circulation.

The SCF (1984), JECFA (1983a,b) and TemaNord (2002) evaluations concluded, based on *in vivo* and *in vitro* studies available at that time, that Azorubine/Carmoisine did not show any genotoxic activity.

In a more recent study, Zaharia and Pavel (2003) tested four colourings (Tartrazine, Azorubine/Carmoisine, Patent Blue and Acid Green 50) on the frequency of divisional cells, mitotic index, mutagenic process, and the probability of these synthetic colourings to induce chromosomal modifications. Using cytogenetic analysis, the researchers claim to have proven that important alterations in the morphology of somatic chromosomes occur in *Secale cereale* (= rye) in the presence of Azorubine/Carmoisine. The Panel notes that this is not a standard genotoxicity assay, and concludes, given that all other genotoxicity tests were negative, and that Azorubine/Carmoisine does not contain a structural alert, that based on the data available there is no concern with respect to genotoxicity.

The conversion of Azorubine/Carmoisine by azo reduction *in vivo*, results in the formation of sulphonated naphthylamines that may not be formed in standard *in vitro* genotoxicity tests. 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 both the sulphonated aromatic amine metabolites, (i.e. naphthionic acid and 2-amino-1-naphtol-4-sulphonic acid) that could, in theory, be formed by azo reduction of Azorubine/Carmoisine, were included in the study, the Panel concludes that the data reviewed by Jung *et al.* (1992) are sufficient to support the conclusion that the sulphonated aromatic amines, formed from Azorubine/Carmoisine by azo reduction, do not give reason for concern with respect to genotoxicity.

The Panel concluded that based on the data available there is no concern with respect to genotoxicity of Azorubine/Carmoisine.

Furthermore, the Panel notes that the specifications on the purity of Azorubine/Carmoisine would allow concentrations of unidentified unsulphonated aromatic amines to be present in concentrations of up to 100 mg/kg Azorubine/Carmoisine. Given the maximal allowed concentration of Azorubine/Carmoisine 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 Azorubine/Carmoisine was negative in *in vitro* genotoxicity as well as in long-term carcinogenicity studies.

There are no indications that Azorubine/Carmoisine induces tumour formation. Various non-neoplastic lesions have been observed after feeding of Azorubine/Carmoisine to mice and rats, but these findings have in the past been disregarded mainly based on the fact that high incidences were also seen in historical controls. In the NTP (1982) studies on carcinogenicity in rats and mice, histopathological examination indicated that Azorubine/Carmoisine was not associated with the incidence of any tumour type. The Panel notes that the adrenal histopathological changes reported are rather common in ageing laboratory rats and, moreover, these lesions generally vary considerably between different studies. Therefore, the Panel concluded that these adrenal histopathological changes are not toxicologically relevant, which is in accordance with the previous evaluation of these data by JECFA and the SCF.

Based on the same dataset for long-term toxicity/carcinogenicity, previous evaluations by SCF, JECFA and the authors of the TemaNord report also concluded that there was no evidence of carcinogenicity for Azorubine/Carmoisine (SCF, 1984; JECFA, 1983a, 1983b; TemaNord, 2002). The ANS Panel agrees with this conclusion.

A study by McCann *et al*. (2007) has concluded that exposure to two mixtures of four synthetic colours plus a sodium benzoate preservative in the diet, both of them, Mix A and Mix B, containing Azorubine/Carmoisine, result in increased hyperactivity in 3- 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 (including Azorubine/Carmoisine) and sodium benzoate in 3-year old children from the Isle of Wight (Bateman *et al.*, 2004).

Recently, EFSA published an opinion (EFSA, 2008a) on this McCann *et al*. study (McCann *et al*., 2007). 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 the activity and attention in some children selected from the general population, despite the effects not being observed for all children in all age groups and not being 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 (EFSA, 2008a).

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 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 ANS Panel concurs with these conclusions.

Overall, the Panel concludes that the present database on genotoxic, semi-chronic, reproductive, developmental and long-term toxicity, and carcinogenicity as well as the McCann *et al.* study (McCann *et al*., 2007) do not give reason to revise the ADI of 4 mg/kg bw/day.

