

SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of Amaranth (E 123) as a food additive¹

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)^{2,3}

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ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion re-evaluating the safety of Amaranth (E 123). Amaranth has been previously evaluated by JECFA in 1972, 1975, 1978 and 1984, and the SCF in 1976, 1979 and 1983. In 1984 the SCF set an ADI for Amaranth of 0-0.8 mg/kg bw/day based on results from a 90-day rat study. In contrast, in 1984 JECFA allocated an ADI of 0-0.5 mg/kg bw/day Amaranth based on a long-term carcinogenicity study in rats. In evaluating the overall toxicological database on Amaranth, the Panel concludes that the point of departure for establishing an ADI for Amaranth can be defined as 15 mg/kg bw/day, taking both the results from the 2-year study and the reproductive and developmental toxicity studies into account. Therefore using an uncertainty factor of 100, the Panel establishes an ADI for Amaranth of 0.15 mg/kg bw/day. The Panel concludes that at the maximum permitted level of use and/or reported use levels of Amaranth (Tier 2), estimates of anticipated exposure for children (1-14 years old) are around 30 times lower than the ADI of 0.15 mg/kg bw/day at the high percentiles (95th/97.5th). However, for adults the anticipated exposure to Amaranth at the 97.5th percentile can be up to 6 times higher than the ADI. The Panel also notes that main contributors to total anticipated exposure to Amaranth for adults were from aperitif wine drinks and Americano. The Panel notes that anticipated exposure to these uses have been made with the maximum permitted levels of use for Americano although no usage value for this beverage was provided by industry, and with the maximum reported levels of use for aperitif wine drinks, which were reported by Industry to be at the same level as the maximum permitted level.

KEY WORDS

Amaranth, E 123, CAS Registry Number 915-67-3, trisodium 2-hydroxy-1-(4-sulphonato-1-naphthylazo)naphthalene-3,6-disulphonate, CI Food Red 9, food colouring substance.

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SUMMARY

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

Amaranth (E 123) is an azo dye authorised as a food additive in the EU and has previously been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1972, 1975, 1978 and 1984, and by the EU Scientific Committee for Food (SCF) in 1976, 1979 and 1983. JECFA and the SCF established different Acceptable Daily Intakes (ADIs) of 0-0.5 and 0-0.8 mg/kg bw/day Amaranth, respectively.

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

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

The Panel evaluated the available data on genotoxicity including an *in vivo* Comet assay in which Amaranth induced significant increases in migration of nuclear DNA in both glandular stomach and the colon of male mice. The Panel considers, in light of the negative carcinogenicity studies and negative results in standard *in vivo* genotoxicity studies, that the biological significance of the positive genotoxicity results is uncertain. Therefore, the Panel concludes that the effects of Amaranth reported in these genotoxicity studies are not expected to result in carcinogenicity. Furthermore, the Panel notes that a dominant lethal test in male rats was reported to be negative.

Overall, based on the weight-of-evidence of the available data, the Panel considers, in line with the opinions expressed by the SCF, JECFA and TemaNord, that there is no concern with respect to the genotoxicity of Amaranth.

The conversion of Amaranth by azo-reduction *in vivo*, results in the formation of sulphonated naphthylamines that may not be formed in the standard *in vitro* genotoxicity tests. In a review by Jung et al. (1992), a range of sulphonated aromatic amines was shown, in general, not to be associated with genotoxicity *in vitro* and *in vivo*. Since all the sulphonated aromatic amine metabolites that could in theory be formed by azo-reduction of Amaranth, including naphthionic acid, were considered in the study, the Panel concludes that the data reviewed by Jung et al. (1992) are sufficiently reassuring to support the conclusion that the sulphonated aromatic amines formed from Amaranth by azo-reduction do not give reason for concern with respect to genotoxicity.

Both JECFA and the SCF concluded that Amaranth is not carcinogenic to rats exposed *in utero* and then subsequently exposed for more than 2 years at doses up to 1250 mg/kg bw/day. The Panel agrees with this conclusion, taking into consideration other available studies on Amaranth, including the most recent, comprehensive 2-year study in rats carried out by British Industrial Biological Research Association (BIBRA) (Clode et al., 1987).

In the 2-year study in rats carried out by BIBRA, renal calcification and hyperplasia were observed at all doses, and therefore no No-Observed-Adverse-Effect-Level (NOAEL) could be identified for Amaranth (Clode et al., 1987). Therefore, and in addition to the afore-mentioned 28-day/90-day study, a re-evaluation of the histology of the renal tissues from the 2-year study reported by Clode et al. (1987) was carried out by Butler and Conning (1983). In the report of this re-evaluation, in respect of renal calcification and hyperplasia a NOAEL of 50 mg/kg bw/day was identified by the authors for

Amaranth. JECFA used this NOAEL to establish an ADI of 0-0.5 mg/kg bw/day. The Panel has re-evaluated this study based on the available data of both sets of histopathological analyses and considers the dose of 50 mg/kg bw/day as a Low-Observed-Adverse-Effect-Level (LOAEL), rather than a NOAEL, for renal pelvic calcification and hyperplasia in female rats. According to the Panel, the treatment-related increase in incidence of these changes in Amaranth-treated rats could be an exacerbation of developing senile nephrosis, as suggested by the authors (Butler and Conning, 1983).

Several studies examined the reproductive or developmental toxicity of Amaranth. Due to methodological insufficiencies, many of them were not conclusive in the determination of any reliable NOAEL in the rat, mouse, hamster and rabbit. Several studies were negative in terms of reproduction toxicity in rats or developmental toxicity in mice, rats, rabbits and dogs. Consequently in these studies, a NOAEL was only considered at the highest dose tested. NOAELs were also determined by considering different endpoints. There have been frequent suggestions of increased resorptions indicating embryotoxicity, but repeating the experiments with improved experimental designs has usually failed to confirm this. Taking all the reproduction and developmental studies into account, NOAELs for Amaranth can be identified in the following species tested: mouse 100 mg/kg bw/day (highest dose tested), rat 15 mg/kg bw/day, rabbit 15 mg/kg bw/day (highest dose tested), cat 50 mg/kg bw/day and dog 75 mg/kg bw/day (approximately).

The Panel concludes that while some sensitivity reactions after Amaranth exposure have been reported, no conclusion on the induction of sensitivity by Amaranth could be drawn from the limited scientific evidence available.

The Panel, in evaluating the overall toxicological database on Amaranth notes that several studies are relevant for establishment of the ADI. Based on these considerations the Panel concludes that the point of departure for establishing an ADI for Amaranth can be defined as 15 mg/kg bw/day, taking both the results from the 2-year study and the reproductive, developmental toxicity studies into account. Therefore using an uncertainty factor of 100, the Panel establishes an ADI for Amaranth of 0.15 mg/kg bw/day.

For children (1-14 years old), Amaranth exposure estimates have been calculated from fish roe consumption for six European countries (Cyprus, Finland, Germany, Greece, UK and Sweden). For the adult population, the Panel has selected the UK population as representative of the EU consumers for Amaranth exposure estimates.

The Panel concludes that at the maximum permitted level of use and/or reported use levels of Amaranth (Tier 2), estimates of anticipated exposure for 1- to 14-year old children are around 30 times lower than the ADI of 0.15 mg/kg bw/day at the high percentiles (95th/97.5th). However, for adults the anticipated exposure to Amaranth at the high percentile (97.5th) can be up to 6 times higher than the ADI.

The Panel also notes that the main contributors to total anticipated exposure for adults were from aperitif wine drinks and Americano. The Panel notes that anticipated exposure to these uses have been made with the maximum permitted level of use for Americano, although no usage value was provided by industry for this beverage, and with the maximum reported levels of use for aperitif wine drinks, which were reported by Industry to be at the same level as the maximum permitted level.

The Panel further notes that the specifications for Amaranth need to be updated with respect to the percentage of material not accounted for that may represent sodium chloride or sodium sulphate as the principal uncoloured components.

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

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

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

According to the Framework Directive 89/107/EEC⁴ on food additives, the Scientific Committee on Food (SCF) should be consulted before the adoption of provisions likely to affect public health, such as the drawing up of lists of additives and the conditions for their use. Accordingly, all food additives, prior to their authorisation, have been evaluated for their safety by the SCF or by its successor the European Food Safety Authority (EFSA).

Directive 89/107EEC as well as Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives⁵ which will apply as from 20 January 2010, require that food additives must be kept under continuous observation and must be re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. In addition Regulation (EC) No 1333/2008 requires that all food additives which were permitted before 20 January 2009 shall be subject to a new risk assessment carried out by EFSA.

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

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

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

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

⁴ OJ L 40, 11.2.1989, p. 27

⁵ OJ L 354, 31.12.2008, p. 16.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of Amaranth when used as a food colouring substance.

Amaranth (E 123) is a mono-azo dye authorised as a food additive in the EU and previously evaluated by the EU Scientific Committee for Food (SCF) in 1976, 1979 and 1983 and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1972, 1975, 1978 and 1984. Amaranth has also been reviewed by TemaNord (2002).

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

2. Technical data

2.1. Identity of the substance

Amaranth is a red mono-azo dye with the chemical formula $C_{20}H_{11}N_2Na_3O_{10}S_3$, CAS Registry Number 915-67-3 and molecular weight of 604.49 g/mol. The chemical name is trisodium 2-hydroxy-1-(4-sulphonato-1-naphthylazo)naphthalene-3,6-disulphonate (Figure 1).

