SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of Sunset Yellow FCF (E 110) 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 Sunset Yellow FCF (E 110). Sunset Yellow FCF has been previously evaluated by JECFA and the SCF. Both committees established an Acceptable Daily Intake (ADI) of 0-2.5 mg/kg bw/day. The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. New studies included studies by Mathur *et al.* reporting significant effects on the testes in rats exposed for 90 days to 250 and 1500 mg Sunset Yellow FCF/kg bw/day. The Panel notes that the Sunset Yellow FCF administered in these studies was obtained at the local market and that its specifications or purity were not defined. The Panel also notes that the 90 day rat study used by JECFA to derive the ADI also reported effects on testes weight, occurring without accompanying histological changes, although sperm morphology and sperm mobility were not analysed. The Panel concludes that these findings do give reason for re-definition of the ADI. The Panel decided to reduce the ADI, by an extra uncertainty factor of 2.5, to 1 mg/kg bw/day and to make the ADI temporary for 2 years. Within this period, clarification of the effects of Sunset Yellow FCF on the testis, sperm morphology and sperm mobility should be provided, based on a 28-day study performed according to the recently updated OECD test guideline 407. The Panel concludes that at the maximum reported levels of use of Sunset Yellow FCF, refined intake estimates are generally below the temporary ADI of 1 mg/kg bw/day, although in 1- to10-year old children the mean and the high percentiles of exposure (95^{th}/97.5^{th}) can be higher than this ADI, at the upper end of the range.

KEY WORDS

Sunset Yellow FCF, E 110, CAS 2783-94-0, Sunset Yellow, Food Yellow No. 5, and FD&C Yellow No. 6, Disodium 2-hydroxy-1-(4-sulphonatophenylazo)naphthalene-6-sulphonate, food colouring substance, EINECS number 220-491-7.

1 On request from the European Commission, Question No EFSA-Q-2008-224, adopted on 24 September 2009.

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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 provide a scientific opinion re-evaluating the safety of Sunset Yellow FCF (E 110) when used as a food colouring substance.

Sunset Yellow FCF (E 110) is an azo dye allowed as a food additive in the EU and previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1982 and the EU Scientific Committee for Food (SCF) in 1984. Both committees established an ADI of 0-2.5 mg/kg bw/day.

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

It is concluded that Sunset Yellow FCF is absorbed from the gastrointestinal tract to only a small extent and thus most of an orally administered dose is excreted via the faeces. As little of the colour was retrieved from the faeces as intact dye, Sunset Yellow FCF is likely to be broken down by intestinal azo-reduction. The urine also predominantly contains azo-reduction products (sulphanilic acid, 1-amino-2-naphtol-6-sulphonic acid, and the *N*-acetylated forms). Following this observation it is noted that systemic exposure to free sulphonated aromatic amines may occur.

The SCF, JECFA and TemaNord evaluations concluded, based on *in vivo* and *in vitro* studies available at that time, that Sunset Yellow FCF did not show any genotoxic activity.

In the Sasaki *et al.* 2002 publication, an *in vivo* Comet assay in mice was used to measure DNA damage in various tissues after gavage of Sunset Yellow FCF at a dose of 0, or 2000 mg/kg bw. At 3 hours and 24 hours after Sunset Yellow FCF administration no DNA damage was noted.

The Panel concluded that the potential genotoxicity of Sunset Yellow FCF has been thoroughly researched both *in vitro* and *in vivo*, and there are no indications of any genotoxic potential of Sunset Yellow FCF or its metabolites.

Eleven studies considering chronic toxicity and carcinogenicity of Sunset Yellow FCF were included in the JECFA 1982 evaluation. The later evaluations from the SCF and TemaNord do not present additional studies on long-term toxicity and no additional long-term studies were conducted since these previous evaluations were published. Altogether it was concluded by SCF, JECFA and the authors of the TemaNord report that there was no evidence for carcinogenicity of Sunset Yellow FCF.

A study by McCann *et al*. concluded that exposure in the diet to two mixtures of four synthetic colours plus the preservative sodium benzoate, Mix A and Mix B, both containing Sunset Yellow FCF, resulted in increased hyperactivity in 3-year old and 8- to 9-year old children in the general population. Recently, the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) published an opinion on this McCann *et al.* study.

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

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

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

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

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

Since the previous evaluations of Sunset Yellow FCF by JECFA in 1982 and the SCF in 1984 some new toxicity studies have been reported.

Mathur *et al.* reported results of a 90 day study on Sunset Yellow FCF in rats at dose levels equivalent to 250 and 1500 mg Sunset Yellow FCF/kg bw/day. There were significant effects on the testes for both dose groups and the Panel concluded that the lowest dose tested is a Lowest Observed Adverse Effect Level (LOAEL). In another paper, Mathur *et al.* reported significant and dose-related elevations in total lipid and various lipid fractions in rats exposed for 90 days to 250 mg and 1500 mg Sunset Yellow FCF/kg bw/day. These results also revealed a LOAEL of 250 mg/kg bw/day. A LOAEL of 250 mg/kg bw/day is lower than the No Observed Adverse Effect Level (NOAEL) of 500 mg/kg bw from the rat and dog study previously used by JECFA to derive the Acceptable Daily Intake (ADI). The Panel noted however that the Sunset Yellow FCF administered in the studies of Mathur *et al.* was obtained at the local market in India and that the specifications or purity of this preparation were not defined. The Panel also noted that the 90-day rat study reported by Gaunt and Gangolli and used by JECFA to derive the ADI also reported effects on testes weight. Although in the rat study reported by Gaunt and Gangolli the effects on testes weight were reported to occur without accompanying histological changes, the parameters investigated did not include sperm morphology and sperm mobility. The Panel concluded that all together these findings do give reason for re-definition of the ADI. In the light of the uncertainties the Panel decided to reduce the ADI for Sunset Yellow FCF, by an extra uncertainty factor of 2.5, to 1.0 mg/kg bw/day and make the ADI temporary for 2 years. Within this period clarification of the effects of Sunset Yellow FCF on the testis, sperm morphology and sperm mobility should be provided, based on a 28 day study performed according to the recently updated OECD test guideline 407, including characterisation of testes histopathology, sperm morphology and sperm mobility.

The Panel concluded that while some sensitivity reactions after Sunset Yellow FCF intake have been reported, mostly when Sunset Yellow FCF is taken within mixtures of other synthetic colours, no conclusion on the induction of sensitivity by Sunset Yellow FCF 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 Sunset Yellow FCF was estimated by the Panel based on the Maximum Permitted Levels (MPLs) of use, by applying the Budget method (Tier 1) with the assumptions described in the report of the 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 the children and adult population according to the Tier 2 and Tier 3 approaches described in the SCOOP Task 4.2., which combines, respectively, detailed individual food consumption information from the population with the MPLs of use as specified in the Directive 94/36/EC on food colours (Tier 2), and with the maximum reported use levels of Sunset Yellow FCF, 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 and the Confederation of the Food and Drink Industries of the EU (Tier 3). For the children population (aged 1-10 years), estimates have been calculated for nine European countries (Belgium, France, the Netherlands, Spain, UK, Czech Republic, Italy, Finland and Germany). For the adult population, the Panel has selected the UK population as representative of EU consumers for Sunset Yellow FCF intake estimates.

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

When considering the maximum reported use levels (Tier 3), the mean dietary exposure to Sunset Yellow FCF for European children (aged 1-10 years) ranged from 0.2 mg/kg bw/day to 2.1 mg/kg bw/day and from 0.6 mg/kg bw/day to 5.8 mg/kg bw/day at the 95th percentile. Estimates reported for the UK adult population give a mean dietary exposure to Sunset Yellow FCF of 0.3 mg/kg bw/day and of 0.9 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks.

The Panel concludes that at the maximum reported levels of use of Sunset Yellow FCF, refined (Tier 3) intake estimates are generally below the temporary ADI of 1 mg/kg bw/day. However, in 1- to 10-year old children the mean and the high percentile of exposure (95th/97.5th) can be 0.2 -2.1 and 0.6-5.8 mg/kg bw/day, respectively, and thus higher than the temporary ADI at the upper end of the range.

The Panel notes that the specifications of Sunset Yellow FCF need to be updated with respect to the level of identified sulphonated subsidiary dyes including the illegal dye Orange II, the level of Sudan I and 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 $\underline{\leq}$ 2 mg/kg whereas the EC specification is $\leq$ 10 mg/kg.

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

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

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

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

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

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

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

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

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

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of Sunset Yellow FCF (E 110) when used as a food colouring substance.

Sunset Yellow FCF (E 110) is an azo dye authorised for use as a food additive in the EU and previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1982 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

Sunset Yellow FCF (E 110) is an azo dye with the formula $C_{16}H_{10}N_2Na_2O_7S_2$. It has a molecular weight of 452.37 g/mol and CAS Registry Number 2783-94-0. Its full chemical name is disodium 2-hydroxy-1-(4-sulphonatophenylazo)naphthalene-6-sulphonate. Its structural formula is:

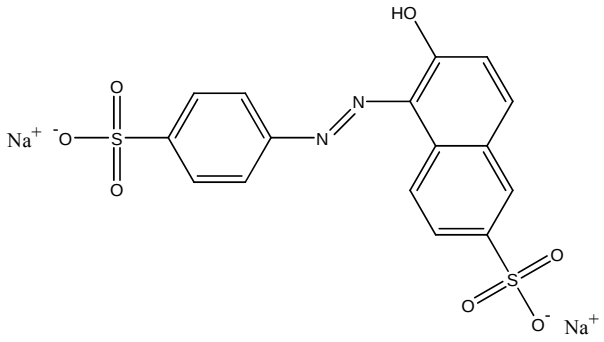

Figure 1. Structural formula for Sunset Yellow FCF

At least 90 synonyms for Sunset Yellow FCF are in use (ChemIDplus Advanced, via internet, 2006). The most commonly used synonyms in published literature are Sunset Yellow FCF, Food Yellow No. 5, and FD&C Yellow No. 6.