Adverse reactions after Azorubine/Carmoisine intake, mostly taken within mixtures of other synthetic colours, have been reported for urticarial and vasculitic reactions. Reports are often characterised by poorly controlled challenge procedures and 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 Azorubine/Carmoisine intake have been reported, mostly when Azorubine/Carmoisine is taken within mixtures of other synthetic colours, no conclusion on the induction of sensitivity by Azorubine/Carmoisine 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 the Third Tiers (Annex A). The dietary exposure to Azorubine/Carmoisine 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 both for adults and for a typical 3 year-old child.

Refined exposure estimates have been performed for both 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 Azorubine/Carmoisine listed in Table 3, as identified by the Panel from the data by the FSA, FSAI, AFSSA, UNESDA, CEPS, ELC, CIAA (Tier 3).

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

When considering MPLs (Tier 2), the mean dietary exposure to Azorubine/Carmoisine for European children (aged 1-10 years), ranged from 0.3 mg/kg bw/day to 2.5 mg/kg bw/day, and from 0.7 mg/kg bw/day to 6.7 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 61%), desserts, including flavoured milk products (14 to 56%), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (16 to 68%). Fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) accounted for 11 to 29% of exposure in 5 countries and surimi accounted for 11% of exposure in one country.

Estimates reported for the UK adult population give a mean dietary exposure to Azorubine/Carmoisine of 0.5 mg/kg bw/day and of 1.1 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 (40%), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (14%) and fruit wines and cider and perry (13%).

When considering the maximum reported use levels from Table 3, the mean dietary exposure to Azorubine/Carmoisine for European children (aged 1-10 years), ranged from 0.25 mg/kg bw/day to 2.4 mg/kg bw/day and from 0.6 mg/kg bw/day to 6.5 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure (>10% in all countries) were soft drinks (17 to 65%), fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (10 to 34%), desserts, including flavoured milk products (15 to 42%) and sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (15 to 70%).

Estimates reported for the UK adult population give a mean dietary exposure of 0.4 mg/kg bw/day and of 1.0 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10%) were at average level soft drinks (50%), sauces and seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (17%).

The Panel further notes that the specifications of Azorubine/Carmoisine 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.

CONCLUSIONS

Azorubine/Carmoisine (E 122) is an azo dye allowed as a food additive in the EU and previously evaluated by JECFA in 1983 and the SCF in 1984. Both committees established an ADI of 0-4 mg/kg bw/day.

The Panel concludes that the present dataset does not give reason to revise the ADI of 4 mg/kg bw/day.

The Panel concludes that at the maximum reported levels of use of Azorubine/Carmoisine, refined intake estimates are below the ADI, although in 1- to 10-year old children the high percentile of exposure (95th) can be slightly higher than the ADI at the upper end of the range.

The Panel concludes that while some sensitivity reactions after Azorubine/Carmoisine intake have been reported, mostly when Azorubine/Carmoisine is taken within mixtures of other synthetic colours, no conclusion on the induction of sensitivity by Azorubine/Carmoisine 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 of Azorubine/Carmoisine 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) data in response to the European Commission request for data "EFSA re-evaluation of food colours"- Southampton colours (SANCO/E3/OS/KM D 5300722, May 2009).
4. 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 foodstuff, 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

ACh	Acetylcholine
ADHD	Attention Deficit Hypersensitivity Disorder
ADI	Acceptable Daily Intake
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.
AFC	Scientific Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
2-ANS	2-amino-1-naphthol-4-sulphonic acid
bw	body weight
CAS	Chemical Abstracts Service
CEPS	European Spirits Organisation
ChE	Cholinesterases
CIAA	Confederation of the Food and Drink Industries of the EU
DBPCFC	Double-blind placebo controlled food challenge study
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
FAO/WHO	Food and Agriculture Organization/World Health Organization
FSA	UK Food Standard Agency (FSA)
FSAI	Food Safety Authority of Ireland
GD	Gestation day
GHA	Global hyperactivity aggregate
HPLC	High Performance Liquid Chromatography
HPLC-DAD	High-performance liquid chromatography with diode-array detection
IC_{50}	The concentration required to inhibit 50% of enzyme activity
JECFA	Joint FAO/WHO Joint Expert Committee on Food Additives
Ki	Enzyme-inhibitor dissociation constant
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
1,2-NQS	1,2-naphthoquinone-4-sulphonate
NOAEL	No Observed Adverse Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
SCF	EU Scientific Committee for Food
SCOOP	A scientific cooperation (SCOOP) task involves coordination amongst Member States to provide pooled data from across the EU on particular issues of concern regarding food safety
UNESDA	Union of European Beverage Associations