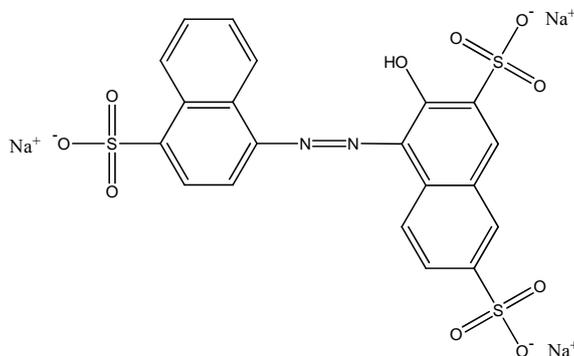


Figure 1. Structural formula of Amaranth (E 123)

Amaranth has among others the following synonyms: CI Food Red 9, Naphthol Red S, CI 16185, E123, Azo Red, Bordeaux S, CI Acid Red 27, Fast Red.

Amaranth is soluble in water (approximately 70 g/l at 25 °C) and slightly soluble in ethanol (up to 4 g/l), but insoluble in vegetable oils. The powder or granules are a red-brown shade. Amaranth is normally supplied as the sodium, potassium or calcium salts. In solution it has a bluish-red colour.

2.2. Specifications

Specifications have been defined in Commission Directive 2008/128/EC⁶ and by JECFA (JECFA, 2006) (Table 1).

Amaranth is described as consisting essentially of trisodium 2-hydroxy-1-(4-sulphonato-1-naphthylazo)naphthalene-3,6-disulphonate and subsidiary colouring matters together with sodium chloride or sodium sulphate as the principal uncoloured components. The purity is specified as not less than 85% total colouring matters and as defined, it might be assumed that most of the remaining 15% may be accounted for by sodium chloride or sodium sulphate, but this is never mentioned explicitly (Commission Directive 2008/128/EC).

Loss on drying at 135°C should be less than 15% and the preparation should not contain more than 0.2% water insoluble matter. Amaranth should not contain more than 3% subsidiary colouring agents and not more than 0.5% (in total) of 4-amino-1-naphthalenesulphonic acid, 3-hydroxy-2,7-naphthalenedisulphonic acid, 6-hydroxy-2-naphthalenesulphonic acid, 7-hydroxy-1,3-naphthalenedisulphonic acid and 7-hydroxy-1,3,6-naphthalenetrisulphonic acid. In addition, Amaranth should not contain more than 0.01% unsulphonated primary aromatic amines (calculated as aniline). The specifications are summarised in Table 1.

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

Table 1: Specifications for Amaranth according to Commission Directive 2008/128/EC and JECFA (2006).

Purity	Commission Directive 2008/128/EC	JECFA (2006)
Water insoluble matter	≤ 0.2%	≤ 0.2%
Subsidiary colouring matters	≤ 3.0%	≤ 3.0%
Organic compounds other than colouring matters:	} ≤ 0.5%	} ≤ 0.5%
- 4-amino-1-naphthalenesulphonic acid		
- 3-hydroxy-2,7-naphthalenedisulphonic acid		
- 6-hydroxy-2-naphthalenesulphonic acid		
- 7-hydroxy-1,3-naphthalenedisulphonic acid		
- 7-hydroxy-1,3,6-naphthalenetrisulphonic acid		
Unsulphonated primary aromatic amines	≤ 0.01% (calculated as aniline)	≤ 0.01% (calculated as aniline)
Ether extractable matter	≤ 0.2% (from a solution of pH 7)	≤ 0.2%
Arsenic	≤ 3 mg/kg	-
Lead	≤ 10 mg/kg	≤ 2 mg/kg
Mercury	≤ 1 mg/kg	-
Cadmium	≤ 1 mg/kg	-
Heavy metals (as Pb)	≤ 40 mg/kg	-

⁶ Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colours for use in foodstuffs. Official Journal of the European Communities, OJ L 6, 10.1.2009, p.20-63.

The Panel noted, as with other azo dyes, that the specifications on the purity of Amaranth permit concentrations of unidentified unsulphonated primary aromatic amines to be present in concentrations of up to 100 mg/kg Amaranth. Given the maximal allowed concentration of Amaranth that can be added to food (100 mg/l beverage), the concentration of these unidentified unsulphonated primary aromatic amines in food could be up to 10 µg/kg food.

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

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

JECFA does not give specifications for aluminium lakes of Amaranth, other than reference to the General Specifications for Aluminium Lakes of Colouring Matters (JECFA, 2006). The Amaranth used in the production process should comply with the specifications as given above, and the aluminium lake should contain not more than 2% water-soluble chlorides and sulphates calculated as sodium salts, not more than 0.5% HCl-insoluble matter, 0.2% ether-extractable matter, 3 mg arsenic/kg and 5 mg lead/kg. Unreacted aluminium oxide may also be present in the final product (not specified).

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

2.3. Manufacturing process

Amaranth is manufactured by coupling 4-amino-1-naphthalenesulphonic acid with 3-hydroxy-2,7-naphthalenedisulphonic acid.

2.4. Methods of analysis in food

A number of different methods have been published for the analysis of Amaranth in various foods including direct analysis of beverages (Vachirapatama et al., 2008), including extraction into dimethyl sulfoxide (DMSO) (Ma et al., 2006) or clean-up by adsorption onto polyamide powder or polyamide columns (Kirschbaum et al., 2006; Morlock and Oellig, 2009) followed in each case by chromatographic determination. High Performance Liquid Chromatography (HPLC) with diode array detection has been mostly employed (Kirschbaum et al., 2006; Yoshioka and Ichihashi, 2008), although other approaches such as High Performance Thin Layer Chromatography (HPTLC) have also been used (Morlock and Oellig, 2009). Although diode array detection does provide some level of confirmation from scanned spectra, the most unequivocal identification of Amaranth in foods was conducted with electrospray Liquid Chromatography-Mass Spectrometry (LC-MS) (negative ionisation) using selected ion monitoring of m/z 537 (Ma et al., 2006). Methods have been applied to soft drinks and confectionery (Yoshioka and Ichihashi, 2008), soft drinks and bakery inks (Morlock and Oellig, 2009) and fish roe and caviar (Kirschbaum et al., 2006) (with recoveries for example of 91% from soft drinks and 93% from confectionery, CV = 2.1-5.4%). It can be concluded that methods of analysis for Amaranth in selected foods appear to be reliable, have received some validation and would be appropriate for use for survey or enforcement purposes.

2.5. Reaction and fate in food

There is a substantial amount of literature concerning the stability of Amaranth, mostly indicating decomposition to identified or unidentified products during heat processing, or reactions with other additives (such as sulphur dioxide, ascorbic acid or nitrite) or reaction with food components such as sugars (BIBRA, 1982b). Although many of the studies have been carried out in solution (Ross, 1975; Bibeau and Clydesdale, 1978; Stephens and Saxby, 1980), there is also much evidence of a lack of stability in processed foods. For example, biscuits containing Amaranth after baking showed a 39-45% loss, which was promoted by baking soda (NaHCO_3), and this was further increased by the presence of sucrose and more so by dextrose. Biscuits with Amaranth added at 217 mg/kg were found after baking to contain naphthionic acid as a degradation product, being equivalent to a loss of 74-94 mg/kg of the added colour. A brownish degradation product was also isolated which had InfraRed (IR) and Ultraviolet (UV) spectra closely matching those of the quinone-sucrose reaction product (Lancaster and Lawrence, 1986). Similarly, commercial biscuits, candy, cereal and ice cream containing Amaranth were also found to contain naphthionic acid equivalent to up to 196 mg/kg Amaranth, representing up to 42% decomposition of the Amaranth (Singh, 1970). A commercial canned chocolate pudding was found to contain 51-58 mg/kg residual Amaranth and 120-150 mg/kg naphthionic acid, with the latter representing 84-88% loss of the added colour (Weissler, 1973). Canned beef containing 7.5-9.1 mg/kg Amaranth before cooking, contained less than 1 mg/kg after cooking (Link, 1974). However, analysis of several fruit drinks before and after pasteurisation showed little or no loss of Amaranth during this process. The Panel notes that most of these studies were carried out 25-30 years ago, and although the observations are nonetheless still valid, with the application of modern instrumental techniques such as LC-MS, identification and characterisation of the degradation products of Amaranth could be more readily achieved.

2.6. Case of need and proposed uses

Permitted use levels have been defined in Directive 94/36/EC⁷ on colours for use in foodstuffs.

Amaranth is a synthetic food colouring substance currently permitted in the EU with a maximal allowed use level of 30 mg/kg in foodstuffs. Amaranth is also permitted in alcoholic beverages at levels up to 100 mg/l. Table 2 summarises those beverages and foodstuffs that are permitted to contain Amaranth up to specified Maximum Permitted Levels (MPLs) of use set by EU legislation (Directive 94/36/EC).

Table 2: MPLs of use of Amaranth in beverages and foodstuffs according to Council Directive 94/36/EC.

Beverages	Maximum Permitted Level of Use (mg/l)
Bitter soda, bitter vino Americano	100
Aperitif wines, spirit drinks including products with less than 15% alcohol by volume	30
Foodstuffs	Maximum Permitted Level of Use (mg/kg)
Fish roe	30

⁷ European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. Official Journal of the European Communities, OJ L 237, 10.9.94, p.13-29.

