SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of Ponceau 4R (E 124) 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 Ponceau 4R (E 124). Ponceau 4R 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. 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. Relevant new studies included a study by Tsuda *et al*. from 2001 reporting effects on nuclear DNA migration in the mouse *in vivo* Comet assay, a study by Tanaka from 2006 on neurobehavioural effects and a study by McCann *et al*. from 2007 that concluded that exposure to a mixture including Ponceau 4R resulted in increased hyperactivity in 3-year old children. The Panel notes that Ponceau 4R was negative in *in vitro* genotoxicity as well as in long term carcinogenicity studies and that the effects on nuclear DNA migration are not expected to result in carcinogenicity. 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 also re-evaluated a long-term mouse study reporting glomerulonephrosis from which they derived a No-Observed-Adverse-Effect Level of 70 mg/kg bw/day. Based on these findings the Panel derives an ADI of 0.7 mg/kg bw/day. The Panel concludes that at the maximum levels of use, intake estimates for adults at the high percentile (97.5th) and for 1- to 10-year old children at the mean and the high percentiles (95th/97.5th) are generally above the ADI even in the refined intake estimates.

KEY WORDS

Ponceau 4R, New Coccine, E 124, CAS 2611-82-7, Trisodium 2-hydroxy-1-(4-Sulphonato-1-naphthylazo)-naphtalene-6,8-disulphonate, food colouring substance, EINECS number: 220-036-2.

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SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety of Ponceau 4R (E 124) when used as a food colouring substance.

Ponceau 4R (E 124) is an azo dye allowed as a food additive in the EU that has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1983 and the EU Scientific Committee for Food (SCF) in 1984. Both committees established an Acceptable Daily Intake (ADI) of 0-4 mg/kg body weight (bw)/day.

Recent results indicated that in an *in vivo* Comet assay, Ponceau 4R induced significant increases in migration of nuclear DNA in glandular stomach, bladder and colon tissue, in the absence of general cytotoxicity in these tissues. The Panel considered in the light of negative carcinogenicity studies, that the biological significance of the Comet assay results is uncertain.

In contrast to this, all *Salmonella* genotoxicity tests with Ponceau 4R have been negative. Because the activation process of these azo dyes in animals is complex, *Salmonella* tests with S9 might not be suitable to detect mammalian genotoxicity.

The conversion of Ponceau 4R by azo reduction *in vivo* results in the formation of sulphonated naphthylamines that may not be formed in the standard *in vitro* genotoxicity tests. Previously, a range of sulphonated aromatic amines was shown to be in general not associated with genotoxicity *in vitro* and *in vivo*. However, not all the sulphonated aromatic amine metabolites that could in theory be formed by azo reduction of Ponceau 4R were included in the study.

The Panel also noted that the specifications on the purity of Ponceau 4R permit concentrations of unidentified unsulphonated aromatic amines to be present in concentrations of up to 100 mg/kg Ponceau 4R. Although some aromatic amines may be associated with genotoxicity or even carcinogenicity, the Panel noted that Ponceau 4R was negative in long term carcinogenicity studies.

Long-term carcinogenicity studies on Ponceau 4R were re-evaluated by the Panel. Several long-term carcinogenicity studies in rats at dose levels up to 1500 mg/kg bw/day, and in mice at dose levels up to 1790 mg/kg bw/day, revealed no evidence of carcinogenicity. This included the absence of neoplasms in the stomach or blood forming tissues, shown to be sensitive organs in the *in vivo* Comet assay in mice. Ponceau 4R induced significant dose-related DNA damage in mice in the glandular stomach and bladder at doses of 100 mg/kg bw and higher, and in the colon at doses of 10 mg/kg bw and higher. In bone marrow cells of male mice, clastogenic activity was noted at a minimum effective dose of 4 mg Ponceau 4R, equivalent to a dose of 80 mg/kg bw. However, carcinogenicity was not observed at dose levels several times higher, up to 1790 mg/kg bw/day for mice, and up to 1500 mg/kg bw/day in rats. The Panel noted that Ponceau 4R was negative in long term carcinogenicity studies and that the effects on nuclear DNA migration observed in the mouse *in vivo* Comet assay are not expected to result in carcinogenicity.

Based on the same dataset for long-term toxicity/carcinogenicity, previous evaluations by JECFA, the SCF and TemaNord also concluded that there was no evidence for carcinogenicity of Ponceau 4R.

A study by McCann *et al*. has concluded that upon exposure to two mixtures of four synthetic colours, plus the preservative sodium benzoate in the diet, one of them, Mix A (containing Ponceau 4R) resulted in increased hyperactivity in 3-year old, but not in 8- to 9-year old children in the general population. In 2008, EFSA also 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 Attention Deficit Hypersensitivity Disorder, 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.

A rat study by Tanaka, reported in 2006, concluded that the No Observed Adverse Effect Level (NOAEL) was presumed to be 0.12% in the diet (approximately 205 mg/kg bw/day) for maze learning by males in the F1 generation. The Panel notes that these neurobehavioural findings were not consistent among sexes and were especially observed because of reduced values in the control group.

The Panel noted that also two studies already available at the time JECFA and the SCF set the ADI, point at NOAEL values lower than 400 mg/kg bw/day. These include:

- a pig study from Gaunt *et al.* from 1969 reporting a NOAEL of 300 mg/kg bw/day based on a slight reduction in the number of erythrocytes at 900 mg/kg bw/day, and,

- the mouse study of Mason *et al.* from 1974 that concluded that the no-effect level, based on the findings of glomerulonephrosis at the 0.25 and 1.25% dietary levels, was 0.05%, equivalent to 70 mg/kg bw/day.

Overall, the Panel concluded that these findings do give reason for re-definition of the ADI. Based on the lowest NOAEL of 70 mg/kg bw/day from the long term mouse study and an uncertainty factor of 100, the Panel derives an ADI of 0.7 mg/kg bw/day.

The Panel concluded that while some sensitivity reactions after Ponceau 4R intake have been reported, mostly when Ponceau 4R is taken within mixtures of other synthetic colours, no conclusion on the induction of sensitivity by Ponceau 4R could be drawn from the limited scientific evidence available. The Panel also noted that sensitive individuals may react at dose levels within the ADI.

The dietary exposure to Ponceau 4R was estimated by the Panel based on the Maximum Permitted Levels (MPLs) of use, by applying the Budget method (Tier 1) with the assumptions described in the report of the Scientific Cooperation (SCOOP) Task 4.2. The Panel calculated a theoretical maximum daily exposure of 8.1 mg/kg bw/day both for adults and for a typical 3 year-old child.

Refined exposure estimates have been performed both forchildren 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 Directive 94/36/EC on food colours (Tier 2) and with the maximum reported use levels, as

identified by the Panel from the data made available 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, the Confederation of the Food and Drink Industries of the EU (Tier 3). For children (aged 1-10 years), estimates have been calculated for nine European countries (Belgium, France, the Netherlands, Spain, UK, Czech Republic, Italy, Finland, Germany). For the adult population, the Panel has selected the UK population as representative of the EU consumers for Ponceau 4R intake estimates.

When considering MPLs (Tier 2), the mean dietary exposure to Ponceau 4R for European children (aged 1-10 years) ranged from 0.3 to 2.5 mg/kg bw/day and from 0.6 to 6.7 mg/kg bw/day at the 95th percentile. Estimates reported for the UK adult population give a mean dietary exposure to Ponceau 4R 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 (Tier 3), the mean dietary exposure of European children (aged 1-10 years) ranged from 0.3 to 2.4 mg/kg bw/day and from 0.7 to 6.2 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 levels of use of Ponceau 4R, intake estimates for adults at the high percentile (97.5th) and for 1- to 10-year old children at the mean and the high percentiles (95th/97.5th) are generally above the ADI of 0.7 mg/kg bw/day even in the refined intake estimates (Tier 2 and Tier 3).