Sunset Yellow FCF is soluble in water and slightly soluble in ethanol (Merck, 2006).

2.2. Specifications

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

Sunset Yellow FCF consists essentially of disodium 2-hydroxy1-(4-sulphonatophenylazo) naphthalene-6-sulphonate and subsidiary colouring matters together with sodium chloride and/or sodium sulphate as the principal uncoloured components. Sunset Yellow FCF is described as the disodium salt, but the calcium and the potassium salt are also permitted (Directive 2008/128/EC).

The purity is specified as not less than 85% total colouring matters, calculated as the disodium salt. The remaining 15% may be accounted for by sodium chloride or sodium sulphate, but this is never mentioned explicitly, ≤ 5% subsidiary colouring matters and ≤ 0.5% 4-aminonaphthalene-1-sulphonic acid, 7-hydroxynaphthalene-1,3-disulphonic acid, 3-hydroxynaphthalene-2,7-disulphonic acid, 6-hydroxynaphtahlene-2-sulphonic acid, 4,4'-diazoaminodi(benzene sulphonic acid) and 6,6'-oxydi(naphthalene-1,3-disulphonic acid), originating from the manufacturing process.

Thus if the existing specifications could be extended to include ≤ 15.0% sodium chloride and/or sodium sulphate as the principal uncoloured components, 99.9% of the material would be accounted for.

Table 1. Specifications for Sunset Yellow FCF according to Commission Directive 2008/128/EC and JECFA (JECFA, 2006)

Purity	Commission Directive 2008/128/EC	JECFA (2006)
Water insoluble matter	≤ 0.2%	≤ 0.2%
Subsidiary colouring matters	≤ 5.0%	≤ 5.0% (of which ≤ 2% colours other than trisodium 2-hydroxy-1-(4-sulphonatophenylazo) naphthalene-3.6-disulphonate)
4-aminonaphtalene-1-sulphonic acid 7-hydroxynaphtalene-1,3-disulphonic acid 3-hydroxynaphtalene-2,7-disulphonic acid 6-hydroxynaphtalene-2-sulphonic acid 4,4'-diazoaminodi(benzene sulphonic acid) 6,6'-oxydi(naphthalene-1,3-disulphonic 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 natural 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 the presence of up to 5% subsidiary colouring matters.

Subsidiary colours have traditionally been divided into "lower sulphonated" and "higher sulphonated" versions. The Panel noted that the illegal dye Orange II (D&C Orange 4, CAS Registry Number 633-96-5, sodium salt of 4-[(2-hydroxy-1-naphthalenyl)azo]benzenesulphonic acid) is one of the normal lower sulphonated subsidiary dyes found in Sunset Yellow FCF. It has essentially the same basic chemical structure but contains one less sulphonate functional group in its structure. The other lower

sulphonated dye is the sodium salt of 6-hydroxy-5-(phenylazo)-2-napthalenesulfonic acid. As such both of these dyes are included in the 5% total subsidiary dyes limit in the EU. The maximum content of Orange II in Sunset Yellow FCF sold within the EU by the Food Additives and Ingredients Association (FAIA) member companies is 1%. Other specifications give more details for the subsidiary dyes where Orange II and other lower sulphonated subsidiary dyes are permitted at up to 2% according to the JECFA specifications; within the USA there is a limit of 1%.

The main subsidiary dye is referred to as a higher sulphonated subsidiary dye which has again the same basic chemical structure as Sunset Yellow FCF but containing three sulphonate functional groups (rather than two for Sunset Yellow FCF). It is known as trisodium salt of 3-hydroxy-4-[(4-sulfophenyl)azo]-2,7-naphthalenedisulphonic acid. This subsidiary dye is important because it improves the solubility of the colour in the presence of calcium ions. If there is a very low level of this subsidiary dye, the Sunset Yellow FCF will precipitate, for instance if it is added to hard water. This subsidiary dye may be present at levels exceeding 4%.

The other higher suphonated subsidiary dye is the trisodium salt of 3-hydroxy-4-[(4-sulfophenyl)azo]-5,7-napthalenedisulfonic acid, which is expected to be present in trace amounts typically <0.05%.

The Panel noted that JECFA also defined a maximum limit for Sudan I (1-(phenylazo)-2-napthalenol), of 1 mg/kg (JECFA, 2009). Sudan I is a known impurity in Sunset Yellow FCF and has been shown to be genotoxic and carcinogenic.

The Panel notes that the specifications on the purity of Sunset Yellow FCF would permit concentrations of unsulphonated aromatic amines to be present in concentrations of up to 100 mg/kg Sunset Yellow FCF. Given the maximal allowed concentration of Sunset Yellow FCF that can be added to food is 500 mg/kg food, the concentration of these unidentified unsulphonated primary aromatic amines could be 50 μg per kg food.

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

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

JECFA does not give specifications for aluminium lakes of Sunset Yellow FCF, other than referring to the General Specifications for Aluminium Lakes of Colouring Matters (JECFA, 2004). The Sunset Yellow FCF used in the production process should comply with the specifications as given above and the aluminium lake should contain not more than 2% water-soluble chlorides and sulphates calculated as sodium salts, not more than 0.5% hydrochloride acid-insoluble matter, not more than 0.2% ether-extractable matter, not more than 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 noted 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 (EFSA, 2008b) and that therefore specifications for the maximum level of aluminium in the lakes may be required.

2.3. Manufacturing process

Sunset Yellow FCF is manufactured by diazotizing 4-aminobenzenesulphonic acid using hydrochloric acid and sodium nitrite or sulphuric acid and sodium nitrite. The diazo compound is coupled with 6-

hydroxy-2-naphthalene-sulphonic acid. The dye is isolated as the sodium salt and dried (HSDB, website accessed 2006; no further information available). Sunset Yellow FCF may be converted to the corresponding aluminium lake under aqueous conditions by reacting aluminium oxide with the colouring matter. Undried aluminium oxide is usually freshly prepared by reacting aluminium sulphate or aluminium chloride with sodium carbonate or sodium bicarbonate or aqueous ammonia. Following lake formation, the product is filtered, washed with water and dried (JECFA, 2004).

2.4. Methods of analysis in food

Several methods for the determination of Sunset Yellow FCF in foods are described in the literature of which variations of High Pressure Liquid Chromatography (HPLC) appear to be most generally employed.

Sunset Yellow FCF can be quantified by HPLC-DAD methods described for water-based foods such as fruit flavoured drinks, alcoholic drinks, jams, sugar confectionery, chilly-salt sweetening, baked green pea, iced black tea and sweets upon dilution or water extraction (Minioti *et al.,* 2007; Vachirapatama *et al.,* 2008). Sunset Yellow FCF in soft drink powder can be detected by double divisor-ratio spectra derivative, inverse least squares and principal component regression methods (Dinç *et al.,* 2002) or capillary zone electrophoresis (Pérez-Urquiza and Beltrán, 2000).

Sunset Yellow FCF in binary mixtures with other synthetic food dyes can be detected by spectrophotometry with absorption onto polyurethane foam (Vidotti *et al*., 2005). Sunset Yellow FCF in ternary mixtures with Tartrazine and Ponceau 4R in commercial foods can be detected by a first derivative spectrophotometric ratio spectrum-zero crossing method (Berzas Nevado *et al*., 1998). Simultaneous determination of Sunset Yellow FCF and Ponceau 4R in gelatine powder can be done by derivative spectrophotometry and partial least-squares multivariate spectrophotometric calibration (Bozdoğan *et al*., 2000).

2.5. Reaction and fate in food

In general, the majority of colour additives are unstable in combination with oxidising and reducing agents in food. Since colour depends on the existence of a conjugated unsaturated system within the dye molecule, any substance which modifies this system (e.g. oxidising or reducing agents, sugars, acids, and salts) will affect the colour.

A Liquid Chromatography - Mass Spectrometry (LC-MS) degradation study of Sunset Yellow FCF in a commercial beverage demonstrated Sunset Yellow FCF to be insensitive to thermal-induced degradation and visible photo-induced degradation, but sensitive to UV-photo-induced conditions in oxidising environment and UV-photo-induced conditions in a reducing environment (Gosetti *et al.,* 2005). 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 use levels

Currently, Sunset Yellow FCF is an authorised synthetic food colouring substance in the EU with a maximal allowed use level of 50 to 500 mg/kg food for various foodstuffs. Sunset Yellow FCF (E

110) is also allowed in alcoholic beverages at levels up to 200 mg/L and non-alcoholic beverages up to 50 mg/L. Table 2 summarises those beverages and foodstuffs that are permitted to contain Sunset Yellow FCF up to specified Maximum Permitted Levels (MPLs) set by EC legislation (EC, 1994).

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

Beverages	**Maximum Permitted Level** (mg/L)
Non-alcoholic flavoured drinks	50
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
Sobrasada	135
Candied fruit and vegetables, Mostarda di frutta Preserves of red fruits Extruded or expanded savoury snack products	200
Pre-cooked crustaceans	250
Mustard Fish roe Solid food supplements/dietary integrators	300
Decorations and coatings Sauces, seasonings, pickles, relishes, chutney and piccalilli Salmon substitutes Surimi	500
Edible cheese rind and edible casings	*Quantum satis*

2.7. Information on existing authorisations and evaluations

Sunset Yellow FCF is permitted as a food additive in the EU under Directive 94/36/EC. Specific purity criteria on Sunset Yellow FCF have been defined in the EU Directive 2008/128/EC.

Sunset Yellow FCF has been evaluated previously by JECFA in 1982 and the SCF in 1984. Both committees established an ADI of 0-2.5 mg/kg bw/day.