Amaranth is not permitted as a food additive in the US but is permitted in other countries such as Canada, Australia, New Zealand and Brazil where it can be used in soft drinks, edible ices, preserves, canned foods and confectionary (range, 30-120 mg/kg).

2.7. Information on existing authorisations and evaluations

Amaranth is permitted as a food additive in the EU under Directive 94/36/EC for use in foodstuffs under the restricted levels of use listed in Table 2.

Amaranth has been evaluated previously by the International Agency for Research on Cancer (IARC, 1975), JECFA (1972, 1975, 1978, 1984), the SCF (1976, 1979, 1983), by US Food and Drug Administration (FDA, 1976) and by the Food Standards Australia New Zealand (FSANZ, 2002, 2005). The SCF allocated a temporary Acceptable Daily Intake (ADI) of 0-0.75 mg/kg bw in 1976 (SCF, 1976). On the basis of a 90-day rat study, the SCF revised the ADI for Amaranth to 0-0.8 mg/kg bw/day (SCF, 1983). JECFA allocated an ADI of 0-0.5 mg/kg bw/day from a long-term carcinogenicity study in rats (JECFA, 1984). Amaranth was a permitted food colour in the United States up until 1976, when the US FDA banned its use as a result of concerns regarding its safety-in-use. These concerns related to the results of carcinogenicity studies conducted by the US FDA itself and by others. The British Industrial Biological Research Association (BIBRA) issued a report on Amaranth in 1982 (BIBRA 1982a, 1982b). In 1998, the Scientific Committee on Medicinal Products and Medical Devices (SCMPMD) issued an opinion on the safety of Amaranth for use in medicinal products and concluded that the intake from such uses was some 20 times lower than the ADI established by the SCF, and was not therefore of concern (SCMPMD, 1998).

2.8. Dietary exposure

2.8.1. Actual levels of use of Amaranth

New information on current use levels of Amaranth in finished products was made available to the Panel.

For aperitif wines, spirit drinks including products with less than 15% alcohol by volume, the Confederation of the Food and Drink Industries of the EU (CIAA) reported for Amaranth typical use levels ranging from 0 to 20 mg/l and maximum use levels ranging from 25 to 30 mg/l (CIAA, 2009).

For bitter soda, bitter vino, Americano and fish roe, no usage values were reported by industry.

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 intakes of food additives. For each successive Tier, this involved a further refinement of intakes. The approach goes from the conservative estimates that form the first Tier (Tier 1) of screening, to progressively more realistic estimates that form the Second (Tier 2) and Third (Tier 3) Tiers (Annex A).

As the maximum reported use levels of Amaranth in beverages and foodstuffs, used for the refined exposure assessment, are the same as the MPLs of use that are summarised in Table 2, only Tier 1 for Budget method and Tier 2 using MPL of use and/or maximum reported use levels were provided by the Panel.

2.8.2.1. Crude estimates (Budget method)

The dietary exposure to Amaranth from the MPLs of use was estimated using the Budget method (Tier 1), with the assumptions described in the report of the SCOOP Task 4.2 (EC, 1998), which is based on the fact that there is a physiological upper limit to the amount of food and drink (for beverages 0.1 l/kg bw and for solids 0.025 kg/kg bw), and thus of food additives, that can be consumed each day. A further assumption is that only a certain proportion of the diet is likely to contain food additives (25%). Full details on the Budget method are described in the report of the SCOOP Task 4.2 (EC, 1998).

In the case of Amaranth, the MPL of use considered for beverages was 100 mg/l. The MPL of use considered for solid foods was 30 mg/kg (Table 2).

The default proportion (25%) of beverages and solid food that could contain the additive was considered adequate even though uses of Amaranth are reported for only a limited number of beverages and solid foods (EC, 1998). This assumes that an adult, weighing 60 kg, consumes daily 1.5 litres of beverages and 375 g of solid foods, containing Amaranth. The theoretical maximum daily exposure to Amaranth for adults would therefore be:

$$(100 \times 0.1 \times 0.25) + (30 \times 0.025 \times 0.25) = 2.5 + 0.2 = 2.7 \text{ mg/kg bw/day.}$$

For children, the level of Amaranth considered in solid food was 30 mg/kg and no level was considered in beverages as Amaranth is not allowed in soft drinks (after exclusion of alcoholic drinks). This assumes that a 3-year old child, weighing 15 kg, consumes daily 94 g of solid foods containing Amaranth.

The overall theoretical maximum daily exposure to Amaranth for children would therefore be:

$$(30 \times 0.025 \times 0.25) = 0.2 \text{ mg/kg bw/day.}$$

2.8.2.2. Refined estimates

As the maximum reported levels of use for Amaranth provided to the Panel were similar to the MPLs of use, presented in Table 2 only the Tier 2 approach has been performed by the Panel for refined estimates.

Exposure estimates for children (1-14 years old) were made by the Panel, based on detailed individual food consumption data for fish roe available for Cyprus, Germany, Finland, Greece and Sweden from the report of the EXPOCHI ("Individual food consumption data and exposure assessment studies for children") consortium (Huybrechts et al., 2010) and for the UK (aged 1.5-4.5 years) from the reports of the Union of European Beverages Associations (UNESDA) and the Natural Food Colours Association (NATCOL) (Tennant, 2006, 2007).

Estimates of Amaranth exposure for adults (>18 years old) have been made by the Panel with the use of the detailed individual food consumption data for the UK population (UK NDNS, 2000 -2001) available from the reports of UNESDA and NATCOL (Tennant, 2006 and 2007) which give more refined adult food consumption data, in comparison to those available to the Panel (i.e. from the EFSA Concise European Food Consumption Database, which gives access to aggregate food categories consumed by 15 European countries). Therefore, it was decided to select UK population as representative of the EU consumers for Amaranth estimates for adults.

Table 3 summarises the anticipated exposure of children and adults to Amaranth.

When considering MPLs of use and/or maximum reported levels of use (Tier 2), the mean dietary exposure to Amaranth for European children (aged 1-14 years) ranged from 0 to 0.0006 mg/kg bw/day and from 0 to 0.04 mg/kg bw/day for high level (95th percentile) consumers of fish roe.

Estimates reported for the UK adult population give a mean dietary exposure to Amaranth of 0.008 mg/kg bw/day, and of 0.88 mg/kg bw/day for high level (97.5th percentile) consumers of alcoholic beverages. The main contributors to the total anticipated exposure to Amaranth (>10%) were aperitif wines drinks (60%) for average consumers and Americano (99%) for high consumers.

Table 3: Summary of anticipated exposures to Amaranth using the tiered approach (EC, 2001) for child and the adult populations.

	Adult UK population (>18 years old) ²	UK and Expochi Children population (1-10 years old, 15-29 ¹ kg body weight) ³
	mg/kg bw/day	
Tier 1. Budget method	2.7	0.2
Tier 2. Maximum Permitted level of use and/or reported use Level		
• Mean exposure	0.008	0- 0.0006
• Exposure 95 th or 97.5 th percentile	0.88	0 - 0.04

¹ Except for the Cypriot children where the reported body weight was 54 kg for 11-14 years old.

² For the UK population, estimates are based on the UNESDA report which gives the 97.5th percentile intake (Tennant, 2006).

³ For EU children, estimates are based on the EXPOCHI report, which gives the 95th percentile intake.

3. Biological and toxicological data

Amaranth has been previously evaluated by JECFA in 1972, 1975, 1978 and 1984, and the SCF in 1976, 1979 and 1983. Amaranth was also reviewed by BIBRA (1982b) and TemaNord (2002). The present opinion briefly reports the major studies evaluated in these opinions and describes the additionally reported new literature data in some more detail.

3.1. Absorption, distribution, metabolism and excretion

In vitro studies

When Amaranth was incubated anaerobically for 4 hours with bacterial suspensions from rat intestinal contents, 85.5% of the compound was metabolised by azo-reduction (Roxon et al., 1967). In whole cell extracts from rat caecal contents, the reduction rate of Amaranth was calculated as 3.3×10^{-6} mmol/min. Comparative studies with other colouring compounds suggested that the degree of sulphonation lowered the reduction rate, a finding attributed to the reduced ability of highly sulphonated compounds to penetrate the bacterial cell wall (Larsen et al., 1976).

Reduction of 7 azo dyes, including Amaranth, was investigated using cell suspensions of predominantly intestinal anaerobes (Chung et al., 1978). Reduction was optimal at pH 7.4 in 0.4 M phosphate buffer and was inhibited by glucose. Flavin mononucleotide caused a marked enhancement of azo-reduction by *Bacteroides thetaiotaomicron*. Other electron carriers, e.g. methyl viologen, benzyl viologen, phenosafranin, neutral red, crystal violet, flavin adenine dinucleotide, menadione and Janus Green B can replace flavin mononucleotide. The authors concluded that these data suggest that an extracellular shuttle was required for azo-reduction.

Like other azo compounds, Amaranth can be anaerobically reduced by liver microsomes via a one or two electron process (Peterson et al., 1977). Amaranth gave a weak Electron Spin Resonance (ESR) signal with liver microsomes and nicotinamide adenine dinucleotide phosphate (NADPH), produced no detectable superoxide at a concentration of 100 μ M and increased the microsomal oxygen consumption only two-fold at a 1 mM concentration. Carbon monoxide inhibited Amaranth reduction

markedly and mersalyl, an inhibitor of thiol containing compounds, completely inhibited the azo-reduction of the dye. Glutathione restored 60% of Amaranth reduction. The authors concluded that Amaranth reduction occurred through liver microsomal cytochromes P450.