The Panel further notes that the specifications for Ponceau 4R 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 on Food (SCF) should be consulted before the adoption of provisions likely to affect public health, such as the drawing up of lists of additives and the conditions for their use. Accordingly, all food additives, prior to their authorization, have been evaluated for their safety by the SCF or by its successor the European Food Safety Authority (EFSA).

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

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

Food colours were among the first additives to be evaluated, therefore many of the evaluations are old. For some of these colours 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 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 Ponceau 4R (E 124) when used as a food colouring substance.

Ponceau 4R (E 124) 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. Identity of the substance

Ponceau 4R (E 124) is an azo dye with the formula $C_{20}H_{11}N_2Na_3O_{10}S_3$. It has a molecular weight of 604.48 and CAS Registry Number 2611-82-7. Its full chemical name is trisodium 2-hydroxy-1-(4-sulphonato-1-naphthylazo)-naphthalene-6,8-disulphonate. Its structural formula is:

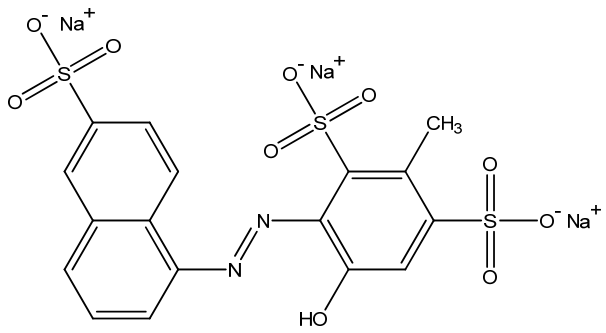

Figure 1. The structural formula of Ponceau 4R

At least 115 synonyms are in use (ChemIDplus advanced, via internet 2006). The most commonly used synonyms in published literature are Ponceau 4R, New Coccine Food Red 102 and Coccine red.

Ponceau 4R 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 in the JECFA (JECFA, 2006) (Table 1).

Ponceau 4R consists essentially of trisodium 2-hydroxy-1-(4-sulpho-1-naphthylazo)-2-naphthol-6,8-disulphonate and subsidiary colouring matters, together with sodium chloride and/or sodium sulphate as the principal uncoloured components. Ponceau 4R is described as the sodium salt. The calcium and potassium salts are also permitted (EC, 2008).

The purity is specified as not less than 80% of total colouring matters, calculated as the sodium salt. The remaining 20% may be accounted for by sodium chloride or sodium sulphate (but this is never mentioned explicitly) and ≤ 1% subsidiary colouring matters and 4-aminonaphthalene-1-sulphonic acid, 7-hydroxynaphthalene-1,3-disulphonic acid, 3-hydroxynaphthalene-2,7-disulphonic acid, 6-hydroxynaphthalene-2-sulphonic acid and 7-hydroxynaphthalene-1,3,6-trisulphonic acid, originating from the manufacturing process together < 0.5%.

Thus, if the existing specifications could be extended to include ≤ 20% sodium chloride and/or sodium sulphate, as the principal uncoloured components, 99.5% of the material would be accounted for.

Table 1. Specifications for Ponceau 4R 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	≤ 1%	≤ 1%
4-aminonaphtalene-1-sulphonic acid 7-hydroxynaphtalene-1,3-disulphonic acid 3-hydroxynaphtalene-2,7-disulphonic acid 6-hydroxynaphtalene-2-sulphonic acid 7-hydroxynaphtalene-1,3,6-trisulphonic acid	total ≤ 0.5%	total ≤ 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 Ponceau 4R permit concentrations of unsulphonated aromatic amines to be present in concentrations of up to 100 mg/kg Ponceau 4R. Given the maximal allowed concentration of Ponceau 4R that can be added to food (500 mg/kg food), the maximum concentration of these unidentified unsulphonated primary aromatic amines in food could be 50 μg/kg food.

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

According to EU legislation (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 (EC, 2008).

JECFA does not give specifications for aluminium lakes of Ponceau 4R other than reference to the General Specifications for Aluminium Lakes of Colouring Matters (JECFA, 2004). The Ponceau 4R used in the production process should comply with the specifications as given above, and the aluminium lake should contain not more than 2% water-soluble chlorides and sulphates calculated as sodium salts, not more than 0.5% HCL-insoluble matter, 0.2% ether-extractable matter, not more that 3 mg arsenic/kg and not more than 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 are required.

2.3. Manufacturing process

Ponceau 4R is manufactured by coupling diazotized naphthionic acid to G acid (2-naphthol-6,8-disulphonic acid) and converting the coupling product to the trisodium salt (HSDB, 2006). Ponceau 4R 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

Ponceau 4R can be quantified by differential pulse polaropgraphy (Combeau *et al.,* 2002) and - Performance Liquid Chromatography with diode-array detection (HPLC-DAD) (Minioti *et al.,* 2007).

Ponceau 4R can also be quantified in soft drinks by differential pulse polarography (Combeau *et al*., 2002), and HPLC-DAD methods described for water-soluble foods, like fruit flavoured drinks, alcoholic drinks, jams, sugar confectionery, chill-salt sweetening, baked green pea, iced black tea and sweets, upon dilution or water extraction (Minioti *et al.,* 2007; Vachirapatama *et al.,* 2008).

Ponceau 4R in ternary mixtures with Tartrazine and Sunset Yellow FCF, in commercial foods, can be detected by a first derivative spectrophotometric ratio spectrum-zero crossing method (Berzas Nevado *et al.,* 1998).

Simultaneous determination of Ponceau 4R and Sunset Yellow FCF, in gelatin powder can be done by derivative spectrophotometry and partial least-squares multivariate spectrophotometric calibration (Bozdogan *et al.,* 2000).

2.5. Reaction and fate in food, stability

No data were available in the published literature specifically on Ponceau 4R. However, in general, the majority of colour additives are unstable in combination with oxidising and reducing agents in food. Since colour depends on the existence of a conjugated unsaturated system within the dye molecule, any substance which modifies this system (e.g. oxidising or reducing agents, sugars, acids, and salts) may affect the colour (Scotter and Castle, 2004).

2.6. Case of need and proposed uses

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

Currently, Ponceau 4R (E 124) 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. Ponceau 4R is also allowed in beverages at levels up to 200 mg/L. Table 2 summarises those beverages and foodstuffs that are permitted to contain Ponceau 4R up to specified Maximum Permitted Levels (MPLs) set by EC legislation (EC, 1994).

Table 2. Maximum permitted use levels of Ponceau 4R in beverages and foodstuffs according to Council Directive 94/36/EC

Beverages	**Maximum Permitted Level** (mg/L)
Non-alcoholic flavoured drinks	50
Americano Bitter soda, bitter vino Liquid food supplements/dietary integrators	100
Spirituous beverages Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails Fruit wines, cider and perry	200
Foodstuffs	**Maximum Permitted Level** (mg/kg)
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 Jam, jellies and marmalades and other similar fruit preparations including low calorie products	100
Candied fruit and vegetables, Mostarda di frutta Preserves of red fruits	200

Extruded or expanded savoury snack products Sobrasada Chorizo sausage	
Pre-cooked crustaceans Salchichon	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

Ponceau 4R has been evaluated previously by JECFA in 1983 and the SCF in 1984. Both committees established an Acceptable Daily Intake (ADI) of 0-4 mg/kg bw.

2.8. Dietary exposure

2.8.1. Actual levels of use of Ponceau 4R

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). Ponceau 4R was found to be present at a level higher than 0.1 mg/L (Limit Of Detection - LOD) in 34 products, with levels varying from 1 to 47 mg/L. In another survey, conducted in 2005 by the Food Safety Authority of Ireland (FSAI), Ponceau 4R was found to be present at a level higher than 1.0 mg/L (Limit Of Quantification - LOQ) in eight out of 54 soft drinks; the concentration in these products ranged from 1 to 36 mg/L (FSAI, 2009). A usage survey conducted by the Union of European Beverage Associations (UNESDA) in 2005 suggests that the highest current use level of Ponceau 4R in beverages is 42 mg/L (Tennant, 2006). A more recent report from UNESDA in 2009, gives a range of use levels from 3 to 40 mg/L (UNESDA, 2009). A current use level of 40 mg/L has also been reported by the Confederation of the Food and Drink Industries of the EU (CIAA) (CIAA, 2009) and by French industries (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 for Ponceau 4R from 6.5 to 50 mg/L (ELC, 2009) .