2.8. Dietary exposure

2.8.1 Actual levels of use of Sunset Yellow FCF

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). Sunset Yellow FCF was found to be present at a level higher than 0.1 mg/L (Limit of Detection - LOD) in 61 products. In three products the concentration was higher than the Maximum Permitted Level. Overall, the concentrations of Sunset Yellow FCF fount to be present in the samples varied from 1 to 61 mg/L. In another survey conducted in 2005 by the Food Safety Authority of Ireland (FSAI), Sunset Yellow FCF was found to be present at a level higher than 1.0 mg/L (Level of Quantification - LOQ) in 20% of the analysed 54 soft drinks; the concentration in these products ranged from 1 to 49 mg/L (unpublished data provided by FSAI). A usage survey conducted by the Union of European Beverage Associations (UNESDA) in 2005 suggested that the highest current use level of Sunset Yellow FCF is 50 mg/L (Tennant, 2006). A more recent report from UNESDA in 2009 gives a range of use levels from 1 to 48 mg/L (UNESDA, 2009). The Confederation of the Food and Drink Industries of the EU (CIAA) also reported other current use levels of Sunset Yellow FCF ranging from 1 to 48 mg/L (CIAA, 2009). French companies reported use levels ranging from 0.6 to 46 mg/L (unpublished data provided by the Agence Française de Sécurité Sanitaire des Aliments (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 Sunset Yellow FCF from 4 to 50 mg/L (ELC, 2009).

For spirituous beverages, including products with less than15% alcohol, in the survey conducted by FSAI (2009) Sunset Yellow FCF was found to be present in one out of 14 retail samples at a level of 17 mg/L (LOD of 1 mg/L). The European Spirits Organisation (CEPS) reported a range of use levels of Sunset Yellow FCF from 0 to 100 mg/L (CEPS, 2009).

For fruit wines (still or sparkling), cider and perry, the CIAA reported a range of typical maximum use levels 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 194 retail samples of brightly coloured packaged sweets selected for being distinctly coloured (FSA, 2002). Sunset Yellow FCF was found to be present in 66 products, with levels varying from 1 to 106 mg/kg. According to the FSAI data, Sunset Yellow FCF was present at a level higher than 1.0 mg/kg in 63 out of 183 confectionery products, with levels varying from 1 to 122 mg/kg (unpublished data provided by the FSAI). Data provided by French industries on Sunset Yellow FCF in sweets showed use levels varying from 0 to

38 mg/kg (unpublished data provided by AFSSA). Data provided by the ELC (2009) give a range of typical low and maximum use levels from 7 to 50 mg/kg; the CIAA reported a range of typical low and maximum use levels from 10 to 50 mg/kg.

For candied fruit, vegetables, mostarda di frutta, to date no uses were reported by the CIAA members.

For preserved red fruits, the FSAI survey gave no detected samples from 10 retail samples with LOD/LOQ range from < 2 to < 5 mg/kg; to date no uses were reported by the CIAA members.

For jams, jellies and marmalades, the FSAI survey gave no detected samples from 5 retail samples with LOD/LOQ range from < 2 to < 5 mg/kg; to date no uses were reported by the CIAA members.

For decorations and coatings, data from the FSAI survey gave no detected samples from 4 retail samples with LOD/LOQ range < 5 mg/kg; the CIAA reported a range of typical use levels of Sunset Yellow FCF from 0.1 to 200 mg/kg.

For fine bakery wares, the CIAA (2009) reported a range of typical use levels of Sunset Yellow FCF from 6 to 50 mg/kg, whereas the ELC gave a range of typical low and maximum use levels from 14 to 30 mg/kg.

For edible ices, the FSAI (2009) survey gave analytical values of Sunset Yellow FCF ranging from 1 to 83 mg/kg for 7 out of 30 retail samples. The ELC has provided further data from the FAIA, which gave typical use levels ranging from 1.5 to 3 mg/kg.

For flavoured processed cheese and edible cheese rind and edible casing, the CIAA reported a typical maximum value for Sunset Yellow FCF of 0.02 mg/kg.

For desserts, including flavoured milk products, the FSAI survey (2009) gave a range of analytical values from 1 to 197 mg/kg for 6 out of 35 retail samples and the CIAA reported a range of typical low and maximum use levels of Sunset Yellow FCF from 1 to 10 mg/kg.

For sauces, seasonings, pickles, relishes, chutney, the FSAI survey (2009) gave a range of analytical values from 2 to 50 mg/kg from 1 detected sample out of 5 retail samples; the CIAA reported a range of typical low and maximum use levels of Sunset Yellow FCF from 50 to 450 mg/kg.

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

For extruded or expanded savoury snack products, savoury snack products and savoury coated nuts, the CIAA reported a maximum use level of 25 mg/kg. The FSAI survey gives a range of analytical values from 13 to 107 mg/kg for 3 detected samples out of 3 retail samples of savoury snack products and savoury coated nuts.

For foods for particular nutritional purposes (PARNUTS) and food supplements, the CIAA's members provided a range of typical low and maximum use levels of Sunset Yellow FCF from 0 to 50 mg/kg.

In order to refine 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 Sunset Yellow. 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 Sunset Yellow FCF in beverages and foodstuffs used for the refined assessment; they have been defined by applying the rules reported in Annex A to the data available to EFSA.

Table 3. Maximum reported use levels of Sunset Yellow FCF in beverages and foodstuffs used for the refined exposure assessment

Beverages	**Maximum reported use level (mg/L)**
Fruit wines, cider and perry	1
Non-alcoholic flavoured drinks Liquid food supplements/dietary integrators	50
Bitter soda, bitter vino Spirituous beverages	100
Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails	200
Foodstuffs	**Maximum reported use level (mg/kg)**
Flavoured processed cheese Edible cheese rind and edible casings*	0.02
Desserts including flavoured milk products Jam, jellies and marmalades and other similar fruit preparations including low calorie products	10
Savoury snack products and savoury coated nuts Extruded or expanded savoury snack products Soups	25
Confectionery Fine bakery wares Edible ices Complete formulae for weight control intended to replace total daily food intake or an individual meal Complete formulae and nutritional supplements for use under medical supervision Solid food supplements/dietary integrators	50
Fish paste and crustaceans paste Smoked fish Meat and fish analogues based on vegetable proteins	100
Sobrasada	135
Candied fruit and vegetables, Mostarda di frutta Preserves of red fruits Decorations and coatings	200
Pre-cooked crustaceans	250
Mustard Fish roe	300
Sauces, seasonings, pickles, relishes, chutney and piccalilli	450
Salmon substitutes Surimi	500

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

2.8.2 Exposure assessment

The Panel agreed to follow the principles of the stepwise approach, which were used in the report of the Scientific Cooperation (SCOOP) Task 4.2 (EC, 1998), to estimate additives' intakes. For each successive Tier, this involved a further refinement of intake estimates. The approach goes from the conservative estimates that form the First Tier (Tier 1) of screening, to progressively more realistic estimates that form the Second (Tier 2) and Third (Tier 3) Tier.

2.8.2.1. Crude estimates (Budget method)

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

In the case of Sunset Yellow FCF, the maximum permitted use level considered for beverages was 200 mg/L. The maximum permitted 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 Sunset Yellow FCF may be used in a variety of solid foods that could represent more than 25% of processed foods, it is unlikely that a person would systematically choose all processed 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 also considered adequate 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 Sunset Yellow FCF. 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 Sunset Yellow FCF considered in beverages was 50 mg/L (after exclusion of alcoholic drinks), and the level considered in solid food was 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 Sunset Yellow FCF.

The overall theoretical maximum daily exposure in children would therefore be:

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

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

2.8.2.2. Refined estimates

Refined exposure estimates have been performed for Tier 2 using maximum permitted use levels presented in Table 2 and maximum practical use levels presented in Table 3 to deal with the specific cases of *quantum satis* authorisation for edible cheese rinds and edible casings, and for Tier 3 using the maximum reported use levels presented in Table 3, for children and adult populations.

Exposure estimates for children (aged 1-10 years) 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 to 4.5 years) were made by the Panel with the use of detailed individual food consumption data (UK NDNS, 1992-1993)

available from the UNESDA report (Tennant, 2006) and with the MPLs of use as specified in the Directive 94/36/EC on food colours (EC, 1994) 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 on 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 Sunset Yellow FCF intake estimates for adults.

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

Table 4 summarises the anticipated exposure of children and adults to Sunset Yellow FCF.

In the case of Sunset Yellow FCF, when considering MPLs of use (Tier 2), the mean dietary exposure of European children (aged 1-10 years and weighing 25-30 kg) considered by the EXPOCHI consortium ranged from 0.3 mg/kg bw/day to 2.5 mg/kg bw/day, and from 0.7 mg/kg bw/day to 6.7 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure (>10% in all countries) were soft drinks (13 to 60%), desserts, including flavoured milk products (11 to 55%), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (12 to 68%). Fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) accounted for 10 to 29% of exposure in 5 countries, and surimi and jams, jellies and marmalade accounted for 11% of exposure in one country.

For UK pre-school children aged 1.5 to 4.5 years and weighing 15 kg, the mean dietary exposure to Sunset Yellow FCF was 1.4 mg/kg bw/day and 3.5 mg/kg bw/day for the high level (97.5th percentile) consumers of beverages. The main contributors to the total anticipated exposure (>10%) for UK pre-school children were soft drinks which accounted for 60% of the exposure.

Estimates reported for the UK adult population give a mean dietary exposure to Sunset Yellow FCF 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%), fruit wines and cider, and perry (13%).

Further data suggest that current use levels of Sunset Yellow FCF 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 estimates of dietary exposure to Sunset Yellow FCF (Tier 3).

When considering the maximum reported use levels from Table 3, the mean dietary exposure of European children (aged 1-10 years and weighing 25-30 kg), considered by the EXPOCHI consortium, ranged from 0.2 mg/kg bw/day to 2.1 mg/kg bw/day and from 0.6 mg/kg bw/day to 5.8 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure (>10% in all countries) were soft drinks (10 to 58%), fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (11 to 40%). Desserts, including flavoured milk products accounted for 10 to 20% of exposure in 4 countries and sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli accounted for 16 to 70% of exposure in 6 countries. Surimi accounted for 13% of exposure in one country.

For UK children aged 1.5 to 4.5 years and weighing 15 kg, the mean dietary exposure to Sunset Yellow FCF was 1.1 mg/kg bw/day and 3.2 mg/kg bw/day for high level (97.5th percentile) consumers

of soft drinks. The main contributors to the total anticipated exposure (>10%) for UK pre-school children were soft drinks, accounting for 75% of the exposure.