In vivo studies

Six adult rats were given a single oral dose of 100 mg Amaranth (corresponding to 222 to 333 mg/kg bw) in aqueous solution per animal (Radomski and Mellinger, 1962). Only 0.45% of the dose administered was found as parent compound in the faeces collected over a period of 48 hours. In the same study, a single oral dose of 50 mg Amaranth per animal was administered to 4 rats. Only 2.8% of the dose was absorbed from the gastrointestinal tract; the metabolites in the urine and bile were predominantly products resulting from the reductive fission of the azo-linkage, such as 1-amino-4-naphthalene sulphonic acid and 1-amino-2-hydroxy-3,6-naphthalene disulphonic acid. The former compound was found also in the faeces. The liver enzyme that reduces azo-linkages plays only a small part in the metabolism, as was shown in experiments in which the colour was given by intrasplenic infusion. Reduction of the compound is therefore most probably effected by the intestinal bacteria.

The conversion of Amaranth to naphthionic acid and the fate of the absorbed naphthionic acid were investigated in rats receiving a single oral dose of 200 mg/kg bw Amaranth or naphthionic acid (Pritchard et al., 1976). The major metabolite of Amaranth found in the plasma was naphthionic acid. The faeces were the major route of excretion of naphthionic acid (43–52% of the dose) with a smaller proportion found in the urine (10–17% of the dose). A similar conversion of Amaranth to naphthionic acid was obtained in rats receiving a similar oral dose for 5 days. There was no evidence of naphthionic acid accumulation since plasma levels attained after repeated administration did not exceed those measured after a single dose.

Female rats were orally dosed at 20 mg/kg bw [$1,4,5,8,1',4',5',8'$ - ^{14}C]-Amaranth and killed after 4, 12 or 24 hours. Total radioactivity was measured in blood, organs and excreta. Concentrations of radioactivity in blood peaked after a mean time of 4.2 hours and then declined exponentially until 16 hours post-dosing. The largest amounts of radioactivity were found in the gastrointestinal tract, followed by the lung, heart, liver, bile and blood. No radioactivity was detected in the respiratory gases or in the brain. After 4, 12 and 24 hours respectively, up to 0.01%, 12–50% and 65–85% of the radioactivity was recovered in the faeces and 0.5–2%, 4–11% and 6–10% in the urine, while the tissues contained 1.5–5%, 1–4% and 0.03–0.1% at these time points. Amaranth, naphthionic acid and 5 unidentified metabolites were detected in intestinal contents and faeces. All samples of urine also contained naphthionic acid and traces of Amaranth were found in urine up to 12 hours post-dosing (Ruddick et al., 1977; Ruddick et al., 1979). When 10 pregnant rats were given the same ^{14}C -labelled Amaranth (1.1 mg/kg bw) on the 19th day of gestation, after 30 or 90 min no radioactivity was detected in the fetuses (Ruddick et al., 1978).

Groups of 5 male and 8 female rats were fed for 9 days diets providing doses of 20, 200 and 2000 mg/kg bw Amaranth or control diet (Willes et al., 1980). Blood samples were collected and analysed for naphthionic acid. The female rats were mated with untreated males and received the same dosages of Amaranth throughout mating and gestation. Compared to controls, a significant increase in plasma naphthionic acid occurred only in animals receiving 200 or 2000 mg/kg bw doses. The naphthionic acid concentration in maternal blood, amniotic fluid and fetal plasma from the 2000 mg/kg bw dose group were substantially less than 10-fold higher than those from the 200 mg/kg bw dosed group. The naphthionic acid concentrations in maternal plasma were about 5-times higher than in plasma of their fetuses. The authors concluded that these data indicated the partial saturation of the metabolism of Amaranth in the rat at 2000 mg/kg bw compared to 200 mg/kg bw doses.

The absorption, metabolism and excretion of [$1,4,5,8,1',4',5',8'$ - ^{14}C]-Amaranth were investigated in the rat, mouse and guinea-pig receiving 2 or 200 mg/kg bw of the colour in aqueous solution (BIBRA 1982b, Phillips et al., 1987). In the rat and mouse, the principal route of excretion was the faeces, whereas in the guinea-pig, urinary excretion accounted for up to 50% of the dose. Only trace amounts

were expired as $^{14}\text{CO}_2$. No marked accumulation was found in any tissue of the rats, mice or guinea-pigs 72 hours post-dosing. Naphthionic acid was found in the faeces of all 3 species along with substantial, but variable, amounts of unchanged dye. Examination of the urine by HPLC showed that 60–80% of the urinary radioactivity was associated with naphthionic acid in all 3 species. In studies of *in situ* absorption from isolated intestinal loops of small intestine of the rat, mouse and guinea-pig, no significant absorption of Amaranth was detected over a 100-fold concentration range (20–2000 $\mu\text{g/ml}$).

The biliary excretion of Amaranth has been investigated in several studies on rats receiving i.v. injections of the dye (Klaassen, 1970; Fisher and Varga, 1974; Takada et al., 1974; Iga and Klaassen, 1979; Gregus and Klaassen, 1982). These studies generally demonstrated that parenterally-administered Amaranth is rapidly excreted in the bile by an active process using the same pathways as other organic ions. Excretion is almost entirely as the unchanged compound and is in part dependent on bile flow. The rapid uptake by the liver appears to be saturable and the rate-limiting factor would be its removal from the biliary tree by bulk-flow.

The hepatobiliary transport of Amaranth was studied in control or carbon tetrachloride-treated rats using single-pass liver *ex vivo* perfusion (Takahashi et al., 1986). Amaranth was metabolised via azo-reduction to naphthionic acid (1-amino-4-naphthalenesulphonic acid) which was not excreted in the bile but effluxed into the effluent. This excretion was decreased in livers from carbon tetrachloride-treated rats.

Overall, it can be concluded that after oral administration of Amaranth, there is little absorption of the intact molecule from the gastrointestinal tract of rats. Faecal contents contain products resulting from the reductive fission of the azo-linkage, such as naphthionic acid (1-amino-4-naphthalenesulphonic acid) and 1-amino-2-hydroxy-3,6-naphthalenedisulphonic acid. Naphthionic acid was excreted in lower amounts into the urine of rats. Furthermore, *in vitro* study results indicate that both caecal contents and liver microsomes of rats could cleave azo-linkages in Amaranth. The major metabolite found in the plasma and urine is naphthionic acid. No marked accumulation was found in any tissue of the rats, mice or guinea-pigs. There is no report on the fate of Amaranth in humans.

3.2. Toxicological data

3.2.1. Acute oral toxicity

The oral LD_{50} for Amaranth is 6 g/kg bw in the rat (Galea et al., 1971) and >10 g/kg bw in the mouse (FDA, 1959).

3.2.2. Short-term and subchronic toxicity

A 28-day/90-day study in rats exposed to Amaranth was carried out by Ford et al. (1983) and further mentioned in Clode et al. (1987). This study investigated clinical condition, body weight changes, food and water consumption and renal parameters only, following the observation of an increased incidence of renal calcification and pelvic epithelial hyperplasia with degenerative changes in female rats in a 2-year dietary study (Clode et al., 1987; described in more detail in section 3.2.4). Groups of 25 male and female Wistar rats, 11 weeks old, were fed diets designed to provide intakes of 0, 20, 40, 80 or 1250 mg/kg bw/day Amaranth for either 28 or 90 days. Males in the 1250 mg/kg bw group gained slightly less weight than controls, but food intake was unaffected, and female body weights were unaffected compared to controls. Relative kidney weights and the renal concentrations of calcium, magnesium and phosphorus were not affected by treatment with Amaranth at any dose level and for either period of time. The overall incidence of histopathological findings in the kidney was low but there was an increase in the number of high dose male animals with renal pelvic hyperplasia and calcification after 90 days (4/25 versus 0/25 in controls, $p < 0.05$), but not after 28 days. According to the authors, the No-Observed-Adverse-Effect-Level (NOAEL) of this study was 80 mg/kg bw/day,

and the SCF established an ADI of 0-0.8 mg/kg bw on the basis of this NOAEL (SCF, 1983). The Panel agrees with the NOAEL of 80 mg/kg bw/day identified in this study.

No compound-related effects on gross pathology or other evidence of systemic toxicity were found in pigs (single group of 4 animals) at a dose of 500 mg/kg bw/day Amaranth for 22 days by gavage followed by 750 mg/kg bw/day for 55 days (Sondergaard et al., 1977).

3.2.3. Genotoxicity

No clear evidence of mutagenicity was found in bacterial and yeast mutation tests conducted in the absence and presence of metabolic activation systems (Al-Mossawi, 1983; Ishidate et al., 1984; Prival et al., 1988). The Panel notes 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 notes that a final conclusion from negative Ames test results obtained under standard conditions cannot be drawn. The Amaranth metabolites 1-amino-4-naphthalene sulphonic acid (naphthionic acid) and 2-naphthol-3,6-disulphonic acid, sodium salt (R-amino salt) were however also judged to be non-mutagenic (Chung et al., 1981).