For spirituous beverages, including products with less than 15% alcohol, in the survey conducted by the FSAI (2009) Ponceau 4R was found to be present in concentrations ranging from 1 to 90 mg/L, in14 retail samples. The European Spirits Organisation (CEPS) reported a range of use levels of Ponceau 4R from 0 to 170 mg/L (CEPS, 2009).

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

2.8.1.2. Foodstuffs

For confectionery products, the Panel was also provided with data from an *ad hoc* survey conducted by the FSA, in which artificial colours were analytically determined in 195 retail samples of brightly coloured packaged sweets, selected for being distinctly coloured (FSA, 2002). Ponceau 4R was found to be present at a level higher than 0.3 mg/kg (LOD) in 48 products, mainly fruit-flavoured, and in particular strawberry-flavoured, with levels varying from 0.3 to 56 mg/kg. According to the FSAI data, Ponceau 4R was present at a level higher than 1.0 mg/kg in 37 out of 183 confectionery products, with levels ranging from 1 to 35 mg/kg (FSAI, 2009). Data provided by French industries on Ponceau 4R in sweets showed use levels ranging from 5 to 40 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.

For decorations and coatings, data from the FSAI (2009) survey gave a range of analytical values of Ponceau 4R from 5 to 6.2 mg/kg for four retail samples; the CIAA reported a range of typical low and maximum use levels from 10 to 500 mg/kg.

For preserved red fruits, the FSAI survey (2009) gave a range of analytical values from 2 to 63 mg/kg for 10 retail samples, and the CIAA reported a range of typical low and maximum use levels from 17 to 200 mg/kg.

For edible ices, the FSAI survey (2009) gave analytical values of Ponceau 4R ranging from 1 to 11 mg/kg for 30 retail samples. No data on use levels in edible ices were provided by industry.

For flavoured processed cheese and edible cheese rind and edible casing, the CIAA reported a range of typical low and maximum use levels of 0.2 to 20 mg/kg.

For desserts, including flavoured milk products, the FSAI survey (2009) gave a range of analytical values from 1 to 49 mg/kg for 35 retail samples, and the CIAA reported a range of typical low and maximum use levels of Ponceau 4R from 1 to 50 mg/kg.

For sauces, seasonings, pickles, relishes, chutney and picalilli, the CIAA reported a range of typical low and maximum use levels from 1 to 450 mg/kg.

For fish paste and crustacean pastes, the CIAA reported a range of typical low and maximum use levels from 2 to 60 mg/kg.

For extruded or expanded savoury snack products, and savoury snack products and savoury coated nuts, the CIAA (2009) reported a range of typical low and maximum use levels of Ponceau 4R from 20 to 70 mg/kg.

For jams, jellies and marmalades, the FSAI survey gave a range of analytical values of Ponceau 4R from 2 to 64 mg/kg for five retail samples, and the CIAA reported a range of typical low and maximum use levels from 17 to 100 mg/kg.

Some other data provided mainly by the CIAA on the typical range of use levels, gave maximum use levels of Ponceau 4R according to the current legislation for candied fruit, vegetables, mostarda di frutta, preserves of red fruit, fine bakery wares, mustard and soups.

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 Ponceau 4R. 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 Ponceau 4R in beverages and foodstuffs used for the refined exposure assessment; they have been defined by applying the rules in Annex A to the data available to EFSA.

Table 3. Maximum reported use levels of Ponceau 4R in beverages and foodstuffs used for the refined exposure assessment

Beverages	**Maximum reported use levels (mg/L)**
Fruit wines, cider and perry	1
Non-alcoholic flavoured drinks	50
Spirituous beverages	170
Americano Bitter soda, bitter vino Liquid food supplements/dietary integrators	100
Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails	200
Foodstuffs	**Maximum reported use levels (mg/kg)**
Edible ices	11
Flavoured processed cheese Edible cheese rind and edible casings*	20
Confectionery Fine bakery wares 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
Fish paste and crustaceans paste	60
Savoury snack products and savoury coated nuts Extruded or expanded savoury snack products	70
Smoked fish Meat and fish analogues based on vegetable proteins Jam, jellies and marmalades and other similar fruit preparations including low calorie products	100
Candied fruit and vegetables, Mostarda di frutta Preserves of red fruits Sobrasada Chorizo sausage	200
Pre-cooked crustaceans Salchichon	250
Mustard Fish roe Solid food supplements/dietary integrators	300
Sauces, seasonings, pickles, relishes, chutney and piccalilli	450
Decorations and coatings Salmon substitutes Surimi	500

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

2.8.2. Exposure assessment

The Panel agreed to follow the principles of the stepwise approach which were used in the report of the Scientific Cooperation (SCOOP) Task 4.2 (EC, 1998), to estimate additives' intakes. For each successive Tier, this involved a further refinement of 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 (Tier 3).

2.8.2.1. Crude estimates (Budget method)

The dietary exposure to Ponceau 4R 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 Ponceau 4R, the maximum permitted use level considered for 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 Ponceau 4R 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 Ponceau 4R. 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 Ponceau 4R 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 Ponceau 4R. The overall theoretical maximum daily exposure to Ponceau 4R 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 Ponceau 4R may be used *quantum satis* in edible cheese rind and edible casings. As this is a very specific food category, which is unlikely to be consumed in high amounts on a daily basis, if at all, it was excluded from the Budget method calculation, since it is not expected to influence the outcome of this exposure calculation to any relevant extent.

2.8.2.2. Refined estimates

Refined exposure estimates have been performed for the Tier 2, using maximum permitted 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 for UK children (aged 1.5 - 4.5 years) were made by the Panel with the use of the detailed individual food consumption data (UK NDNS, 1992-1993) available from the UNESDA report (Tennant, 2006) and with the MPLs of use as specified in the Directive 94/36/EC on food colours from Table 2 (Tier 2 approach), and with the maximum reported use levels from Table 3 (Tier 3 approach).

Since the UK population is considered to be one of the highest consumers of soft drinks in Europe and as estimates were 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 Ponceau 4R intake estimates for adults.

Estimates of Ponceau 4R 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 as specified in Directive 94/36/EC (EC, 1994) for Tier 2 approach (Table 2), and with the maximum reported use levels for Tier 3 approach (Table 3).

Table 4 summarises the anticipated exposure of children and adults to Ponceau 4R.

In the case of Ponceau 4R, 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 to 2.5 mg/kg bw/day and from 0.7 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 (11 to 48%), desserts, including flavoured milk products (10 to 53%), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (13 to 73%). Fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) accounted for 10 to 29% of exposure in five countries. Jams, jellies and marmalade, and Chorizo sausage; Salchichon accounted for 11% and 13% of exposure in one country, respectively.

For UK children aged 1.5 to 4.5 years and weighing 15 kg, the mean dietary exposure 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%) were soft drinks (60%).

Estimates reported for the UK adult population give a mean dietary exposure to Ponceau 4R of 0.5 mg/kg bw/day and of 1.1 mg/kg bw/day for the high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10%) were soft drinks (40%), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (14%) and fruit wines and cider and perry (13%).

Further data suggest that current use levels of Ponceau 4R 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 Ponceau 4R (Tier 3).