Estimates reported for the UK adult population give a mean dietary exposure to Sunset Yellow FCF of 0.3 mg/kg bw/day and of 0.9 mg/kg bw/day for the high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10%) were soft drinks (60%), sauces and seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (18%).

Table 4. Summary of anticipated exposure to Sunset Yellow FCF using the tiered approach (EC, 2001) in the children and adult population

	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 • Exposure 95th* or 97.5th percentile **	 0.5 1.1	 1.4 3.5	 0.3-2.5 0.7-6.7
Tier 3. Maximum reported use levels • Mean exposure • Exposure 95th* or 97.5th percentile**	 0.3 0.9	 1.1 3.2	 0.2-2.1 0.6-5.8

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

3. Biological and toxicological data

Sunset Yellow FCF has been evaluated previously by JECFA in 1982 (1982), the SCF in 1984 (1984) and by TemaNord in 2002 (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 reports five studies on the toxicokinetic aspects of Sunset Yellow FCF.

In a study in rats given a single oral dose of Sunset Yellow FCF, 0.8% of the administered dose was recovered from the faeces as intact colour. In bile and urine these percentages were 3% and 0.8% respectively. In the urine of rats given large doses of Sunset Yellow FCF (no detail on route of exposure) the azo-reduction products sulphanilic acid and 1-amino-2-naphtol-6-sulphonic acid were found. No qualitative or quantitative measurement of reduction products in the faeces was carried out. The authors concluded that breakdown of Sunset Yellow FCF to (sulphonated) aromatic amines is due to reduction by intestinal bacteria rather than by liver enzymes (Radomski and Mellinger, 1962).

In rats that had received ^{14}C-Sunset Yellow FCF (labelled at the C-8 position of the naphthalene ring) by gavage at doses of 2.7 mg (equivalent to 13.5 mg/kg bw), 94.5% of the total radioactivity was eliminated in the faeces and 8.5% excreted in the urine. Intact dye in urine accounted for 1-2% of the total dose and was mainly excreted in the first 24 hours. The remainder of the urinary radioactivity were naphthol sulphonic acid metabolites arising from cleavage of the azo bond (Honohan *et al.*, 1977). The same study reported that following gavage doses of 2-25 mg Sunset Yellow FCF

(equivalent to 10-125 mg/kg bw), 0.3% and 1.5% of it was excreted as intact colour in urine and bile respectively

After an intravenous injection of Sunset Yellow FCF in rats (no specification on dose) 20-30% of the dose was found in the bile after 6 hours (Ryan and Wright, 1961).

The urine of rabbits which were fed a single dose of Sunset Yellow FCF contained unchanged colour (2%), and the two azo-reduction products sulphanilic acid (54%), and 1-amino-2-naphtol-6-sulphonic acid (55% in 24 hours). In addition the *N*-acetylated form of sulphanilic acid p-acetamidobenzene-sulphonic acid was present in the urine (23%) (percentages indicate the ratio of the amount of the metabolite found to the theoretical amount, assuming complete breakdown) (Daniel, 1962).

In the SCF and TemaNord evaluations no additional studies on the toxicokinetic aspects of Sunset Yellow FCF are mentioned.

Kuno and Mizutani (2005) have investigated the influence of Sunset Yellow FCF on the activities of phase I and phase II drug-metabolising enzymes (CYP2A6, UGT1A6, and UGT2B7). Their findings indicate that Sunset Yellow FCF is neither substrate, nor inhibitor of the enzymes studied.

The Panel noted that the data available on ADME characteristics for Sunset Yellow FCF were not generated using state-of-the-art methodology.

3.2. Toxicological data

3.2.1 Acute oral toxicity

The JECFA evaluation contains information on acute toxicity. Based on studies by Gaunt *et al.* (1967), LD_{50} values were determined after oral administration of Sunset Yellow. Oral administration to mice and rats gave LD_{50} values of > 6000 and >10 000 mg/kg bw respectively. In addition Lu and Lavallée (1967) report that in an oral acute toxicity test in rats the LD_{50} was > 2000 mg/kg bw.

In a study by Sasaki *et al.* (2002), in order to set appropriate doses for a Comet assay, LD_{50} values were determined for several food additives including Sunset Yellow FCF. As in their simple acute toxicity experiments on four to five mice no death was observed at 2000 mg/kg Sunset Yellow FCF, the LD_{50} was defined as > 2000 mg/kg.

3.2.2 Short-term and subchronic toxicity

In the JECFA evaluation several short-term toxicity studies on Sunset Yellow FCF are described.

The colour was given to a group of 16 young rats as a 2% solution in the drinking-water for 10 months (amounting to approximately 2 g/kg bw/day assuming a bodyweight of 250 g and an intake of 25 mL/day). The diet was suboptimal in vitamin B_2. In comparison to controls the colour accelerated growth of the young rats and improved the survival rates of these animals. No histopathological changes of the liver were observed (Manchon and Lowy, 1964).

In another study, groups of rats (15 per sex) were fed a diet containing 0, 0.5, 1, 2, or 3% Sunset Yellow FCF (equivalent to 0, 250, 500, 1000, or 1500 mg/kg bw/day) for 90 days. No adverse effects were observed regarding haematological and histological parameters, and terminal liver and kidney function. Growth and food consumption were normal although slight diarrhoea was observed at the highest concentrations. At autopsy, the caecum was enlarged at the 2 and 3% levels and the testes were

enlarged at the 3% level (Gaunt *et al.,* 1967). The Panel assumes that this study was used by JECFA to derive an ADI.

In a dog study, groups of four animals were fed the colour at 1.0% or 5.0% of the diet (equivalent to 250 or 1250 mg/kg bw/day) (no details on duration, or sex ratio). Two of the four animals given 5%, and one animal given 1.0%, lost weight progressively and had to be sacrificed after 2-3 months. In general, the dietary levels of 5.0% and 1.0% in the diet of dogs were considered moderately and slightly toxic respectively. Weight loss and diarrhoea were the chief clinical effects. Gross and microscopic pathological changes were present but were not characteristic (no further details) (no reference). The Panel assumes that this study was used by the SCF to derive an ADI taking the lowest dose level of 250 mg/kg bw as the NOAEL and an uncertainty factor of 100.

In a study in pigs the colour was fed to groups of six animals (3/sex) at levels of 0, 250, 500, and 1000 mg/kg bw/day for 98 days. No aberrations were observed in weight gain, haematological indices, urinalysis, organ weights, serum levels of transaminases and urea, or microscopic examination of tissues (no detail on which exact tissues were examined) (Gaunt *et al.*, 1969).

In the TemaNord review one additional study on the subject of short-term and sub-chronic toxicity is described.

In a rat study (Aboel-Zahab *et al*., 1997), male rats received in the diet two mixtures containing Tartrazine, Brilliant Blue FCF, Sunset Yellow and Carmoisine (sample A and B) for 30- and 60-day periods (no specification on dosage). The compositions of mixtures A and B, containing Sunset Yellow, Tartrazine, Carmoisine and Brilliant Blue were not specified (unknown concentration of each colour). The effects on body weight, blood picture, liver and kidney functions, blood glucose, serum and liver lipids, liver nucleic acids (DNA and RNA), thyroid hormones (T3 and T4) and growth hormone, and histopathological examinations of liver, kidney and stomach sections were evaluated. These parameters were also investigated 30 days after colourant stoppage (post-effect).

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

As Sunset Yellow FCF was not administrated alone, these data can not be used for the evaluation of Sunset Yellow FCF specifically.

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

The SCF evaluation (1984), states that short-term tests showed no obvious toxic effects.

Several more recent studies were identified.

In a 30-days study 10 rats received a daily dose of 5 mg/kg bw/day Sunset Yellow FCF. After treatment 5 animals were killed and examined, and the other 5 were allowed a 2-week recovery period before examination. Significant but small increases in comparison to controls were observed in aspartate transaminase (AST) activity (119% of control; $p<0.05$) and alanine transaminase (ALT)

activity (113% of control; $p<0.05$), indirect bilirubin (175% of control; $p<0.05$) but not in total bilirubin, and urea (132% of control; $p<0.01$). Total protein and serum globulin were significantly decreased. Apart from AST activity (114% of control; $p<0.05$) and urea (111% of control; $p<0.05$), all parameters recuperated during the recovery period (Helal *et al.*, 2000; Mekkawy *et al.*, 2001). The Panel concludes that the effects observed were not toxicologically relevant because they were small.

In a 30-days rat study the animals received an oral dose of 0.5 mg/kg bw/day in combination with 10 mg of the food preservative sodium nitrite. Exposure to the mixture significantly decreased rat body weight, red and white blood cell count, percentages of haemoglobin and haematocrit, serum inorganic phosphorus, serum protein and serum albumin. Significant increases were observed in serum glucose, T3 and T4, calcium, gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), alkaline phosphatase and cholesterol. After a 15-day recovery period most biochemical and haematological parameters were completely recovered (Helal, 2001; Helal and Abdel-Rahman, 2005). The Panel considers these effects on blood parameters of little relevance as these were observed in combination with exposure to sodium nitrate and the effects can therefore not be assigned specifically to Sunset Yellow FCF.