A study in *Drosophila* showed evidence for Amaranth-induced sex-linked recessive lethal damage and loss of X and Y chromosomes (Oster, 1976; Springer, 1976). However, the results of this study were not reproduced in two subsequent studies (Abrahamson, undated; Anon undated). Amaranth was not mutagenic in somatic and germ-line cells of *Drosophila* (Tripathy et al., 1995). A number of *in vitro* studies performed in mammalian cells produced equivocal results regarding the genotoxicity of Amaranth (Kawashi et al., 1980; Ishidate et al., 1984). For instance, Amaranth at concentrations up to 1 mg/ml only produced a marginally positive effect in the chromosome aberration test with Chinese hamster fibroblasts (Ishidate and Odashima, 1977). In the same cell system and the same laboratory Amaranth has been associated with clastogenicity (Ishidate et al., 1984). *In vivo* cytogenetic studies showed that Amaranth produced marginal chromosomal aberrations in the bone marrow cells in rats with some increases in polyploidy at 30 mg/kg bw. A dominant lethal test in male rats with up to 5000 mg/kg bw Amaranth by gavage for 5 days was reported to be negative (Stanford Research Institute, 1972).

More recently, Tsuda et al. (2001) used an *in vivo* Comet assay to measure DNA damage after gavage feeding Amaranth to groups of male mice at doses of 0, 1, 10, 100, 1000 and 2000 mg/kg bw. Three hours after administration, Amaranth induced significant increases in migration of nuclear DNA in the glandular stomach at doses of 1000 mg/kg bw and higher. In the colon, significant differences between the treatment group and controls were observed at doses from 10 mg/kg bw and higher; the effect was dose-related. DNA damage in the other organs was sporadic. In pregnant mice, a modest but significant effect was observed also 3 hours after treatment with a dose of 2000 mg/kg bw in the colon and 6 hours after treatment in the lung. Amaranth did not damage DNA in the other organs or the embryo. Necropsy and histopathological examinations revealed no treatment-related effect on the colon and glandular stomach. The authors therefore concluded that the effect observed was not likely to be due to general cytotoxicity. The Panel considers that the indications provided by the study of Tsuda et al. (2001) should not be disregarded.

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

Recently, Poul et al. (2009) demonstrated a lack of genotoxicity of Amaranth in the gut micronucleus assay in mice after administration by oral gavage of 20, 200 or 1000 mg/kg bw twice at 24-hour intervals, and examination 24 hours later. The authors assessed the DNA damaging effect by recording

the frequency of micronucleated cells and cell toxicity by identifying the apoptotic and mitotic cells. The concentrations of parent compound and its main metabolite (i.e. sulphanilic acid) were measured in faeces during a 24-hour period after single oral administrations of the food dye. Parent dye compound and its main aromatic amine metabolites were detected in significant amounts in the environment of colonic cells. Acute oral exposure to Amaranth did not induce genotoxic effects 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. According to the authors, these results suggest that the increase in Comet tail length previously observed in the colon of mice treated with Amaranth in the *in vivo* Comet assay (Tsuda et al., 2001) represents transient DNA damage that is unable to be fixed in stable genotoxic lesions, and might be partly explained by local cytotoxicity of the dye.

Azo-reduction of Amaranth may produce sulphonated aromatic amines. However, sulphonation of the ring of azo dyes is considered to prevent the activation of the resulting aromatic amines to genotoxic and carcinogenic products (EFSA, 2005). In this respect, Jung et al. (1992) have reviewed the genotoxicity of a range of sulphonated aromatic amines including naphthionic acid. To provide insight in the effect of sulphonation on the genotoxic potential of phenylamines and naphthylamines, the genotoxicity of sulphonated aromatic amines was compared with their unsulphonated analogues. It was found that, in general, sulphonated phenylamines and naphthylamines are non-mutagenic to *Salmonella* in Ames tests. For some sulphonated aromatic amines no genotoxicity was also demonstrated with a variety of other test systems *in vitro* and *in vivo* (no details given). Based on the available data, the authors concluded that sulphonated aromatic amines, in contrast to their unsulphonated analogues, have no or very low genotoxic potential. Hence, the authors concluded that exposure to sulphonated aromatic amines, derived from metabolic cleavage or present as contaminants in colourings is unlikely to induce any significant genotoxic risk.

Overall, based on the weight-of-evidence of the available data, the Panel considers, in line with the opinions expressed by the SCF, JECFA and TemaNord, that there is no concern with respect to the genotoxicity of Amaranth

3.2.4. Chronic toxicity and carcinogenicity

A number of studies in mice and numerous studies in rats have been reviewed to evaluate the carcinogenic potential of Amaranth (BIBRA, 1982b, JECFA, 1972, 1975, 1978, 1984). However, many studies were deemed limited or confounded by insufficient information about the purity of the substance, or by very low purity of the substance or by deficiencies in study design and execution (low number of animals, low survival rates and autolysis). Amaranth was also reviewed by the IARC (1975) which concluded for similar reasons, that the carcinogenicity of this compound could not be evaluated.

No evidence of carcinogenicity was seen in rats fed diets containing 0, 0.03, 0.3 or 1.5% Amaranth for up to 64 weeks (Mannell et al., 1958). In a study conducted by the US FDA (FDA, 1964), in which rats were fed diets containing 0, 0.5, 1, 2 or 5% Amaranth, no effects were seen other than a reduction in body weight gain at the highest dose level and a non-statistically significant increase in mammary tumours in female rats of all treated groups. Following this initial observation, more extensive studies were carried out by the US FDA involving groups of 100 Osborne-Mendel or Sprague-Dawley rats fed 1% or 2% Amaranth in the diet did not show any evidence of carcinogenicity (FDA, 1964), and this result was supported by the absence of any carcinogenic effect in parallel studies in C3Hf and C57Bl mice (FDA, 1964). An increase in tumours of the peritoneum and intestine was reported when diets containing up to 1.6% Amaranth, of unknown purity, were fed to rats for 25 months (Baigusheva, 1968). In this study no tumours were seen in controls. In a similar study, rats given diets containing 2% “chemically pure” Amaranth for their lifespan (up to 33 months) developed a variety of malignant tumours while no tumours were recorded in controls (Andrianova, 1970). The Panel has reservations regarding the validity of the results reported by Baigusheva (1968) and Andrianova (1970), as the

tumours were not related to a specific organ and were representative of the background neoplasia seen in aged rats and considers the absence of tumours in control animals in both studies very unusual.

However, as a result of evaluation of the available data on carcinogenicity, concerns were expressed in the early 1970s by the US FDA and others regarding the possible carcinogenicity of Amaranth. A further US FDA study was carried out on large numbers of rats fed Amaranth in the diet at doses up to 1500 mg/kg bw/day for 2.5 years (Gordon and Taylor, 1975). These rats were obtained from the F2A litters of parents also treated with Amaranth. Although this study suffers from an uncertain feeding history for some of the animals, there was a statistically significant increase in the total number of malignant tumours in the female rats receiving 1500 mg/kg bw/day which was no longer significant when all types of tumours were aggregated. In addition, there were no unusual or unique tumour types other than those common for rats of that strain. Nevertheless, the US FDA concluded on the basis of the results of all the available studies that there were concerns regarding the safety-in-use of Amaranth and banned its use in 1976 (FDA, 1976). The Panel considers that there was no convincing evidence of Amaranth-induced carcinogenicity based on the limited description of this study, noting that evaluation of total malignant tumour incidences as evidence of carcinogenic potential of the test compound can only be performed based on a thorough consideration of all tumour data including onset, and data on non-neoplastic, hyperplastic and preneoplastic lesions but these data were not considered by the authors nor available from the publication.

In order to further address the concerns regarding the possible carcinogenic potential of Amaranth, an additional long-term study with in utero exposure in rats was carried out with Amaranth (Clode et al., 1987). Groups of 90 (control) and 54 (treated) rats of each sex were given Amaranth in their diet to provide intakes of 0 (control), 50, 250 or 1250 mg/kg bw/day for 2 years after weaning. The parental generation (F0) rats had been exposed to the respective dose levels for 60 days before mating, and dams were continuously exposed during gestation and lactation.

Amaranth had no adverse effects on fertility, haematological parameters, serum chemistry or incidence of tumours in the F0 generation. All treated animals showed contamination of the fur and red colouring of the faeces and at the high dose only the faecal pellets were poorly formed. Rats in the high-dose group produced more pups, and the average pup weight was lower than that of the controls. Rats of the F1 generation given the highest dose level had an average body weight slightly lower than the controls despite a small increase in food and water intake. The F1 generation of both sexes given the highest dose level and males given 250 mg/kg bw/day had markedly enlarged caeca on macroscopic examination, and caecal weights were increased.

The incidence and organ distribution of tumours found in the study were typical for the strain of rat used in the study and did not show a treatment-related effect. The authors concluded that Amaranth fed to rats at dose levels of up to 1250 mg/kg bw/day in the diet did not have any carcinogenic effect, and the Panel agrees with this conclusion.

Apart from the effects of Amaranth on body weight and on caecal weight in the F1 generation, the main treatment-related effects seen in the study were on the kidneys of female rats (Clode et al., 1987). High-dose females excreted more protein in the urine after 18 months and on histopathological examination females in all treated groups showed an increased incidence of renal calcification and renal pelvic epithelial hyperplasia. The incidence of renal pelvic calcification in females reported by the authors was 8/90, 11/54, 17/54 and 21/54 in the 0 (control), 50, 250 or 1250 mg/kg bw/day intake groups respectively, while the incidence of renal pelvic epithelial hyperplasia in these groups was 0/90, 7/54, 15/54 and 14/54 respectively (Clode et al., 1987). The renal changes appeared specific for female rats as no treatment-related renal changes were observed in male rats. According to the authors of the study, because of the effects of Amaranth on the kidneys of the females, it was not possible to identify a no-untoward-effect level in this study (Clode et al., 1987).