When considering the maximum reported use levels from Table 3, the mean dietary exposure to Ponceau 4R for European children (aged 1 to 10 years old and weighing 25-30 kg) considered by the EXPOCHI consortium ranged from 0.3 to 2.4 mg/kg bw/day, and 0.7 to 6.2 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure (>10% in all countries) were soft drinks (11 to 50%), desserts, including flavoured milk products (10 to 53%), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (11 to 70%). Fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) accounted for 11 to 32% in five countries. Jams, jellies and marmalade, and Chorizo sausage; Salchichon, were contributing for 11% and 13% in one country, respectively.

For UK children, aged 1.5 to 4.5 years and weighing 15 kg, the mean dietary exposure was 1.3 mg/kg bw/day, and 3.3 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 (66%) and desserts, including flavoured milk products (10%).

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 soft drinks (52%), sauces and seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (16%).

Table 4 Summary of anticipated exposure to Ponceau 4R using the tiered approach (EC, 2001) in children and adult populations

	Adult UK population (>18 years old)	**Pre-school UK children** (1.5- 4.5 years old, 15 kg body weight)	**Children EXPOCHI population** (1-10 years old, 25-30 kg body weight)
	mg/kg bw/day		
Tier 1. Budget method	8.1	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	0.6 - 6.7
Tier 3. Maximum reported use levels			
• Mean exposure	0.4	1.3	0.3 - 2.4
• Exposure 95th* or 97.5th percentile**	1.0	3.3	0.7 - 6.2

* For EU children, estimates are based on the EXPOCHI report, which gives the 95th percentile intake.
** For UK, estimates are based on 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

Ponceau 4R has been previously evaluated by JECFA in 1983 and the SCF in 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

The JECFA evaluation of 1983 gives a brief understanding of the biochemical fate of Ponceau 4R. Single oral dose studies of uniformly ^{14}C-labeled Ponceau 4R of 0.5 or 50 mg/kg bw in rats, mice and

guinea-pigs show that substantially all of an orally administered dose of Ponceau 4R-related material (e.g. ^{14}C-label) is excreted in the urine, bile and faeces, with the majority being accounted for in the faeces (90%; 25-35% parent compound); metabolites are found in the urine (mainly naphthionic acid) and faeces (naphthionic acid and 7-hydroxy-8-aminonaphtalene-1,3-disulphonic acid); and finally, apart from some retention in foetuses, there is no marked accumulation in any tissue. Only some Ponceau 4R was absorbed by isolated intestinal loops (Phillips *et al.*, 1982).

In a study in which rats received an intravenous dose of Ponceau 4R, 30-45% of the dye was excreted unchanged in the bile within six hours (Ryan and Wright, 1961).

Furthermore, it was found that after intraperitoneal administration of the dye, the bile was coloured in mice and rats (Gaunt *et al.*, 1967).

Finally, a study by Walker (1968) indicates that Ponceau 4R is reduced *in vitro* by rat caecal contents.

The TemaNord report (2002) mentions a more recently conducted study on azo-reductase activity but does not give experimental details or results (Singh *et al.*, 1997).

Recently, Kuno and Mizutani (2005) have investigated the influence of Ponceau 4R on the activities of phase I and II drug-metabolizing enzymes (CYP2A6, UGT1A6, and UGT2B7). Their findings indicate that Ponceau 4R is neither substrate, nor inhibitor of the enzymes studied.

Overall, it can be concluded that Ponceau 4R is hardly absorbed, and that the major part of excreted material in the faeces is not the parent compound but products resulting from azo reduction. These products (naphthionic acid = 1-amino-naphtyl-4-sulphonic acid, and 7-hydroxy-8-aminonaphthalene-1,3-disulphonic acid) could also be demonstrated in the urine, pointing at absorption and systemic exposure to free sulphonated aromatic amines.

3.2. Toxicological data

3.2.1. Acute oral toxicity

The JECFA evaluation contains summary information on acute toxicity. After oral administration of Ponceau 4R to mice and rats (no specification if by gavage or diet), the LD_{50} values were 8000 mg/kg bw and > 8000 mg/kg bw, respectively (Gaunt *et al.*, 1967).

In addition, in four cats a negative Heinz bodies test was obtained after administration of a 5% aqueous solution of Ponceau 4R by stomach tube (no further details) (DFG, 1957).

The SCF concludes that no significant acute toxic effects have been demonstrated.

In a recent study, Sasaki *et al.* (2002) determined approximate LD_{50} values for several food additives, including Ponceau 4R. As in their simple acute toxicity experiments on 4-5 mice, no death was observed at 2000 mg/kg Ponceau 4R, the LD_{50} was defined as > 2000 mg/kg.

Altogether, it can be concluded that the acute oral toxicity of Ponceau 4R is low.

3.2.2. Short-term and subchronic toxicity

The JECFA evaluation describes two sub-chronic studies.

In a 90-day study, rats (16/group) treated with 0, 0.5, 1, or 2% Ponceau 4R in their feed (equivalent to 0, 250, 500 and 1000 mg/kg bw/day, respectively), slight (31-59%) but significant ($p < 0.05$) increases in aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) values, and a significant decrease in liver weight and haemoglobin concentrations were demonstrated at the highest dose level. No adverse effects were seen in appearance, behaviour, growth, food consumption, red blood cell counts, most organ weights, renal function, or gross pathology and histopathology (Gaunt *et al.*, 1967). The authors concluded that the NOAEL in this study was 1% Ponceau 4R in the diet, equivalent to 500 mg/kg bw/day.

In a 3-month study with pigs (3/sex/group) fed Ponceau 4R at doses of 0, 100, 300 and 900 mg/kg bw/day, there was report of a slight reduction in the number of erythrocytes in the highest dose group. No abnormalities were observed concerning growth, composition of urine and serum, organ weights or histopathology (Gaunt *et al.*, 1969). The Panel notes that this study indicates a NOAEL of 300 mg/kg bw/day, but that it was performed with only a limited number of animals.

3.2.3. Genotoxicity

JECFA refers to three studies on mutagenicity. These studies comprised two bacterial mutagenicity tests using *Escherichia coli* at a maximum concentration of 5000 mg/L (Lück and Rickerl, 1960), and a *Bacillus subtilis* "rec -assay" (Kada *et al*., 1972). Additionally, they describe a rat fetal hepatocyte assay determining cytotoxicity (Sako *et al*., 1980). Although no details on the outcome of these studies were presented, JECFA concluded that Ponceau 4R had no mutagenic potential.

The Panel notes that the "rec-assay" by Kada *et al*. (1972) is a bacterial DNA repair assay which has little predictive value for mutagenicity, whereas the study by Sako *et al*. (1980) is a cytotoxicity, rather than a genotoxicity test.

Several *in vitro* studies investigating the genotoxicity of Ponceau 4R are available and were reported by TemaNord (Izbirak *et al*., 1990; Cameron *et al*., 1987; Hayashi K *et al*., 1988; Ishidate *et al*., 1984; Kornbrust and Bartknecht, 1985).

Cameron *et al*. (1987) tested the mutagenicity of Ponceau 4R (Acid Red 18) in the *Salmonella typhimurium* assay with strains TA1535, TA1537, TA1538, TA98 and TA100 with and without S9, as well as in the mouse lymphoma TK+/- assay. Ponceau 4R was negative in all *Salmonella typhimurium* test strains at dose levels up to 10 mg/plate and reported to be negative in the mouse lymphoma assay (Cameron *et al*., 1987).

Ponceau 4R was also reported to be negative in the *Salmonella* plate test by others (Longstaff *et al*., 1984; Haveland-Smith and Combes, 1980; Izbirak *et al*., 1990), and for mitotic gene conversion in *Saccharomyces cerevisiae* (Sankaranarayanan and Murthy, 1979) as well as in *in vitro* and *in vivo*/*in vitro* rat hepatocyte primary culture DNA repair assays (Kornbrust and Bartknecht, 1985).

Ponceau 4R (New Coccine) was also reported to be negative in the dominant lethal test with the fresh water fish *Oryzias latipes* at dose levels of 200 mg/kg (Shimada and Egami, 1984).