Mathur *et al.* (2005a) administered Sunset Yellow FCF (obtained from the local market in India, purity not specified) to groups of 10 rats in concentrations of 0 (control), 0.5 or 3% of the feed (equivalent to 0, 250, and 1500 mg/kg bw/day) for 90 days. Histologically the testes of the 0.5% dose group showed degenerative changes in some seminiferous tubules. Spermatogonia forming basal layer of seminiferous tubules were found to be distorted. Maturation arrest was observed in many tubules. Mature sperms were absent but Leydig cells and Sertoli cells were normal in appearance. Testes of the 3% Sunset Yellow FCF treated rats showed an increase in the degenerative changes. The necrosed area appeared irregular involving many tubules, the affected tubules displayed extensive desquamation and sloughing off of almost all the seminiferous epithelium lining the basement membrane. Seminiferous tubules near to the degenerated ones appeared normal. In most of the tubules, pycnotic spermatocytes at the germinal elements were seen. In some other tubules, pycnotic spermatogenesis was arrested at the spermarogonial or spermatocyte stage, while in a few tubules transformation into spermatozoa could be seen. Sertoli cells had virtually obliterated the lumen in some degenerating tubules and were highly vacuolated. The Leydig cells and blood vessels appeared normal. The histological observations on testes revealed that almost 50% of the tubules displayed signs of toxicity. At both dose levels the activity of alkaline phosphatase and cholesterol in serum was significantly increased and serum protein significantly decreased. The effect on serum alkaline phosphatase amounted to +151% of control ($p<0.001$) and +128% of control ($p<0.001$) at the low and high dose level respectively. The Panel concluded that the lowest dose tested, equivalent to 250 mg/kg bw/day, is a Lowest Observed Adverse Effect Level (LOAEL). The Panel also noted the undefined specifications and source of the Sunset Yellow FCF tested in this study.

Mathur *et al.* (2005b) also reported that this sub lethal dose of Sunset Yellow FCF administered to rats for 90 days at 0 (control), 0.5 or 3.0% in the diet (amounting to approximately 0, 250 and 1500 mg/kg bw/day) produced significant and dose-related elevations in total lipid and various lipid fractions. The maximum increase was seen in triglycerides, the lowest increase was observed in cholesterol. The authors concluded that changes in the lipid metabolism were caused by liver damage. The Panel concluded that the lowest dose tested equivalent, to 250 mg/kg bw/day, is a LOAEL.

Finally, Ching *et al.* (2005) investigated the acute *in vivo* histological effects of Egg Yellow, which is a mixture containing Sunset Yellow FCF, by determining histopathological changes in some rat tissues. The colour was orally administered (dissolved in 5 ml distilled water) to rats at doses of 500, 1000, or 2000 mg/kg bw/day for 3 days. Treated rats showed remarkable differences compared to controls. Gross examination of tissues revealed marked ulcerative lesions and haemorrhage on the antra of stomach of rats given the colourant at 2000 mg/kg bw. Gross examination also revealed mild splenomegaly, hepatomegaly and enlarged pale kidneys in the rats administered the colourant at 1000 mg/kg bw or 2000 mg/kg bw. Histopathological examination of sections from the liver, kidney, spleen, stomach and ileum of rats treated with Sunset Yellow FCF revealed a variety of dose-related

degenerative, inflammatory and proliferative lesions which included necrosis, especially in the liver. Necrosis was observed in the kidneys, glomeruli and renal papillae necrosis and also in splenic tissue. Tissues from the control rats showed normal morphology which was remarkably different from those of the treated rats. The Panel concludes that since the material tested is a mixture, the study cannot be taken into account for the evaluation of Sunset Yellow FCF.

3.2.3 Genotoxicity

The JECFA evaluation describes five studies on mutagenicity.

No mutagenic effects were found at a concentration of 0.5 g Sunset Yellow FCF/100 mL in *Escherichia coli* (Lück and Rickerl, 1960).

Sunset Yellow FCF was not mutagenic in three strains of *Salmonella typhimurium*, with or without metabolic activation. Various sulphonated naphthylamines (although none a possible metabolite of Sunset Yellow FCF) were also without mutagenic activity (Garner and Nutman, 1977).

In an Ames test, no reverse mutations were observed in four strains of *Salmonella typhimurium*. Tests were conducted in the presence or absence of liver microsomal fractions obtained from phenobarbitone pre-treated rats (Viola and Nosotti, 1978).

No increases in mitotic gene conversion in *Saccharomyces cerevisiae* were induced following exposure to Sunset Yellow FCF (Sankaranarayanan and Murthy, 1979).

In a rec assay with *Escherichia coli*, and fluctuation assays with *Escherichia coli* and *Salmonella typhimurium* either with or without metabolic activation, no sign of genotoxicity was demonstrated (Haveland-Smith and Combes, 1980).

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

In the TemaNord evaluation nine *in vitro* and three *in vivo* mutagenicity/genotoxicity studies are mentioned which were published since the JECFA evaluation.

In vitro data from Sweeney *et al.* (1994), indicate that direct-acting oxidative genotoxicity may be induced by reaction products of azo dyes (including Sunset Yellow FCF). However, in the TemaNord report this finding is considered to be of questionable relevance.

Regarding the other eight *in vitro* studies mentioned in the TemaNord evaluation no experimental details are presented. The overall conclusion is that these studies provide no evidence for genotoxicity (Ashby *et al.*, 1988; Benigni, 1989; Izbirak *et al.*, 1990; McGregor *et al.*, 1988; Rafii *et al.*, 1997; Tennant *et al.*, 1986, 1987; Yoshimoto *et al.*, 1984).

No mutagenic effects were noted in an *in vivo* bone marrow micronucleus tests in mice and rats after a single oral exposure to 2000 mg/kg bw Sunset Yellow FCF (Westmoreland and Gatehouse, 1991).

Sunset Yellow FCF was administered to rodent species by gavage to check for possible mutagenic activity in the Ames test, or for clastogenicity in bone marrow cells using cytogenetic test systems. It was concluded by the authors that no genotoxic harm is to be expected from the ingestion of Sunset Yellow FCF (Wever *et al.*, 1989).

Mice orally exposed to 0.17 or 1.7 mg/kg bw of Sunset Yellow FCF did not display any increase in the number of cells with chromosomal damages (Durnev *et al.*, 1995).

Without giving details, the SCF also concludes that there is no indication of genotoxic activity associated with Sunset Yellow FCF. Sasaki *et al.* (2002) used an *in vivo* Comet assay in mice to measure DNA damage in various tissues after gavage of Sunset Yellow FCF at a dose of 0 or 2000 mg/kg bw. At 3 hours and 24 hours after administration no DNA damage was noted.

Recently Poul *et al*. (2009) demonstrated a lack of genotoxicity of Sunset Yellow FCF in the gut micronucleus assay in mice after administration by oral gavage 20, 200 or 1000 mg/kg bw twice at 24 hour intervals examination 24 hours later. The authors assessed the genotoxic effects by recording the frequency of micronucleated cells and cell toxicity by identification of the apoptotic and mitotic cells. The concentrations of parent compound and its main metabolites were measured in faeces during a 24-hour period after single oral administrations of the food dye. Parent dye compounds and their main aromatic amine metabolites were detected in significant amounts in the environment of colonic cells. Acute oral exposure to Sunset Yellow FCF did not induce a genotoxic effect in the micronucleus gut assay at doses up to 2000 mg/kg bw. Food dye administration increased the mitotic cells at all dose levels when compared to controls. Azo-reduction of Sunset Yellow FCF produces sulphonated aromatic amines of which the genotoxicity has been reviewed by Jung *et al.* (1992). Although the paper was published over a decade ago, it is discussed in this section as it has not been referred to in previous evaluations. To provide insight in the effect of sulphonation on the genotoxic potential of phenyl- and naphthylamines, the genotoxicity of a range of sulphonated aromatic amines was compared with their unsulphonated analogues. It was found that sulphonated phenyl- and naphthylamines in general (and the Sunset Yellow FCF metabolites sulphanilic acid and 1-amino-2-naphtol-6-sulphonic acid in specific), 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 provided). Based on the available data, the authors conclude that sulphonated aromatic amines, in contrast with their unsulphonated analogues, have no or very low genotoxic potential. Furthermore, 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.

The Panel concluded that Sunset Yellow FCF is not genotoxic.

3.2.4 Chronic toxicity and carcinogenicity

Eleven studies considering the chronic toxicity and carcinogenicity of Sunset Yellow FCF were included in the JECFA (1982) evaluation. The Panel noted that these studies were performed before OECD guidelines and Good Laboratory Practice (GLP) were established.

Groups of 60 mice (30 per sex) were provided diets containing 0.2, 0.4, 0.8 or 1.6% Sunset Yellow FCF (equivalent to 100, 200, 400, or 800 mg/kg bw/day) for 80 weeks. No adverse effects were noted in terms of death rate, body weight gain, organ weights, haematological findings, or histopathological findings. There was no increase in tumour incidence (Gaunt *et al.,* 1974).

In a long-term study, 30 mice were given 0.05% Sunset Yellow FCF in their drinking water for 52 weeks, and were observed for life. Weekly and total ingestion of the colour were about 17 and 884 mg per mouse respectively. In the exposed animals nine lymphomas and one benign intestinal tumour were found in seven survivors. In controls, five lymphomas and one intestinal tumour were found in 13 survivors (Bonser *et al.*, 1956).

Two strains of mice (C_{57} black, and C_3H; 100 animals per strain at both doses and 100 animals for controls) were fed Sunset Yellow FCF at concentrations of 1 or 2% of the diet (equivalent to 1429 and 2858 mg/kg bw/day) for 2 years. No effects on tumour formation were noted (FDA, 1964).

In a preliminary rat study, 5 animals per sex received diets containing 4% (2000 mg/kg bw/day) of the colour for periods up to 18 months. The only effects observed were some staining of the glandular stomach and small intestine in some animals, which was accompanied in these organs with granular deposits. No compound related tumours were observed (Willheim and Ivy, 1953).

Groups of rats (15 per sex) were given diets with 0, 0.03, 0.3, or 1.5% of Sunset Yellow FCF dye (equivalent to 0, 15, 150, or 750 mg/kg bw/day) for 64 weeks. Compared to controls no differences were observed regarding mortality, food intake, growth, organ weights, histopathology, or blood picture. No significant difference in tumour incidence was found (Mannell, 1958).

Groups of 20 rats (both sexes) were fed Sunset Yellow FCF at levels of 0, 0.5, 1, or 2% of the diet (equivalent to 0, 250, 500, or 1000 mg/kg bw/day). No adverse effects were observed concerning growth rates, food consumption or survival. Autopsy at the 79th and 102nd weeks revealed no aberrations in liver histopathology. No neo-plastic change or carcinogenicity was detected (Kanisawa, 1967).