A re-evaluation of the histopathology of the renal tissues from the 2-year study reported by Clode et al. (1987) was conducted by Butler and Conning (1983). This re-evaluation was carried out without

prior knowledge of the treatment group, and broadly confirmed the findings of the initial pathology, although the authors also evaluated calcification at other sites in the kidney, in addition to renal pelvic calcification. The incidences of renal pelvic calcification in females reported by the authors was 13/89, 14/51, 18/54 and 24/53 in the 0 (control), 50, 250 or 1250 mg/kg bw/day intake groups respectively, while the incidence of renal pelvic epithelial hyperplasia in these groups was 7/89, 9/51, 17/54 and 20/53 respectively (Butler and Conning, 1983). Statistical analysis of the re-evaluated data indicated that incidences of renal pelvic epithelial hyperplasia and renal pelvic calcification in females receiving 250 or 1250 mg/kg bw/day were significantly different from control, but that neither incidences of renal pelvic epithelial hyperplasia nor renal pelvic calcification in female rats were statistically significantly different from control at the dose level of 50 mg/kg bw/day. The study also examined adrenal pathology, given the possibility that the renal calcification changes could be due to increased blood calcium levels, which would be expected to result in adrenal medullary hyperplasia. However no treatment-related adrenal changes were detected.

JECFA (1984) concluded that the re-evaluation by Butler and Conning (1983) of the long-term study indicated that the NOAEL for renal pelvic calcification and renal pelvic epithelial hyperplasia was 50 mg/kg bw/day Amaranth. On this basis and applying an uncertainty factor of 100, JECFA established an ADI of 0-0.5 mg/kg bw/day (JECFA, 1984).

The Panel has re-evaluated this study and has noted that the study does not show a definitive NOAEL for renal pelvic hyperplasia and renal pelvic calcification in the female rats, although the dose level of 50 mg/kg bw/day was a NOAEL for renal calcification at other sites in the kidney. The Panel notes that the incidence of renal pelvic calcification in low dose females was 14/51 compared with 13/89 for controls, $p=0.053$. Moreover, the Panel notes that even in the absence of statistical significance as established by Butler and Conning (1983), the incidence of renal pelvic hyperplasia was over two times higher in low dose females (50 mg/kg bw/day, 9/51) than in controls (7/89). The Panel also notes that Von Kossa staining of the kidneys for calcium showed the presence of positive material in the renal pelvis in an additional female at 50 mg/kg bw/day Amaranth, as well as in small numbers of additional females at 250 and 1250 mg/kg bw/day, not diagnosed from the routine slides. These were not included by Butler and Conning (1983) in the overall incidences for renal pelvic calcification.

The Panel notes that the number of animals/kidneys examined in the Butler and Conning (1983) re-analysis were slightly different than those given by Clode et al. (1987). No explanation was provided for these minor discrepancies, but the Panel concludes that these might be anticipated in such a reanalysis, as might the differences in incidences between the two sets of analyses. Overall, taking into consideration the results of both sets of histopathological analyses, the Panel considers that the intake level of 50 mg/kg bw/day Amaranth should be considered to be a Low-Observed-Adverse-Effect-Level (LOAEL) rather than a NOAEL, based on the renal changes in female rats. According to the Panel, the treatment-related increase in incidence of these changes in Amaranth-treated rats could be an exacerbation of developing senile nephrosis, as suggested by the authors (Butler and Conning, 1983).

In relation to the carcinogenicity of Amaranth, the Panel considers that the available studies, including the most recent, comprehensive study carried out by BIBRA (Clode et al., 1987), taken overall do not indicate that Amaranth has a carcinogenic potential.

3.2.5. Reproductive and developmental toxicity

3.2.5.1. Reproduction studies

Rat

Two studies have been performed by Shtenberg and Gavrilenko (1970, 1972) on rats. Amaranth in a water solution was given orally at doses of 1.5 or 15 mg/kg bw/day for 12-14 months (parental generation). Rats (groups of one male and four females) were mated 3 times. A significantly higher

percentage of unsuccessful pregnancies with no alive born pups were found at 3rd mating and increased percentages of stillborns at 1st and 2nd matings (20.7 and 33.8% respectively vs. 0% in controls) were reported for the group treated with 1.5 mg/kg bw/day. The treatment with the higher dose did not result in an apparent increase of the adverse effect on fertility and pregnancy outcome. The treatment of the F1 generation with either 1.5 or 15 mg/kg bw/day Amaranth did not affect the fertility and pregnancy outcomes of the matings at 4-5 or 7-8 months, as well as the postnatal development of the progenies (Shtenberg and Gavrilenko, 1970). In a second study (Shtenberg and Gavrilenko, 1972), 320 sexually mature Wistar rats (derived from an initial F0 generation of 8 animals for each treated group and 4 animals for the control group) were given orally in the drinking water 1.5 or 15 mg/kg bw/day Amaranth for up to 12 months. Impaired sperm quality and increased oestrus cycle length and intervals were detected. These changes, even though statistically significant, were not systematic, and no consistent evidence of a dose-effect relationship was seen. No effect on histology of the testes or on fertility was observed. No consistent dose-effect relationship was found regarding the severity of the histological changes in the ovaries described by the authors such as follicular atrophy and interstitial tissue growth. At the end of the treatment period studies for prenatal and postnatal development revealed in the first one statistically significant increased rate of post-implantation death in both treatment groups (without dose-effect relationship) and in the second one a higher incidence of stillbirth at 15 mg/kg bw/day and a significant increase in mortality at one month post-partum in both treatment groups (without dose-effect relationship).

The Panel notes that the low number of animals per group, the lack of information on sham-gavage for the control animals, the lack of details on the different methods applied and inconsistency between the different parameters under study as well as the general lack of a dose-effect and length of the exposure-effect relationship preclude the use of these studies for establishing a NOAEL or LOAEL.

Five groups of 10 male and 20 female rats were fed 1.5, 15, 45 and 150 mg/kg bw/day Amaranth in their diets for 2 weeks and then mated twice to produce the F1 generation. There was no statistically significant effect on growth of the parent generations or on litter, weaning and teratological findings in either F1, F2 or F3A generations (Haley et al., 1972; Smith et al., 1974a,b). The Panel considers that the NOAEL of this study is 150 mg/kg bw/day Amaranth, the highest dose tested.

In a three-generation reproduction and teratology study, Osborne-Mendel rats were fed 30, 300, 3000 and 30 000 mg Amaranth (90% pure)/kg diet, equivalent to 2.4-2570 mg/kg bw/day for males and 2.8-3083 mg/kg bw/day for females. F0 rats were fed Amaranth in their diet beginning at the time of weaning. At 3, 5, 7 and 10-11 months of age F0 rats were mated to produce F1 generations. There was no mortality related to ingestion of Amaranth. No effects related to treatment were observed on the reproductive ability, fertility indices, average litter size per dam, viability of the offspring, the average number of live born pups per pregnant female or the viability index (number of live born/total number born). Randomly selected F1 animals were mated to produce F2 generations and so were F2 animals to produce F3 generations, all of them consisting of controls and 4 dose levels groups with number of females per group ranging from 35 to 40 animals. Compared to controls the reproductive ability was not affected by treatment as seen from the similar levels of fertility indices and average litter size per dam. There was also no apparent effect on the viability of the offspring as there was no significant difference in the average number of live born pups per dam or the viability index. A significantly lower 14 or 21 day survival index was reported for some of the litters but without an apparent dose-related trend. The authors concluded that the NOAEL was 3000 mg Amaranth/kg diet equivalent to 150 mg/kg bw/day if sporadic decreases in the survival index were disregarded (Collins et al., 1975a). The Panel agrees with this NOAEL.

3.2.5.2. Developmental studies

Mouse

On gestational days 6 to 15 female albino CD-1 outbred mice were treated by oral tube at dose levels of 27, 90, 300 or 1000 mg/kg bw/day Amaranth (FDRL, 1972). The control females were sham-gavaged. There was no negative effect of the treatment on maternal weight, number of implantations,

dead and alive fetuses and the weight of the fetuses. The percentage of resorptions was higher in all treatment groups compared to controls and increases in the number of skeletal and soft tissue abnormalities were seen.

Considering the absence of both statistical analysis of the data and a dose-effect relationship, the Panel considers that no NOAEL can be derived from this study.

NMRI mice were given Amaranth in water at 7.5, 30 or 100 mg/kg bw/day by gavage on gestational days 0-7 or 6-18 and examined on day 18. No effect was observed on the number of implantations, frequency of fetal deaths or resorptions, gross, skeletal or internal malformations, or fetal weight. Rib and vertebral malformations occurred in one fetus of each of the Amaranth groups treated on gestational days 0-7, whereas none were found in 94 control fetuses examined; however this finding was not considered to be evidence for teratogenicity (Larsson, 1975).

The Panel considers that the NOAEL of this study is 100 mg/kg bw/day Amaranth, the highest dose tested.