Ishidate *et al.* (1984) tested the genotoxicity of Ponceau 4R (Food Red 102; new coccine) in the Ames test with *Salmonella typhimurium* strains TA92, TA1535, TA100, TA1537, TA94 and TA98 in the presence of S9, and in a chromosomal aberration test using Chinese hamster fibroblasts. Ponceau 4R was reported to be negative in all Ames tests at a maximal dose level of 5 mg/plate, and positive in the chromosomal aberration test with 13% incidence of cells with chromosomal aberrations at 24 hours and 12% at 48 hours.

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.

Ponceau 4R was reported to be negative in the mouse micronucleus assay (Hayashi M *et al.,* 1988).

In addition, TemaNord mentions an *in vivo* cytogenetic study in bone marrow cells of male mice in which clastogenic activity (chromosome aberrations) was noted at a minimum effective intraperitoneal dose of 4 mg/kg bw Ponceau 4R (Agarwal *et al*., 1993). Nonetheless, TemaNord concluded that no mutagenic potential was demonstrated for Ponceau 4R. The Panel noted that dosing in this study was not by the oral route and therefore considered this study of limited value since Ponceau 4R is poorly absorbed when dosed by the oral route.

Tsuda *et al.* (2001) have used an *in vivo* Comet assay to measure DNA damage after gavage feeding Ponceau 4R (coccine red) to groups of 4 male mice at doses of 0, 1, 10, 100 and 2000 mg/kg bw. Three hours after administration, Ponceau 4R induced significant increases in migration of nuclear DNA in the colon, bladder and glandular stomach. In the colon, significant differences between the treatment group and controls were observed at doses of 10 mg/kg bw and above. In the bladder and glandular stomach, significant differences were found at doses of 100 mg/kg bw and above. In the 2000 mg/kg bw dose group, nuclear DNA migration was also examined at 6 and 24 hours after administration. After 6 hours, a significant increase in the magnitude of migration of nuclear DNA was found in the bladder, whereas after 24 hours a significant increase in the magnitude of migration of nuclear DNA was found in the bladder, liver, kidney and lung. After both 6 and 24 hours, the magnitude of migration of nuclear DNA was no longer visible in the colon and glandular stomach. Necropsy and histopathological examination revealed no treatment-related effect on the colon, bladder and glandular stomach. The authors therefore conclude that the effect observed was not likely to be due to general cytotoxicity. The Panel considered that the indications provided by the study of Tsuda *et al*. (2001) should not be disregarded.

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

Azo reduction of Ponceau 4R may produce sulphonated aromatic amines. Jung *et al*. (1992) have reviewed the genotoxicity of a range of sulphonated aromatic amines including naphthionic acid. 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 are non-mutagenic to *Salmonella* in Ames tests. For some sulphonated aromatic amines no genotoxicity was also demonstrated with a variety of other test systems *in vitro* and *in vivo* (no details given). Based on the available data, the authors concluded that sulphonated aromatic amines, in contrast to their unsulphonated analogues, have no or very low genotoxic potential. Hence, the authors concluded that exposure to sulphonated aromatic amines, derived from metabolic cleavage or present as contaminants in colourings is unlikely to induce any significant genotoxic risk.

3.2.4. Chronic toxicity and carcinogenicity

The JECFA evaluates a total of eight studies on this subject. The Panel noted that these studies were performed before OECD guidelines and Good Laboratory Practice were established.

In a long-term study in mice (30/sex/group), the test animals were fed diets containing 0, 0.01, 0.05, 0.25 and 1.25% Ponceau 4R for 82 weeks (equivalent to 0, 14, 70, 357 and 1790 mg/kg bw/day) (Mason *et al.*, 1974). The colouring was converted to a yellow metabolite in the gastrointestinal tract, but no effect was observed on mortality, body weight gain or organ weight. It was found that at the two highest dose levels (0.25 and 1.25% in the diet) mice suffered mild anaemia (in the form of either reduced erythrocyte counts or haemoglobin concentrations) in the first six months of the study, although this was statistically significant ($p < 0.01$) only at the highest dose level. There was an increased incidence of foamy reticulo-endothelial cells in the liver at the 1.25% level, and glomerulonephrosis at the 0.25 and 1.25% levels. Granulosa-cell tumours of the ovary were found only in treated mice (one at each of the two lower doses, two at 0.25% and four at 1.25%), but the authors stated that the incidence was not statistically significant, either in any single group or when all treated females were compared to controls. Moreover, in controls from another study, three such tumours were found in 42 mice (Grasso *et al.,* 1974). It was therefore considered unlikely by BIBRA that this finding was related to Ponceau 4R administration. Other tumours, not found in controls, included a single interstitial cell tumour in the testis of a single mouse fed 1.25% Ponceau 4R, which was again similar to the 1/46 incidence in another control group (Brantom *et al.,* 1973). It was concluded (BIBRA, 1982a; BIBRA, 1982b) that Ponceau 4R was not carcinogenic at levels up to 1.25% in mice and that the no-effect level, based on the findings of glomerulonephrosis, was 0.05%, equivalent to 70 mg/kg bw/day. The Panel agrees with this conclusion.

JECFA considered the increased incidence of foamy reticulo-endothelial cells and glomerulonephrosis observed in the study by Mason *et al.* (1974) to be adverse effects and used these findings as the base for setting the ADI. The JECFA evaluation indicates that the 'no-untoward-effect level' was 0.05% in the diet. In spite of this, JECFA concluded that the level causing no toxicological effect was the 0.25% dietary level, calculated by JECFA to be equivalent to 375 mg/kg bw/day and leading to an ADI of 0-4 mg/kg bw/day.

The Panel considers the level of 0.05% in the diet, equivalent to 70 mg/kg bw/day, as the NOAEL of the study based on the occurrence of glomerulonephrosis at the two higher dose levels.

In another study, 10 rats/sex/group were given diets containing 0, 0.03, 0.3 and 3% (equivalent to 0, 15, 150, and 1500 mg/kg bw/day) of the colour for 64 weeks. Females at the highest level had lower food consumption and a decrease in body weight. Females also displayed increases of relative weights of heart, liver and kidney. No effects were found on histopathology and haemoglobin levels (Allmark *et al.,* 1957).

In another study, 66 rats/sex/group were given diets to provide up to 1250 mg/kg bw/day for a maximum treatment period of 118 weeks. No increase in tumour incidence was observed (BIBRA unpublished report by Stevenson *et al.*, 1981; data published by Brantom *et al.,* 1987b).

The Deutsche Forschungsgemeinschaft (DFG) has conducted a set of four oral carcinogenicity studies in rats:

In the first study, 10 rats were fed 0.2% Ponceau 4R in their diet (equivalent to 100 mg/kg bw/day) for 417 days and observed for 1011 days. No tumours were found (DFG, 1957).

In a similar study, 11 rats received a 1% solution of the dye in the drinking water (equivalent to a dose of 1000 mg/kg bw/day) for 216 days and were observed for 791 days. One rat developed a sarcoma in the liver. No comments were given on the significance of this finding (DFG, 1957).

In an experiment with 75 rats fed 0.1% Ponceau 4R in the diet (50 mg/kg bw/day) (no detail on period of exposure or observation), no tumours were observed (DFG, 1957).

After providing 10 rats with a diet of 0.2% of the colour (100 mg/kg bw/day) in their feed during life-span, no signs of carcinogenic potential were observed (DFG, 1957).

In addition, a carcinogenic study was conducted after subcutaneous injection of 13 rats with 0.5 mL of a 1% solution Ponceau 4R for 365 days and a 857-days observation period. No tumours were found (DFG, 1957).

No new literature on Ponceau 4R induced-long-term toxicity was published since these previous evaluations.

Overall, although some of these studies are limited, the available carcinogenicity studies with Ponceau 4R do not show any carcinogenic effect.