In a long-term rat study, 24 litter-mated Osborne-Mendel rats (12 per sex) were fed diets containing 0, 0.5, 1, 2, and 5% of the colour (equivalent to 0, 250, 500, 1000, or 2500 mg/kg bw/day). An increase in the number of mammary tumours was observed but not statistically significant (no reference).

In another long-term carcinogenicity study, two strains of rats (100 animals per strain and 200 animals for controls) were fed 1 or 2% (equivalent to 500 or 1000 mg/kg bw/day) Sunset Yellow FCF in the diet. Gross and microscopic pathology showed no effect on tumour formation (FDA, 1964).

In another study, 20 rats received a subcutaneous injection of 1mL of a 1% solution of the food colour twice a week for seven months (total of 55 injections). One rat developed an intraperitoneal tumour (observation period not mentioned) (DFG, 1957).

In a dog study, 5 female dogs received diets with 2% of the colour which is equivalent to 500 mg/kg bw/day for seven years. There were no signs of compound related histopathology (FDA, 1964). This dog study may have been the second study used by JECFA to derive the ADI.

Subcutaneous or intraperitoneal injection of the colour in suckling hamsters (no detail on amount of injections) did not increase mortality or tumour incidence over a period of 330 days (Price *et al.*, 1978).

The SCF and TemaNord do not present additional studies on long-term toxicity and no additional long-term studies were conducted since the previous evaluations.

There were no recent studies on carcinogenicity available.

3.2.5 Reproductive and developmental toxicity

JECFA very briefly describes two reproductive studies. In a study, animals (no details on which animals were used in this specific study) received doses of Sunset Yellow FCF based on multiples of the ADI or "of the projected safe dose determined from data from previous long-term feeding studies in rats and dogs", with a maximum dose of 1000 mg/kg bw/day. There was no indication of adverse effects on reproductive performance (no details on which parameters were studied are given) (Pierce *et al.*, 1974).

Groups of 10 immature female rats received a subcutaneous injection of 250 mg/kg bw Sunset Yellow FCF twice daily for 3 days and were sacrificed on day 4. No estrogenic activity was detected (based on uterine weight) (Graham and Allmark, 1959).

Furthermore, the JECFA evaluation mentions two other studies (one in rats, one in rabbits) in which developmental toxicity were investigated.

In the rat study, animals were gavage fed Sunset Yellow FCF at dose levels of 100, 300, or 1000 mg/kg bw on gestational days 6 to 15. The mean body weight of the offspring of the exposed dams was decreased although with doubtful significance. No other effects were observed in dams regarding body weight, corpora lutea, empty implantation sites, early- and late resorptions, and life or dead fetuses. Litter showed no aberrations in terms of sex ratio, or external, internal, and skeletal abnormalities. No fetuses with malformation were observed (IRDC, 1972a).

Rabbits received doses of 100, 300, or 1000 mg/kg bw of the food colour by gavage on gestational days 6-18. Dams displayed no anomalies with regard to body weight, corpora lutea, early and late resorptions, and life or dead term fetuses. No foetal effects were observed in terms of mean body weight, sex ratio, or external and skeletal abnormalities. At the highest dose an incomplete twin was found but this was considered not to be treatment-related (IRDC, 1972b).

In the TemaNord report one additional mouse study is described in which several developmental and neurobehavioral effects of Sunset Yellow FCF were examined.

Sunset Yellow FCF was given to mice at levels of 0 (controls), 0.15, 0.30 and 0.60% in the diet, 10 animals per sex and per group, from 5 weeks of age in the F0 generation to 9 weeks of age in the F1 generation (Tanaka, 1996) F0 (parent) generation exploratory behaviour in an animal movement analysing system was measured at 8 weeks of age. The same was carried out at 3 and 8 weeks of age for F1 animals and in addition they were tested at 7 weeks of age for 3 days in a multiple water T-maze. The F1 pups functional and behaviour development was measured and scored also in the early lactation period in five different tests.

The results showed an absence of adverse effects related to treatment in F0 (parent) generation. There were no differences from controls for average body weight in preconceptional, gestational and lactation periods. No adverse effects were recorded on movement activity in exploratory behaviour. There were no significant differences from control values for reproductive parameters as litter size, litter weight and sex ratio at birth. In the lactation phase of F1 postnatal development there were certain statistically significant differences from controls but they were neither dose related nor permanent, such as increased average body weight in the low- and mid-dose groups during the late lactation (days 14 and 21) and reduced survival in the mid-dose group in the mid lactation (days 7 and 14).

Regarding neurobehavioral parameters in the early lactation period, data which might be interpreted as a delay in functional development with an indication of a dose-relation were seen only in one of the five tests, only in one sex, and detected only at one of the two periods of examination. Score frequencies for swimming direction at Post Natal Day (PND) 4 (but not on PND 14) were significantly depressed in both males and females but were only dose-dependent in females. The score for swimming head angle was also affected in a dose-dependent manner in females. Scores for surface righting at PND 7, but not at PND 4, and negative geotaxis at PND 4, but not at PND 7, were affected in males only at mid-dose (0.30% Sunset Yellow in the diet). These effects were not dose-related.

In the F1 generation no difference was observed in spontaneous motor activity of exploratory behaviour of the animals from the treatment groups compared to controls as measured in an animal movement analysing system at 3 and 8 weeks of age.

The male offspring from the treated groups performed in a multiple water T-maze at 7 weeks of age in a similar way as the control animals. Time taken to complete the maze was reduced significantly on

the second and third trial as compared to the first trial in controls and the highest dose group. A similar tendency was seen for the number of errors. In females, a reduction in the parameters of the treated groups was seen mainly in the second trial but without any indications for a dose-response relationship. The control group did not react as expected by reduction of time taken and number of errors in the second and third trial, which would be a demonstration of learning ability. The author's conclusions that the above differences in maze learning might not have been caused by Sunset Yellow FCF exposure seems well grounded.

The overall conclusion of the Panel is that this study does not demonstrate an adverse effect of Sunset Yellow FCF on reproductive parameters and physical and behaviour development of the offspring in the postnatal life. The Panel concluded that the NOAEL of the study can be defined as the highest dose level tested which was 0.60% in the diet amounting to about 1000 mg/kg bw/day.

The SCF states that reproductive function was not affected and no teratological potential noted.

No new literature on the subject of reproductive and developmental toxicity has been published since the TemaNord evaluation.

3.2.6 Hypersensitivity

The JECFA evaluation briefly describes two studies on allergenicity/sensitisation. In these two studies no sensitisation activity was observed in experiments with Sunset Yellow FCF in guinea pigs (Bär and Griepentrog, 1960) and in a skin test with patients sensitive to p-phenylene-diamine Sunset Yellow FCF produced eczematous hypersensitivity (Baer *et al.*, 1948).

In a study by Schultz-Ehrenburg and Gilde (1987) of 90 patients with chronic or chronic relapsing urticaria, 4% of cases presented intolerance which was found to be caused by food additives (benzoates, sorbic acid, and Sunset Yellow).

TemaNord very briefly describes one additional study in humans in which a small subgroup of patients with atopic dermatitis responded to oral provocation with a mixture of food additives including Sunset Yellow FCF. However as the mixture contained 23 different food additives no discrimination between the different pseudo-allergens can be made (Worm *et al.*, 2000).

In a placebo controlled, double blind clinical study (Supramaniam and Warner, 1986) a group of 43 children with angioedema and/or chronic urticaria and who responded to an additive free diet was challenged with individual food additives 'hidden' in opaque capsules. Ten out of 36 challenged children responded to Sunset Yellow FCF at a dose of 0.1 mg. In a less well controlled clinical study (Wilson and Scott 1989) in which the diagnosis of additive sensitivity was made by the parents, 1/13 challenged children reacted in a blind fashion to a Tartrazine/Sunset Yellow drink combination each at a dose of 8.5 mg/250 mL.

It is also noted that cross-hypersensitivity towards p-phenylene-diamine and Sunset Yellow FCF has been observed in 2 adult patients with eczema taking a coloured antihistamine preparation (Sornin de Leysat, 2003).

Reactions to food colourings, including those triggered by immune (immediate and delayed type hypersensitivity) and non-immune (intolerance) mechanisms are assumed to be infrequent in the population, and prevalence of 0.14 to around 2% have been reported (Young *et al.,* 1987; Hannuksela and Haahtela, 1987; Fuglsang, 1993, 1994). Adverse reactions after Sunset Yellow FCF intake, mostly taken within mixtures of other synthetic colours, have been reported for vasculitic and urticarial reactions (Lowry *et al.,* 1994; Mikkelsen *et al.,* 1978). Reports are often characterised by poorly controlled challenge procedures. Recent studies performed under properly controlled conditions imply

that sensitivity to food additives in patients with chronic urticaria/angioedema or asthma is uncommon (Simon, 2003; Supramaniam and Warner, 1986).

The Panel concluded that there are no indications that Sunset Yellow FCF has intolerance-inducing or allergenic properties. It is noted however that cross-hypersensitivity towards p-phenylene-diamine has been observed.

3.2.7 Other studies

TemaNord describes a study on Sunset Yellow FCF related aberrant behaviour in children.

In this study Pollock and Warner (1990) conducted a study on Sunset Yellow FCF-related aberrant behaviour in children. The behavior of 39 children was observed by their parents to improve on an artificial food additive-free diet and to deteriorate with dietary lapses. Nineteen children completed a double-blind placebo controlled challenge study with a combination of synthetic food colours (Azorubine (25 mg), Tartrazine (50 mg), Sunset Yellow (25 mg) and Amaranth (25 mg)) and these colours were shown to have an adverse effect on a daily Conners' rating of behaviour, although most parents could not detect these changes.