In two studies from the same laboratory (Tanaka 1992, 1993), Amaranth was given to mice from 5 weeks of age in the F0 generation (10 animals per sex per group) for 4 weeks, during a mating period of 5 days and during the gestation and lactation of dams. In the first study (A) the animals were treated at dietary levels of 0, 0.03, 0.09 and 0.27% (equivalent to 0, 50, 150 and 450 mg/kg bw/day, respectively). In the second study (B) Amaranth was provided with drinking water at levels of 0, 0.025, 0.075 and 0.225%, (equivalent respectively to 0, 54, 148 and 495 mg/kg bw/day during gestation period and to 180, 720 and 1709 mg/kg bw/day during lactation period). No treatment-related toxicity was seen in maternal animals (F0 dams). In both studies (A and B) there were no statistically significant differences in the litter parameters (i.e. litter size, pup weight and sex ratio) at birth. In study B the average body weight of both sexes was significantly increased on day 21 and the survival index on day 21 was significantly reduced. The only behavioural developmental test in which there was a consistent treatment-related effect throughout both studies was the olfactory orientation. The Panel notes the low number of dams per group, respectively litters, the lack of consistency between the two studies regarding differences in the performance of control animals in the different behaviour tests and the lack of dose-effect relationships.

The Panel concludes that there is no consistent evidence for an adverse effect of Amaranth and these studies cannot be used for establishment of a NOAEL.

Rat

On gestational days 6 to 15, female albino Wistar rats were treated by oral intubation with dose levels of 27, 90, 300 or 1000 mg/kg bw/day Amaranth (FDRL, 1972). The control females were sham-gavaged. No negative effect on maternal weight, no clearly discernible differences from controls for the average implantation sites and viable fetuses per pregnant female were observed. The percentage of resorptions was higher in all dose-groups than that for controls without a clear dose-effect relationship. No cases of fetuses with external or soft tissues malformations were reported. The incidence of skeletal anomalies or variants in treatment groups did not differ from control and was without a dose-effect trend. No statistical analysis data were provided.

Considering the absence of both statistical analysis of the data and a dose-effect relationship, the Panel considers that no NOAEL can be derived from this study.

Amaranth was administered by stomach tube to Osborne-Mendel female rats on gestation days 0-19 in doses of 0 (controls), 7.5, 15, 30, 150 or 200 mg/kg bw/day dissolved in distilled water (Collins and McLaughlin, 1972). No adverse clinical signs were observed in any control or treated dams during the experiment. There were no differences from controls for the mean number of corpora lutea and percentages of implantation loss and mean number of fetuses per dam. The percentage of non-viable implantations increased with increasing doses of Amaranth and consequently the number of live

fetuses decreased in direct relation with the dose level. Statistically significant increases in the percentage of litters with one resorption, the percentage of litters with two or more resorptions as well as the mean number of resorptions per litter were seen in the three highest dose groups (30, 150 or 200 mg/kg bw/day) and there was an indication of a dose-related trend. At 15 mg/kg bw/day the above parameters were also higher than control values but without statistical significance ($p > 0.1$). The average body weight of fetuses was not affected by the administration of Amaranth. Neither gross nor skeletal and soft tissue abnormalities showed any dose relationship nor did their incidence differ markedly from that in the control. Collins and McLaughlin (1972) concluded that 7.5 mg/kg bw/day appears to be a no-effect level under the conditions of their study.

The Panel notes that the fetotoxic effects at 15 mg/kg bw/day Amaranth were not statistically significant and concludes that 15 mg/kg bw/day Amaranth is the NOAEL for this study.

In an extension of the last study (Collins and McLaughlin, 1973), female Osborne-Mendel rats were given the Amaranth metabolites sodium 1-amino-4-naphthalene sulphonate (sodium naphthionate) (96.7 % pure), 2-naphthol-3.6-disulphonic acid, sodium salt (R-amino salt) (97% pure) and the intermediate and common Amaranth contaminant R salt (2-naphthol-3.6-disulphonic acid, sodium salt; 38.3% pure), dissolved in water, at dose levels of 15, 30, 100 or 200 mg/kg bw/day by stomach tube on gestation days 0-19. No adverse clinical signs were observed in any dams, and no statistically significant effects on implantation occurred after treatment with any compound. There were no decreases in the total number and number of live fetuses per litter or in fetal mean weight. However, the proportion of non-viable implantations was significantly increased by sodium naphthionate at all dose levels and by R salt at 15, 100 and 200 mg/kg bw/day. Sodium naphthionate at 200 mg/kg bw/day and R salt at 100 and 200 mg/kg bw/day significantly increased the number of litters with one or more resorptions and with two or more resorptions, and the number of resorptions per litter. A statistically significant increase in the incidence of litters with one or more fetuses bearing skeletal abnormalities occurred only at 200 mg/kg bw/day R-amino salt and at 30 mg/kg bw/day sodium naphthionate, while with rats given the latter compound at 100 mg/kg bw/day, there were statistically significant increases in the percentage of fetuses with sternebral abnormalities, and in the number of such abnormalities per litter. It was concluded that neither of the Amaranth metabolites were as embryotoxic as Amaranth (Collins and McLaughlin, 1973).

Amaranth from 3 different origins (87-92% purity) was given to Wistar rats at dose levels of 15, 30, 100 or 200 mg/kg bw/day on gestation days 0-18, either by gavage or in a semi-synthetic diet, and the dams were killed on day 19 (Khera et al., 1974). There was no evidence of maternal toxicity, and the number of corpora lutea and live fetuses per litter, the incidence of decidualoma and fetal weight were unaffected.

The Panel considers that the NOAEL of this study is 200 mg/kg bw/day Amaranth, the highest dose tested.

Charles River rats (17-22/group) were exposed to Amaranth dissolved in aqueous methylcellulose by gavage at doses of 15, 50 or 150 mg/kg bw/day on gestation days 6-15, and killed on day 20 (Keplinger et al., 1974). There was no effect on maternal weight gain or on the incidence of resorptions, viable fetuses or females with one or more resorption sites. The incidence of external, skeletal and internal abnormalities was also unaffected.

The Panel considers that the NOAEL of this study is 150 mg/kg bw/day Amaranth, the highest dose tested.

Five groups of Charles River rats were given Amaranth by gavage in doses of 15, 150, 450 or 1500 mg/kg bw/day on gestation days 6-15, and killed on day 20 (Burnett et al., 1974). No abnormal effects were seen on maternal weight gain, litter size, average fetal weight or number of resorptions, and there were no gross abnormalities related to the compound.

The Panel considers that the NOAEL of this study is 1500 mg/kg bw/day Amaranth, the highest dose tested.

Two litters from the 3-generation reproduction study of Collins et al. (1975a) were used to study effects on fetal development (Collins et al., 1975b). The dams from F1A and F2B were also fed 30, 300, 3000 or 30 000 mg Amaranth/kg diet. The intakes were calculated to range from approximately 2.3 to approximately 2462.0 mg/kg bw/day. Average weight gain and food consumption of the dams were unaffected by treatment. In F1A animals the number of corpora lutea was significantly lower at 30 000 mg/kg diet, and this reduced the number of fetuses and live fetuses per litter, but pre-implantation loss per litter was unaffected. The mean fetal weight was significantly lower for F1A1 animals exposed to 30 mg Amaranth/kg diet than the controls, although no decrease was observed in the animals from any other generation or other dose group. Only at the 3000 mg/kg diet level in F1A animals was there a statistically significant increase in the proportion of litters with one or more resorptions. No specific skeletal or soft-tissue abnormality could be correlated to Amaranth treatment in either the F1A1 or F3B litters. The number of sternebral abnormalities per litter was significantly increased at 30 mg/kg diet in F3B, but significantly decreased only at 3000 mg/kg diet in the F1A1 litter. The number of soft tissue abnormalities per litter was significantly decreased only at 3000 mg/kg diet in the F1A1 and F3B litters (Collins et al., 1975b).

By considering these sporadic changes and the absence of any clear dose-response relationship, the Panel considers that no NOAEL can be derived from this study.

Three different laboratories undertook a collaborative study using Osborne-Mendel rats (Collins et al., 1976a,b), Charles River rats (Keplinger et al., 1976), and with both rat strains (Holson et al., 1976a,b). Amaranth (89.2% pure) was given at a dose level of 200 mg/kg to groups of at least 20 rats by gavage on gestation days 0-19, 6-15 or 7-9, or in the drinking water at 0.2% on gestation days 0-19 (providing a dose of 264-300 mg/kg). Control groups were gavaged with a saline solution of the same osmolarity and pH as the Amaranth solution, with distilled water, or sham-gavaged (insertion of tube without solution), or given distilled water to drink (Collins et al., 1976a).

In none of the studies were there statistically significant effects on maternal weight, mean numbers per female of corpora lutea, implantations, early or late resorptions or viable fetuses, on females with one or more resorptions, or on fetal weights or fetal sex distribution, and no statistically significant increases in skeletal or visceral abnormalities were seen.

With Osborne-Mendel rats, pre-implantation loss was significantly increased in the group treated by gavage on days 0-19, but the relevant control group (gavaged with saline on gestation days 0-19) showed an abnormally low incidence (Collins et al., 1976b). This study was conducted in the same strain of rat for the same period and at the highest dose level used by Collins and McLaughlin (1972) failed to replicate its findings.

Charles River rats gavaged on days 0-19 showed statistically significant increases both in pre-implantation loss and in litters with two or more resorptions; however, the former was again considered to be related to a low value in the relevant control group, and the latter was regarded as of questionable biological significance in view of the normal number of viable pups in that group (Keplinger et al., 1976).