3.2.5. Reproductive and developmental toxicity

JECFA describes one study specifically referred to as a reproductive study. In this study, rats (36/sex) received doses of 0, 50, 500, or 1250 mg Ponceau 4R/kg bw/day over three generations (BIBRA unpublished report by Stevenson *et al.*, 1980; data published by Brantom *et al.* in 1987a). Except for the F0 generation, the animals were also exposed throughout gestation and lactation. Observed differences compared to controls were a pink coloration of the fur, softer more yellow-coloured faeces at the two higher doses, enlarged caeca and decreased liver weights at the two higher dose levels, and a slightly more advanced development of the skeleton in all the dose groups.

No anomalies were observed concerning body weight or food and water intake during the three pre-mating periods, pre- and post implantation losses, or the weight and appearance of the foetuses, and developmental parameters (survival, body weight, and histological examination). Based on this study, the authors concluded that Ponceau 4R lacks any adverse reproductive or developmental effects up to a dose of 1250 mg/kg bw/day.

In addition, JECFA describes three studies under the heading of teratogenicity. In the first teratogenicity study, mice which were gavage-fed Ponceau 4R at doses of 0, 7.5, 30, or 100 mg/kg bw/day during gestational days 0 to 7 or 6 to 18 showed no treatment-related effects in terms of number of implantations, frequency of fetal death and resorptions, gross malformations, skeletal or internal malformations, and fetal weight (Larsson, 1975). The Panel notes that this indicates a NOAEL of at least 100 mg/kg bw/day, the highest dose tested.

The second teratogenicity study in which rats were fed doses of 0, 1000, 2000 and 4000 mg/kg bw/day by gavage during gestational days 1 to 20, no treatment-related teratogenic effects were observed with regard to the number of *corpora lutea*, the number of implantations and fetuses dead or alive, gross malformations, skeletal and internal malformations, and fetal weight (Meyer and Hansen, 1975). The Panel concludes that the NOAEL in this study is at least 4000 mg/kg bw/day, the highest dose tested.

In the third study, feeding of 0, 0.01, 0.1, or 1% Ponceau 4R through the diet (equivalent to 0, 5, 50, or 500 mg/kg bw/day) to rats throughout the gestational period caused no deleterious effects in terms of embryonic death and intra-uterine growth, gross skeletal and visceral abnormalities, body weight gain, postnatal skeletal development and external differentiation, and the histology of the kidneys (Kihara *et al.*, 1977).

Furthermore, JECFA describes a long-term study which considers some reproductive and developmental parameters (BIBRA unpublished report by Stevenson *et al.*, 1981; data published by Brantom *et al.* in 1987b).

In this study, groups of 66 animals of both sexes were fed a diet of 0, 50, 500 or 1250 mg/kg bw/day for 9 weeks. Each female was paired with a male of the same dose group, and treatment continued during gestation and lactation. The young were exposed until approximately 20% of the animals

survived. Apart from pink coloration of the fur, none of the observations on the parental animals, including fertility and pup rearing, could be related to treatment.

No changes were observed regarding survival, haematological examinations, serum and plasma analysis, renal concentrations, renal cell excretion, urinary pH, other organ weights, or histopathology.

Considering the young, a few differences compared to controls were observed. In both sexes the highest dose caused a slightly lower weight gain that was not due to lower food intake, and caecum weight was increased which was considered to be the cause of the observed soft and unformed faeces. In females the highest dose increased the incidence of high concentrations of proteins in the urine. Finally, in males, kidney weight was increased in a non-dose-related manner, and testes weights were increased at the two highest dose levels. The NOAEL was determined by the study authors to be 500 mg/kg bw/day.

In a recent study conducted by Tanaka (2006), groups of 20 mice (10/sex) received a diet containing 0, 0.12, 0.24 or 0.48% Ponceau 4R (equivalent to 212, 423 and 819 mg/kg bw/day; average of both sexes combined). Animals were fed from 5 weeks of age of the F0 generation to 9 weeks of age of the F1 generation, during which period selected reproductive and neurobehavioral parameters were measured. The animals from the F0 generation were 5 weeks of age at the start of the study. At 9 weeks of age, each female was paired with one male from the same treatment group for a period of 5 days. The males were removed from females after 5 days, and the females were allowed to carry their litters to term, deliver and rear all of their offsprings. In the F1 generation litter size, litter weight and sex ratio were measured at birth. The functional and behavioural developmental parameters were measured and scored for all individual offsprings during the lactation period in the F1 generation.

There was no adverse effect of Ponceau 4R on litter size, litter weight or sex ratio at birth. The average body weight of both male and female offsprings was significantly increased at the highest dose level at postnatal days 0, 4 and 21, but not at days 7 and 14. No adverse effects on litter size, litter weight, or sex ratio at birth were noted. With regard to neurobehaviour, there was a significant effect of Ponceau 4R on surface righting at postnatal day 4, and on water T-maze performance in males at 7 weeks. In multiple T-maze performance in the F1 generation, the time taken was significantly longer than the control in the mid-dose and high-dose groups in males, and those effects were reported to be significantly dose-related ($p < 0.01$). The authors concluded that the dose levels of Ponceau 4R tested produced no adverse effects on reproduction, and a few adverse effects on neurobehavioural parameters in mice. Tanaka (2006) concluded that the NOAEL was presumed to be 0.12% in the diet (approximately 205 mg/kg bw/day) for maze learning by males in the F1 generation.

The Panel notes that these neurobehavioural findings were not consistent among the sexes and were especially observed because of reduced values in the control group.

3.2.6. Hypersensitivity

In a guinea-pig test, Ponceau 4R was found not to have sensitisation potency (no details on experimental design) (Bär and Griepentrog, 1960).

In a series of 51 patients showing signs of general allergy, 16% reacted to an oral dose of Ponceau 4R. No evidence was presented to show that sensitisation occurred due to the colour rather than due to a cross-reaction with some other material (Mikkelsen *et al.*, 1978).

A patch test carried out on a group of 50 people diagnosed presumptive allergic contact dermatitis to colouring matters, failed to demonstrate sensitisation to Ponceau 4R (Rapaport, 1980).

Weber *et al.* (1979) investigated azo colour sensitivities in aspirin-sensitive asthmatics (ASA). Of 43 tested to the azo dyes (Mix No. l), four were positive on open challenge. All four were re-tested double-blind to the individual dyes, and in one patient was there a positive test. This patient underwent double-blind challenge with Ponceau 4R twice and reacted on both occasions.

Higher sensitisation rates have been described in a study of 25 patients experiencing allergic reactions after food intake, aged between 1.5 and 12.5 years (Ibero *et al.*, 1982). After a 48-hour avoidance of colours, patients were challenged. The challenge consisted of Tartrazine, Sunset yellow FCF, New Coccine (Ponceau 4R) and Erythrosine, and was positive for dyes in around 58% of patients.

Veien and Krogdhal (1991), in a case report, describe a 24-year old woman who responded with development of a leukoclastic vasculitis after a placebo controlled challenge with 50 mg of Ponceau 4R dye.

Lindemayer and Schmidt (1979) exposed 26 ASA-positive and 18 ASA-negative patients suffering from urticaria in a provocation test to eight different food additives (preservative and colouring matters). Altogether, 31 tests were exaluated and five patients responded to Ponceau Rouge (Ponceau Red) (abstract only available).

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 (Fuglsang, 1993, 1994) Adverse reactions after Ponceau 4R intake, mostly taken within mixtures of other synthetic colours, have been reported for urticarial and vasculitic reactions (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).

3.2.7. Other studies

Carter *et al*. (1993) showed in a double-blind placebo controlled challenge study in 19 food colouring-sensitive children with Attention-Deficit Hyperactivity Disorder (ADHD) (identified in an open challenge study) a significant deterioration of behaviour after intake of a mixture of dyes in capsule form. Each capsule contained 6.5 mg mixed colours (1 mg Tartrazine, 1 mg Sunset Yellow, 1 mg Quinoline Yellow, 0.5 mg Carmoisine, 0.5 mg Brilliant Blue, 0.5 mg Erythrosine, 0.5 mg Green S, 0.5 mg Indigo carmine, 0.5 mg Amaranth and 0.5 mg Ponceau 4R with glucose as filler).