In a study by Osman *et al.* (2004), the effect of Sunset Yellow FCF and sulphanilic acid (a metabolite of Sunset Yellow FCF) on both true (erythrocyte) and pseudo-(plasma) cholinesterases (ChEs) was investigated *in vitro* (human erythrocytes and plasma) and *in vivo* (rat feeding study). Both Sunset Yellow FCF and sulphanilic acid inhibited true and pseudo-ChE *in vitro*, but inhibition by Sunset Yellow FCF was greater. Inhibition by Sunset Yellow FCF was of mixed type (competitive and non-competitive), whereas sulphanilic acid inhibition is only non-competitive. The *in vivo* effect of feeding rats a diet supplemented with Sunset Yellow FCF or sulphanilic acid (both at 4 mg/kg bw/day amounting to 0.2 mg/kg bw/day) resulted in a significant decrease in both true and pseudo-ChE activity. However, it appears that the role of acetylcholine (ACh) in hyperactivity has not yet been elucidated (on the contrary, ChE inhibitors have been clinically studied and shown to alleviate ADHD in children) and therefore the contribution of altered plasma-ChE levels on the behavioural changes noted cannot be decisively concluded upon. Furthermore, the effects amounted to values of 86% ($p<0.05$) and 77% ($p<0.05$) of controls for true (erythrocyte) and pseudo (plasma) ChE respectively upon *in vivo* exposure to 0.2 mg/kg bw/day and were considered to be of limited toxicological relevance.

A study by McCann *et al*. (2007) has concluded that exposure to two mixtures of 4 synthetic colours plus the preservative sodium benzoate in the diet result in increased hyperactivity in 3-year old and 8- to 9-year old children in the general population. In an earlier study by the same research team, there was some evidence for adverse behavioural effects of a mixture of 4 synthetic colours and sodium benzoate in 3-year old children on the Isle of Wight (Bateman *et al.,* 2004). In the 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, class room observers and parents, plus, for 8- to 9- year old children, a computerised test of attention.

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

Mix A significantly increased the GHA scores for all 3-year old children compared to the placebo control GHA scores (effect size 0.20 [CI 0.01 to 0.39], $p<0.05$). This result persisted when analysis was restricted to 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.

Sunset Yellow FCF (E 110) is an azo dye authorised for use as a food additive in the EU and previously evaluated by JECFA in 1982 and the SCF in 1984. Both committees established an ADI of 0-2.5 mg/kg bw/day.

Specifications have been defined in the Directive EC 2008/128/EC and by JECFA (2006). The purity is specified as not less than 85% total colouring matters, calculated as the sodium salt. The remaining 15% may be accounted for by sodium chloride or sodium sulphate, but this is never mentioned explicitly, $\leq$ 5% subsidiary colouring matters and $\leq$ 0.5% 4-aminonaphthalene-1-sulphonic acid, 7-hydroxynaphtahlene-1,3-disulphonic acid, 3-hydroxynaphthalene-2,7-disulphonic acid, 6-hydroxynaphtahlene-2-sulphonic acid, 4,4'-diazoaminodi(benzene sulphonic acid) and 6,6'-oxydi(naphthalene-1,3-disulphonic acid). Thus if the existing specifications could be extended to include $\leq$ 15.0% sodium chloride and/or sodium sulphate as the principal uncoloured components, 99.9% of the material would be accounted for.

The Panel noted that the illegal dye Orange II (D&C Orange 4, CAS Registry Number 633-96-5, sodium salt of 4-[(2-hydroxy-1-naphthalenyl)azo]benzenesulphonic acid) is one of the normal lower sulphonated subsidiary dyes found in Sunset Yellow FCF. The maximum content of Orange II in Sunset Yellow FCF sold within the EU by FAIA member companies is 1%. Other specifications give more details for the subsidiary dyes where Orange II and other lower sulphonated subsidiary dyes are permitted at levels up to 2% according to the FAO/WHO specifications; within the USA there is a limit of 1%.

The Panel also noted that JECFA also defined a maximum limit for Sudan I (1-(phenylazo)-2-napthalenol), of 1 mg/kg (JECFA, 2009). Sudan I is a known impurity in Sunset Yellow FCF and has been shown to be genotoxic and carcinogenic.

The JECFA evaluation appears to have established the current ADI for Sunset Yellow FCF of 0-2.5 mg/kg bw/day on the basis of two NOAELs obtained from two different studies; one in rats, the other in dogs. In the rat study the NOAEL was determined to be 1% in the diet or 500 mg/kg bw/day. This value is probably derived from the 90-day rat study by Gaunt *et al.* (1967) in which at levels of 2% and 3% Sunset Yellow FCF in the diet, enlargement of caecum and at 3% enlargement of the testes was observed, with the latter possibly related to chronic diarrhoea. An uncertainty factor of 200 was applied probably because the study dossier was incomplete. In the dog study no effects were observed

at a dose level of 2% of the colour in the diet, which is equivalent to 500 mg/kg bw/day. This NOAEL is probably derived from the chronic toxicity study conducted by the FDA (1964). In this study the dose of 500 mg/kg bw/day was the only dose administered. An uncertainty factor of 200 was applied probably because the study dossier was incomplete

The SCF determined the ADI based on a long-term dog study for which no reference is given. Plausibly, it is the subchronic toxicity study (no reference) in which dietary levels of 1.0 and 5.0% in the diet of dogs were considered slightly and moderately toxic respectively. Weight loss and diarrhoea were the chief clinical effects. Gross and microscopic pathological changes were present but were not characteristic (no further details) Application of an uncertainty factor of 100 to a NOAEL of 250 mg/kg bw/day would then lead to an ADI of 0-2.5 mg/kg bw.

The SCF (1984) and also the JECFA (1982) and TemaNord (2002) evaluations concluded, based on *in vivo* and *in vitro* studies available at that time, that Sunset Yellow FCF did not show any genotoxic activity.

In another study, Sasaki *et al.* (2002) used an *in vivo* Comet assay in mice to measure DNA damage in various tissues after gavage of Sunset Yellow FCF at a dose of 0, or 2000 mg/kg bw. At 3 hours and 24 hours after administration no DNA damage was noted. Poul *et al*. (2009) did not observe genotoxicity of Sunset Yellow in the gut micronucleus assay in mice given Sunset Yellow FCF by oral gavage twice within a 24-hour interval.

The Panel concluded that the potential genotoxicity of Sunset Yellow FCF has been thoroughly researched both *in vitro* and *in vivo*. There are no indications of any genotoxic potential of Sunset Yellow FCF or its metabolites.

It is concluded that Sunset Yellow FCF is absorbed from the gastrointestinal tract to only a small extent and thus most of an orally administered dose is excreted via the faeces. As little of the colour was retrieved from the faeces as intact dye, Sunset Yellow FCF is likely to be broken down by intestinal azo-reduction. The urine also predominantly contains azo-reduction products (sulphanilic acid, 1-amino-2-naphtol-6-sulphonic acid, and the *N*-acetylated forms). Following this observation it is noted that systemic exposure to free sulphonated aromatic amines may occur.

The Panel concurs with the view expressed in previous evaluations (JECFA, 1982; TemaNord 2002) that the absorption of Sunset Yellow FCF is limited, but that after reduction in the gastrointestinal tract free sulphonated aromatic amines may reach the systemic circulation.

The conversion of Sunset Yellow FCF by azo-reduction *in vivo*, results in the formation of sulphonated naphthylamines that may not be formed in 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 to be in general not associated with genotoxicity *in vitro* and *in vivo*. Since all the sulphonated aromatic amine metabolites that could in theory be formed by azo-reduction of Sunset Yellow FCF were included in the study, the Panel concludes that the data reviewed by Jung *et al.* (1992) are sufficiently re-assuring to support the conclusion that the sulphonated aromatic amines formed from azo-reduction of Sunset Yellow FCF do not give reason for concern with respect to genotoxicity.

Furthermore, the Panel notes that the specifications on the purity of Sunset Yellow FCF would allow concentrations of unidentified unsulphonated aromatic amines to be present in concentrations of up to 100 mg/kg Sunset Yellow FCF. Given the maximal allowed concentration of Sunset Yellow FCF that can be added to food (500 mg/kg food), the maximum concentration of these amines in food could be 50 µg/kg food. Although some aromatic amines may be associated with genotoxicity or even carcinogenicity, the Panel noted that Sunset Yellow FCF was negative in *in vitro* genotoxicity as well as in long term carcinogenicity studies. Eleven studies considering chronic toxicity and carcinogenicity of Sunset Yellow FCF were included in the JECFA evaluation. The SCF and TemaNord do not present additional studies on long-term toxicity and no additional long-term studies

were conducted since these previous evaluations. Altogether it was concluded by SCF, JECFA and the authors of the TemaNord report that there was no evidence for carcinogenicity of Sunset Yellow FCF (SCF, 1984; JECFA 1982; TemaNord 2002).

A study by McCann *et al*. (2007), has concluded that exposure to two mixtures of four synthetic colours plus a sodium benzoate preservative in the diet, both of them, Mix A and Mix B, containing Sunset Yellow FCF, resulted in increased hyperactivity in 3-year old and 8- to 9-year old children in the general population. In an earlier study by the same research team there was some evidence for adverse behavioural effects due to a mixture of four synthetic colours (including Sunset Yellow FCF) and sodium benzoate in 3-year old children on the Isle of Wight (Bateman *et al*,. 2004).

Recently EFSA published an opinion (EFSA, 2008a) on this McCann *et al*. (2007) study. 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.* (2007) study provides limited evidence that the two different mixtures of synthetic colours and sodium benzoate tested had a small and statistically significant effect on activity and attention in some children selected from the general population, although the effects were not observed for all children in all age groups and were not consistent for the two mixtures. The AFC Panel also concluded that the findings may thus be relevant for specific individuals within the population, showing sensitivity to food additives in general or to food colours in particular.

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

The AFC Panel also concluded that:

- since mixtures and not individual additives were tested in the study by McCann *et al*. (2007), 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.

Since the previous evaluations of Sunset Yellow FCF by JECFA in 1982 and the SCF in 1984 several new toxicity studies have been reported.

It is concluded that in the JECFA (1982) and SCF (1984) evaluations the major adverse effects observed after administration of Sunset Yellow FCF were enlargement of the caecum and testes at doses higher than 500 mg/kg bw/day (Gaunt *et al.,* 1967). More recent literature (Mathur *et al.,* 2005a,b) revealed significant effects on the testes and lipid profiles in rats exposed for 90 days to 250 mg/kg bw/day.

Mathur *et al.* (2005a) reported results of a 90 day study on Sunset Yellow FCF in rats at dose levels equivalent to 250 and 1500 mg Sunset Yellow FCF/kg bw/day. There were significant effects on the testes in groups given both doses and the Panel concluded that the lowest dose tested is a LOAEL. In another paper Mathur *et al.* (2005b) reported significant and dose-related elevations in total lipid and various lipid fractions in the rats exposed for 90 days to 250 and 1500 mg Sunset Yellow FCF/kg

bw/day. These results also reveal a LOAEL of 250 mg/kg bw/day. A LOAEL of 250 mg/kg bw/day points at a NOAEL lower than the NOAEL of 500 mg/kg bw/day from the rat (Gaunt *et al.,* 1967) and dog study previously used by JECFA to derive the ADI. The Panel noted however that the Sunset Yellow FCF administered in these studies of Mathur *et al.* (2005a, 2005b) was obtained at the local market in India and that the specifications or purity of this preparation were not defined. The Panel also noted that the 90-day rat study reported by Gaunt *et al.* (1967) also reported effects on testes weight. Although in the rat study reported by Gaunt *et al.* (1967) the effects on testes weight were reported to occur without accompanying histological changes, the parameters investigated did not include sperm morphology and sperm mobility. The Panel concluded that altogether these findings do give reason for re-definition of the ADI. In light of the uncertainties the Panel decided to reduce the ADI for Sunset Yellow FCF, by an extra uncertainty factor of 2.5, to 1.0 mg/kg bw/day and to make the ADI temporary for 2 years. Within this period clarification of the effects of Sunset Yellow FCF on the testis, sperm morphology and sperm mobility should be provided, based on a 28 day study performed according to the recently updated OECD test guideline 407 (OECD, 2006), including characterisation of testes histopathology, sperm morphology and sperm mobility.

The Panel noted that in spite of the effects on testes no effects on reproduction were found in earlier studies, which could be due to the fact that perhaps only part of the tubules were affected, whereas other cells of importance, including Leydig cells and Sertoli cells were not affected.

. Adverse reactions after Sunset Yellow FCF intake, mostly taken within mixtures of other synthetic colours, have been reported for urticarial and vasculitic reactions. Reports are often characterized by poorly controlled challenge procedures 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 Sunset Yellow FCF intake have been reported, mostly when Sunset Yellow FCF is taken within mixtures of other synthetic colours, no conclusion on the induction of sensitivity by Sunset Yellow FCF could be drawn from the limited scientific evidence available. The Panel also notes that sensitive individuals may react at dose levels within the ADI.

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

Refined exposure estimates have been performed both for the children and adult population according to the Tier 2 and Tier 3 approaches described in the SCOOP Task 4.2., which combines, respectively, detailed individual food consumption information from the population with the MPLs of use as specified in the Directive 94/36/EC on food colours (Tier 2), and with the maximum reported use levels of Sunset Yellow FCF 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 (aged 1-10 years), estimates have been calculated for nine European countries (Belgium, France, the Netherlands, Spain, UK, Czech Republic, Italy, Finland and Germany). For the adult population, the Panel has selected the UK population as representative of EU consumers for Sunset Yellow FCF intakes estimates.

When considering MPLs (Tier 2), the mean dietary exposure to Sunset Yellow FCF for European children (aged 1-10 years), ranged from 0.3 mg/kg bw/day to 2.5 mg/kg bw/day and from 0.7 mg/kg bw/day to 6.7 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure (>10% in all countries) were soft drinks (13 to 60%), desserts, including flavoured milk

products (11 to 55%), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (12 to 68%). Fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) accounted for 10 to 29% of exposure in 5 countries and surimi accounted for 11% of exposure in one country.

Estimates reported for the UK adult population give a mean dietary exposure to Sunset Yellow FCF 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 with 40%, sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (14%), fruit wines and cider and perry (13%).

When considering the maximum reported use levels from Table 3, the mean dietary exposure of European children (aged 1-10 years) ranged from 0.2 mg/kg bw/day to 2.1 mg/kg bw/day and from 0.6 mg/kg bw/day to 5.8 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated exposure (>10% in all countries) were soft drinks (10 to 75%), fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (11 to 40%). Desserts, including flavoured milk products accounted for 10 to 20% of exposure in 4 countries and sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli accounted for 16 to 70% of exposure in 6 countries. Surimi accounted for 13% of exposure in one country.

Estimates reported for the UK adult population give a mean dietary exposure of 0.3 mg/kg bw/day and of 0.9 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10%) were soft drinks (60%), sauces and seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney, piccalilli (18%).

The Panel notes that the specifications of Sunset Yellow FCF need to be updated with respect to the level of identified sulphonated subsidiary dyes including the illegal dye Orange II, the level of Sudan I and the percentage of material not accounted for that may represent sodium chloride and/or sodium sulphate as the principal uncoloured components.

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

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

CONCLUSIONS

Sunset Yellow FCF (E 110) is an azo dye authorised as a food additive in the EU and previously evaluated by JECFA in 1982 and the SCF in 1984. Both committees established an ADI of 0-2.5 mg/kg bw/day.

The Panel concludes that the present data base gives reason for re-definition of the ADI. In light of the uncertainties defined, the Panel decided to reduce the ADI for Sunset Yellow FCF, by an extra uncertainty factor of 2.5, to 1.0 mg/kg bw/day and to make the ADI temporary for 2 years. Within this period clarification of the effects of Sunset Yellow FCF on the testis, sperm morphology and sperm mobility should be provided, based on a 28 day study performed according to the recently updated OECD test guideline 407, including characterisation of testes histopathology, sperm morphology and sperm mobility.

The Panel concludes that at the maximum reported levels of use of Sunset Yellow FCF, refined (Tier 3) intake estimates are generally below the temporary ADI of 1 mg/kg bw/day. However, in 1- to 10-

year old children, the mean and the high percentile of exposure (95^{th}/97.5^{th}) can be 0.2 -2.1 and 0.6 - 5.8 mg/kg bw/day, respectively, and thus higher than the temporary ADI at the upper end of the range.

The Panel concludes that while some sensitivity reactions after Sunset Yellow FCF intake have been reported, mostly when Sunset Yellow FCF is taken within mixtures of other synthetic colours, no conclusion on the induction of sensitivity by Sunset Yellow FCF 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 notes that the specifications of Sunset Yellow FCF need to be updated with respect to the level of identified sulphonated subsidiary dyes including the illegal dye Orange II, the level of Sudan I and 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 note 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 on Sunset Yellow (E 110) prepared by the Dutch National Institute for Public Health and 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), 2009. CIAA data in response to the Commission request for data "EFSA re-evaluation of food colours"-Southampton colours (SANCO/E3/OS/km D 53007 - 22 May 2009).

4. ELC (Federation of European Food Additives, Food Enzymes and Food Cultures 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 8 April 2009: Use of certain colour additives in non-alcoholic beverages (26 may 2009).

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ANNEX A

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

1. Decision rules taken to deal with QS authorisations:

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

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

   i. If a value is available for only one colour, this value is used for all the colours (except if this value is available only for annatto-cf point c)

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

c. At the colour level: if there is no available value or if there is just a single value for annatto, the available value for a similar food group for the same colour is used. If there is no similar food group, the highest MPL of 500 mg/kg is used.

Particular cases:

- **Edible casings**: if available use the pork-based products use level; if not available, the highest MPL of 500 mg/kg is used.
- **Edible cheese rinds:** 100 mg/kg (as the flavoured processed cheese category) is used, except for the E 120 (Cochineal) colour whose level is 125 mg/kg for red marbled cheese.

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

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

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

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

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

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

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

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

3. Tiered approach to intake estimation.

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

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

a. Tier 1: Estimates are based MPLs of use, as specified in the Directive 94/36/EC on food colours and the principles of the Budget method.
b. Tier 2: Estimates are based on MPLs of use, as specified in the Directive 94/36/EC on food colours, adjusted for *quantum satis* usages, and national individual food consumption data.
c. Tier 3: Estimates are based on maximum reported use levels and national individual food consumption data.

GLOSSARY/ABBREVIATIONS

ACh	Acetylcholine
ADI	Acceptable Daily Intake
ADHD	Attention-Deficit/Hyperactivity Disorder
AFSSA	Agence Française de Sécurité Sanitaire des Aliments
ANS	Panel on Food Additives and Nutrient Sources added to Food
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
ALT	Alanine transaminase
AST	Aspartate transaminase
CAS	Chemical Abstracts Service
ChEs	Cholinesterases
CEPS	The European Spirits Organisation
CI	Confidence Interval
CIAA	Confederation of the Food and Drink Industries of the EU
CPK	Creatine Phosphokinase
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
EU	European Union
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
FMN	Flavin Mononucleotide
FSA	UK Food Standard Agency
FSAI	Food Safety Authority of Ireland
GGT	Gamma-glutamyl transferase
GHA	Global Hyperactivity Aggregate
HPLC-DAD	High-Performance Liquid Chromatography - Diode Array Detection
HPLC-MS	High-Performance Liquid Chromatography - Mass Spectrometry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC-MS	Liquid Chromatography - Mass Spectrometry
LD_{50}	Lethal Dose, 50% i.e. dose that causes death among 50% of treated animals

LDH	Lactate Dehydrogenase
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit of Detection
LOQ	Level of Quantification
MPL	Maximum Permitted Level
NOAEL	No-Observed-Adverse-Effect Level
OECD	Organisation for Economic Co-operation and Development
PARNUTS	Foods for Particular Nutritional Purposes
PND	Post Natal Day
SCF	Scientific Committee for Food
SCOOP	A scientific cooperation (SCOOP) task involves coordination amongst Member States to provide pooled data from across the EU on particular issues of concern regarding food safety
UNESDA	Union of European Beverage Associations