The study by McCann *et al*. (2007) has concluded that exposure to two mixtures of four synthetic colours, plus the preservative sodium benzoate in the diet, result in increased hyperactivity in 3-years old and 8- to 9-years 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-years old children on the Isle of Wight (Bateman *et al.*, 2004). In the McCann *et al*. (2007) study, the effects of two combinations of Tartrazine (E 102), Quinoline Yellow (E 104), Sunset Yellow FCF (E 110), Ponceau 4R (E 124), Allura Red AC (E 129), Carmoisine (E 122) and sodium benzoate (E 211) on children's behaviour were studied.

The study involved 153 3-year old and 144 8- to 9-year old children. A Global Hyperactivity Aggregate (GHA) score was the main outcome of the study, and this parameter was based on aggregated z-scores of observed behaviours and ratings by teachers, 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, Carmoisine and sodium benzoate. Mix B in this study contained Allura Red AC, Sunset Yellow, Carmoisine, Quinoline Yellow, and sodium benzoate.

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

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.

Ponceau 4R (E 124) 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.

Specifications have been defined in the EU legislation (Directive 2008/128/EC) and by JECFA (JECFA, 2006). The purity is specified as not less than 80% of total colouring matters, calculated as the sodium salt. The remaining 20% may be accounted for by sodium chloride or sodium sulphate (but this is never mentioned explicitly), and ≤ 1% subsidiary colouring matters and 4-aminonaphthalene-1-sulphonic acid, 7-hydroxynaphthalene-1,3-disulphonic acid, 3-hydroxynaphhtalene-2,7-disulphonic acid, 6-hydroxynaphthalene-2-sulphonic acid and 7-hydroxynaphthalene-1,3,6-trisulphonic acid, originating from the manufacturing process together < 0.5%. Thus, if the existing specifications could be extended to include ≤ 20% sodium chloride and/or sodium sulphate, as the principal uncoloured components, 99.5% of the material would be accounted for.

The ADI as defined by JECFA was based on the results from a long-term study in mice which revealed increased incidence of foamy reticulo-endothelial cells in the liver at the 1.25% level (equivalent to 1790 mg/kg bw/day), and glomerulonephrosis at the 0.25 and 1.25% levels (equivalent to 375 and 1790 mg/kg bw/day) (Mason *et al.,* 1974). The JECFA evaluation indicates that the 'no-untoward-effect level' was 0.05% in the diet. In spite of this, the JECFA concluded that the level causing no toxicological effect was the 0.25% dietary level, calculated by JECFA to be equivalent to 375 mg/kg bw/day, and leading to an ADI of 0-4 mg/kg bw/day.

The SCF also established an ADI of 0-4 mg/kg bw/day based on the NOAEL in this long-term mouse study.

Several other subchronic, reproductive, developmental and long-term studies did not report increased incidence of foamy reticulo-endothelial cells in the liver or glomerulonephrosis and revealed NOAEL values that amount to respectively 500 mg/kg bw/day in rats (Gaunt *et al.,* 1967), 300 mg/kg bw/day (only 6 animals) in pigs (Gaunt *et al.*, 1969), 1250 mg/kg bw/day (highest dose tested) in rats (Stevenson *et al.,* 1980; data published by Brantom *et al.,* 1987a), 100 mg/kg bw/day (highest dose

tested) in mice (Larsson, 1975), 4000 mg/kg bw/day in rats (highest dose tested) (Meyer and Hansen, 1975), 500 mg/kg bw/day (highest dose tested) in rats (Kihara *et al.*, 1977), 500 mg/kg bw/day in rats (Brantom *et al.,* 1988), and 205 mg/kg bw/day in mice (Tanaka, 2006).

The Panel concurs with the view expressed in previous evaluations (JECFA, 1983, TemaNord, 2002) that the absorption of Ponceau 4R is limited, but that after reduction in the gastrointestinal tract, its metabolites in the form of free sulphonated aromatic amines may reach the systemic circulation.

The SCF, the JECFA and the TemaNord evaluations concluded, based on studies available at that time, that Ponceau 4R did not show any genotoxic activity.

Results obtained by Tsuda *et al.* (2001) suggest that in an *in vivo* Comet assay, Ponceau 4R induced significant increases in migration of nuclear DNA in glandular stomach, bladder and colon. Necropsy and histopathological examination revealed no treatment-related effects on the colon, bladder and glandular stomach, and the authors therefore concluded that the effect observed was not likely due to general cytotoxicity. The Panel considered in the light of negative carcinogenicity studies that the biological significance of the Comet assay results is uncertain.

The conversion of Ponceau 4R by azo reduction *in vivo*, results in the formation of sulphonated naphthylamines that may not be formed in the standard *in vitro* genotoxicity tests (Prival and Mitchell 1998. In a review by Jung *et al.* (1992), a range of sulphonated aromatic amines was shown in general not to be associated with genotoxicity *in vitro* and *in vivo*, in contrast to their unsulphonated analogues. Although not all the sulphonated aromatic amine metabolites that could in theory be formed by azo reduction of Ponceau 4R 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 Ponceau 4R do not give reason for concern with respect to genotoxicity.

Furthermore, the Panel notes that the specifications on the purity of Ponceau 4R permit concentrations of unidentified unsulphonated aromatic amines to be present in concentrations of up to 100 mg/kg Ponceau 4R. Given the maximal allowed concentration of Ponceau 4R that can be added to food (500 mg/kg food), the concentration of these amines in food are allowed to be 50 µg/kg food. Although some aromatic amines may be associated with genotoxicity or even carcinogenicity, the Panel notes that Ponceau 4R was negative in long term carcinogenicity studies.

Long-term carcinogenicity studies on Ponceau 4R were re-evaluated by the Panel. Several long-term carcinogenicity studies in rats at dose levels up to, respectively, 1250 mg/kg bw/day (Brantom *et al*., 1988), 50, 100 and 1000 mg/kg bw/day (DFG, 1957), and 1500 mg/kg bw/day (Allmarck *et al*., 1957), and in mice at dose levels up to 1790 mg/kg bw/day (Mason *et al*., 1974), revealed no evidence of carcinogenicity. This included the absence of neoplasms in the stomach, shown to be one of the most sensitive organs in the *in vivo* Comet assay in mice (Tsuda *et al*., 2001). Ponceau 4R induced significant dose-related DNA damage in mice in the glandular stomach and bladder at doses of 100 mg/kg bw and higher, and in the colon at dose of 10 mg/kg bw and higher (Tsuda *et al*., 2001). In bone marrow cells of male mice, clastogenic activity was noted at a minimum effective intraperitoneal dose of 4 mg/kg bw Ponceau 4R (Agarwal *et al*., 1993). The Panel noted that dosing in this study was not by the oral route and therefore considered this study of limited value because Ponceau 4R is poorly absorbed when dosed by the oral route. Furthermore, carcinogenicity was not observed at dose levels several times higher up to 1790 mg/kg bw/day for mice (Mason *et al.,* 1974) and up to 1000, 1250 and 1500 mg/kg bw/day in rats. Therefore, the Panel concludes that the DNA damage observed in the mouse *in vivo* Comet assay does not result in carcinogenicity.

Based on the same dataset for long-term toxicity/carcinogenicity, previous evaluations by SCF, JECFA and the TemaNord report also concluded that there was no evidence for carcinogenicity of Ponceau 4R (SCF, 1984; JECFA, 1983; ThemaNord, 2002).

A study by McCann *et al*. (2007) has concluded that upon exposure to two mixtures of four synthetic colours plus the preservative sodium benzoate in the diet, one of them, Mix A (containing Ponceau 4R) resulted in increased hyperactivity in 3-year old, but not in 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 Ponceau 4R) and sodium benzoate in 3-year old children on the Isle of Wight (Bateman *et al.,* 2004).

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

In its opinion (EFSA, 2008a), the AFC Panel concluded that the McCann *et al.* study provides limited evidence that the two different mixtures of synthetic colours and sodium benzoate tested had a small and statistically significant effect on activity and attention in some children selected from the general population, although the effects were not observed for all children in all age groups and were not consistent for the two mixtures. The AFC Panel also concluded that the findings might thus be relevant for specific individuals within the population, showing sensitivity to food additives in general, or to food colours in particular.

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

The AFC Panel also concluded that:

- since mixtures, and not individual additives, were tested in the study 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.

The rat study by Tanaka (2006) concluded that the NOAEL was presumed to be 0.12% in the diet (approximately 205 mg/kg bw/day) for maze learning by males in the F1 generation. The Panel noted that these neurobehavioural findings were not consistent among sexes and were especially observed because of reduced values in the control group.

The Panel noted that also two studies already available at the time JECFA and the SCF set the ADI, point at NOAEL values lower than 400 mg/kg bw/day. These include:

- the pig study (Gaunt *et al.* 1969) reporting a NOAEL of 300 mg/kg bw/day, based on a slight reduction in the number of erythrocytes at 900 mg/kg bw/day; and,

- the mouse study of Mason *et al. (*1974) that concluded that the no-effect level, based on the findings of glomerulonephrosis at the 0.25 and 1.25% dietary levels, was 0.05%, equivalent to 70 mg/kg bw/day.

The Panel concludes that overall these findings do give reason for re-definition of the ADI of 4 mg/kg bw/day. Based on the lowest NOAEL of 70 mg/kg bw/day from the long term mouse study and an uncertainty factor of 100, the Panel derives an ADI of 0.7 mg/kg bw/day.

Adverse reactions after Ponceau 4R intake, mostly taken within mixtures of other synthetic colours, have been reported, including 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 Ponceau 4R intake have been reported, mostly when Ponceau 4R is taken within mixtures of other synthetic colours, no conclusion on the induction of sensitivity by Ponceau 4R could be drawn from the limited scientific evidence available. The Panel also notes that sensitive individuals may react at dose levels within the ADI.

The exposure assessment approach goes from the conservative estimates that form the First Tier of screening, to progressively more realistic estimates that form the Second and Third Tier (Annex A). The dietary exposure to Ponceau 4R 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 both for children and the adult population according to the Tier 2 and Tier 3 approaches described in the SCOOP Task 4.2, which combines, respectively, detailed individual food consumption information from the population with the MPLs of use as specified in Directive 94/36/EC (Tier 2) and with the maximum reported use levels of Ponceau 4R listed in Table 3, as identified by the Panel from the data made available by the FSA, FSAI, AFSSA, UNESDA, CEPS, ELC, CIAA (Tier 3).

For children (1-10 years old), estimates have been calculated for nine European countries (Belgium, France, the Netherlands, Spain, UK, Czech Republic, Italy, Finland, Germany). For the adult population, the Panel has selected the UK population as representative of the EU consumers for Ponceau 4R intake estimates.

When considering MPLs (Tier 2), the mean dietary exposure to Ponceau 4R for European children (aged 1-10 years) ranged from 0.3 to 2.5 mg/kg bw/day and from 0.6 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 (11 to 60%), desserts, including flavoured milk products (10 to 53%) sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (13 to 73%). Fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) accounted for 10 to 29% in five countries. Jams, jellies and marmalade, and Chorizo sausage; Salchichon, accounted for 11% and 13%, respectively, in one country.

Estimates reported for the UK adult population give a mean dietary exposure to Ponceau 4R 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 Ponceau 4R for European children (aged 1-10 years) ranged from 0.3 to 2.4 mg/kg bw/day, and from 0.7 to 6.2 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure to Ponceau 4R (>10% in all countries) were soft drinks (11 to 66%), desserts (including flavoured milk products) (10 to 53%), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (11 to 70%). Fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) accounted for 11 to 32% of exposure in five countries. Jams, jellies and marmalade, and Chorizo sausage; Salchichon accounted for 11% and 13% of exposure, respectively, in one country.

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 soft drinks (52%) and sauces and seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (16%).

The Panel further notes that the specifications of Ponceau 4R 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 ≤ 2 mg/kg whereas the EC specification is ≤ 10 mg/kg.

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

CONCLUSIONS

Ponceau 4R (E 124) is an azo dye previously allowed to be used as a food additive in the EU and 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 database does give reason for re-definition of the ADI and derives an ADI of 0.7 mg/kg bw/day.

The Panel concludes that at the maximum levels of use of Ponceau 4R, intake estimates for adults at the high percentile (97.5th) and for 1- to 10-year old children at the mean and the high percentiles (95th/97.5th) are generally above the ADI of 0.7 mg/kg bw/day even in the refined intake estimates (Tier 2 and Tier 3).

The Panel concludes that while some sensitivity reactions after Ponceau 4R intake have been reported, mostly when Ponceau 4R is taken within mixtures of other synthetic colours, no conclusion on the induction of sensitivity by Ponceau 4R 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 Ponceau 4R 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 ≤ 2 mg/kg whereas the EC specification is ≤ 10 mg/kg.

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

DOCUMENTATION PROVIDED TO EFSA

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

2. CEPS (European Spirits Organisation), 2009. Letter sent to DG SANCO, dated 17 September 2009/GP.TS-006-2009.

3. CIAA (Confederation of the Food and Drink Industries of the EU), 2009. CIAA data in response to the Commission request for data "EFSA re-evaluation of food colours - Southampton study colours" (SANCO/E3/OS/km D 53007, May 22, 2009).
4. ELC (Federation of European Food Additives, Food Enzymes and Food Culture Industries), 2009. ELC comments to EFSA in response to a written request from DG Sanco "EFSA re-evaluation of food colours" – (DG Sanco's additional call for data dated 8 April 2009, letter to EFSA on 20 May 2009).
5. UNESDA (Union of European Beverage Associations), 2009. Comments to the CIAA/DG Sanco in response to a written request from DG Sanco to the CIAA, dated April 8 2009: 'Use of certain colour additives in non-alcoholic beverages' (May 26, 2009).

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ANNEX A

Rules defined by the Panel to deal with *quantum satis* (QS) authorisation, usage data or observed analytical data for all regulated colours to be re-evaluated (30 July 09) and intake estimates

1. Decision rules taken to deal with QS authorisations:

a. In the category 'All other 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

ADHD	Attention-Deficit Hyperactivity Disorder
ADI	Acceptable Daily Intake
AFC	Scientific Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials
AFSSA	Agence Française de Sécurité Sanitaire des Aliments
ALAT	Alanine Aminotransferase (GPT)
Aluminium lakes	Aluminium lakes are produced by the absorption of water soluble dyes onto a hydrated aluminium substrate rendering the colour insoluble in water. The end product is coloured either by dispersion of the lake into the product or by coating onto the surface of the product
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
ASA	Aspirin-sensitive asthmatics
ASAT	Aspartate Aminotransferase (GOT)
CAS	Chemical Abstracts Service
CEPS	European Spirits Organisation
CIAA	Confederation of the Food and Drink Industries of the EU
DG SANCO	The Directorate General for Health and Consumers
EC	European Commission
EFSA	European Food Safety Authority
ELC	The Federation of European Food Additives, Food Enzymes and Food Culture Industries
EXPOCHI	Refers to EFSA Article 36 2008 call for Proposals Focused on Children and Food Consumption
FAO/WHO	Food and Agriculture Organization/World Health Organization
FSA	UK Food Standard Agency
FSAI	Food Safety Authority of Ireland
GHA	Global Hyperactivity Aggregate
HPLC-DAD	High-Performance Liquid Chromatography with Diode-Array Detection
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	Limit of Detection
LOQ	Limit of Quantification
MPL	Maximum Permitted Levels
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
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