In the study performed on both species of rats, Charles River rats treated by gavage on gestation days 0-19 showed statistically significant increases in litters with two or more resorptions and in the percentage of resorptions per litter. Fetal weight in Osborne-Mendel rats gavaged on gestation days 0-19 was significantly less than in control groups sham-gavaged or gavaged with distilled water, but not less than in controls gavaged with saline, and the finding was therefore regarded as spurious. In both strains gavaged on gestation days 0-19, the intestinal anaerobic flora was increased, suggesting that the gut flora's ability to convert Amaranth to its metabolites was also increased; however, no Amaranth or

naphthionic acid could be detected in the plasma of these animals 24 hours after the last treatment (Holson et al., 1976a).

Statistical analysis of the combined data from these studies revealed that in the Osborne-Mendel strain the only statistically significant finding was a decrease in the average number of implants per litter in those given Amaranth in drinking water as compared to controls given distilled water to drink, and this was evident only in a single study (Holson et al., 1976b). In Charles River rats the average number of live fetuses per litter was significantly lower in rats gavaged on gestation days 7-9 than in controls given distilled water to drink, while in rats gavaged on gestation days 0-19 there were statistically significant increases in the number and incidence of resorptions as compared with controls gavaged with saline or distilled water.

Because of the absence of increases in abnormalities or effects on fetal weight or viability, the existence of considerable variability in the incidence of resorptions, and the lack of a corroborating study in the Charles River strain, it was concluded that there was “reason to doubt that” the apparent effect on resorptions was “either biologically significant or reproducible” (Holson et al., 1976b).

Female rats pre-treated for 9 days with dietary Amaranth at doses of 20, 200 or 2000 mg/kg bw/day were mated with untreated males, and given the same doses throughout mating and gestation (Willes et al., 1980). No effects on maternal body weight were observed, and the number of litters, litter size and fetal weight on gestation day 21 were unaffected.

The Panel considers that the NOAEL of this study is 2000 mg/kg bw/day Amaranth, the highest dose tested.

Hamster

Female Golden hamsters were exposed on gestation days 6-10 by oral tube at dose levels of 27, 90, 300 or 1000 mg/kg bw/day Amaranth (FDRL, 1972). No modifications of maternal weight, number of alive litters per dam, average number of implantations and alive fetuses per dam and alive fetuses per litter, number of resorptions, percentage of litters with one or more resorptions or fetal body weight were demonstrated. There were few litters from treatment groups with one or two dead fetuses and increased incidence in skeletal anomalies such as incomplete sternebral ossification.

Considering the absence of both statistical analysis of the data and dose-effect relationship, the Panel considers that no NOAEL can be derived from this study.

Rabbit

Two studies are available in rabbit. In the first one (FDRL, 1972), an increased incidence in skeletal anomalies including the most frequent ones such as incomplete sternebral ossification was described at 90, 300 or 1000 mg/kg bw/day Amaranth dosed daily from gestation day 6 to 18. The Panel notes that the small number of fetuses (14) in the control group does not allow any adequate evaluation of possible induction of abnormalities related to Amaranth treatment. Consequently, no NOAEL can be derived from this study.

Rabbits (17 per group) were given Amaranth in gelatine capsules on gestation days 6-18, at doses of 1.5, 5 or 15 mg/kg bw/day, and sacrificed on gestation day 29 (Keplinger et al., 1974). Numbers of corpora lutea, implantation sites, does with resorptions and does which aborted were unaffected. At all levels there was a decrease in the number of viable fetuses and an increase in the number of resorption sites, but these were neither dose-related nor statistically significant. External abnormalities were also slightly increased in treated groups, again however without relation to dose or statistical significance. Skeletal abnormalities were unaffected, as were body weights and 24 hour survival of progeny, and no internal abnormalities were found.

The Panel considers that the NOAEL of this study is 15 mg/kg bw/day Amaranth, the highest dose tested.

Cat

Groups of 12 female cats were fed 300, 900, or 3000 mg Amaranth/kg diet prior to and during breeding and gestation, and were mated with males fed 3000 mg Amaranth/kg diet. The number of resorption sites compared to controls was increased at 3000 mg Amaranth/kg diet, and 24 hour fetal viability was reduced at 300 mg Amaranth/kg diet. Mean body weight at birth was lower at 900 mg Amaranth/kg diet, but no effects were seen after 8 weeks. None of the parameters examined could be interpreted with certainty as evidence of adverse effects (Korinke et al., 1974).

The Panel considers that the NOAEL of this study is 300 mg/kg diet, equivalent to 50 mg/kg bw/day Amaranth.

Groups of 19-22 female cats were given 1, 2 or 3 gelatine capsules, each containing 305 mg Amaranth (85% pure), equivalent to doses of 92, 187 and 264 mg/kg bw/day respectively (Khera et al., 1976). Treatment was continued from 0-22 days before the onset of gestation to days 61-62 of gestation, after which the progeny were delivered by Caesarean section. There was a lower incidence of pregnancy at 264 mg/kg bw/day Amaranth, but the difference was not statistically significant. Maternal weight gain was unaffected, as were the numbers of corpora lutea, total implants, live fetuses at term, fetuses viable after 24 hours in an incubator and prenatally dead fetuses plus deciduoma. However, the number of deciduoma expressed as a proportion of the number of corpora lutea was significantly greater at the highest dose level (264 mg/kg bw/day) than in the controls. Fetal weight, fetal sex ratio and the incidence of anomalies were unaffected by treatment.

The Panel considers that the NOAEL of this study is 187 mg/kg bw/day Amaranth.

Dog

Four groups of 12 adult female Beagles were fed 300, 900 or 3000 mg Amaranth/kg diet and after 45-382 days mated with males fed 3000 mg Amaranth/kg diet (Mastalski et al., 1975a,b). Six females from each group were examined by Caesarean section on gestation day 60, while the remaining six were allowed to whelp their young and nurse them for 8 weeks. There were no effects of treatment on breeding performance, adult body weight, food consumption, progeny viability, survival, gross pathology or skeletal development. Compared to controls, the number of resorption sites was increased and the body weight of pups was reduced in the Caesarean-sectioned dogs fed 900 mg Amaranth/kg diet, but these effects were not seen at 3000 mg Amaranth/kg diet. After weaning the first litter, the adults were rebred, and all were allowed to whelp their young and nurse them for 8 weeks. No adverse effects were noted in either the adult dogs or the second progeny litter.

The Panel considers that the NOAEL of this study is 3000 mg/kg diet equivalent to 75 mg/kg bw/day Amaranth, the highest dose tested.

In summary, there have been frequent observations of increased resorptions indicating embryotoxicity of Amaranth, but repeating the experiments with improved experimental designs has usually failed to confirm this. Taking all the reproduction and developmental studies into account, NOAELs for Amaranth can be identified for the following species tested: mouse 100 mg/kg bw/day (highest dose tested), rat 15 mg/kg bw/day, rabbit 15 mg/kg bw/day (highest dose tested), cat 50 mg/kg bw/day and dog 75 mg/kg bw/day (approximately).

Amaranth has undergone extensive teratogenicity and multigeneration reproduction studies. Due to methodological insufficiencies, many of them were not conclusive for the determination of any reliable NOAEL in the rat (Shtenberg and Gavrilenko, 1970; 1972; Collins et al 1976), the mouse (Tanaka 1992, 1993), the hamster and the rabbit (FDRL, 1972). Several studies were negative in terms of reproduction toxicity in rat (Haley et al., 1972; Smith et al., 1974a,b; Collins et al., 1975a) or developmental toxicity in mouse (Larsson, 1975), rat (Khera et al., 1974; Keplinger et al., 1974; Burnett et al., 1974; Willes et al., 1980), rabbit (Keplinger et al., 1974) and dogs (Mastalski et al., 1975a,b). Consequently, the highest dose tested in these studies was considered to be the NOAEL.

3.2.6. Allergenicity, hypersensitivity and intolerance

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 prevalences of 0.14 to around 2% have been reported (Young et al., 1987; Hannuksela and Haahtela, 1987; Fuglsang, 1993, 1994).

A few isolated cases of sensitivity to Amaranth have been reported, usually describing urticaria, but these were usually associated with exposure to other colours (Mikkelsen et al., 1978).

In vitro studies showed no effect of 100 µM Amaranth on IgG and IgM production but a suppression of IgE production by rat lymphocytes *in vitro* (Kuramoto et al., 1997). Likewise, by using peripheral blood lymphocytes purified from normal human volunteers, Amaranth at non-cytotoxic millimolar concentrations caused limited though statistically significant inhibition of activation of cell proliferation by phytohaemagglutinin, and natural killer cell activity (Koutsogeorgopoulou et al., 1998). At similar millimolar concentrations, Amaranth was reported to inhibit serotonin release from RBL-2H3 cells; the cytotoxic effect of Amaranth was not documented in the text (Tanaka et al., 1995).

The immunological effect of oral administration of food colouring agent was recently studied in rats given oral doses of 47 mg/kg bw/day Amaranth for 4 weeks (Hashem et al., 2010). After 2 weeks all animals were immunostimulated by i.p. injection of sheep red blood cells 10% (1 ml/rat). Results revealed that the treatment had no effect on the body weight gain. The authors concluded that Amaranth exerted a suppressing effect on the cellular but not on the humoral immune response. However, the effects reported, such as a decrease in circulating neutrophils and monocytes, an increase in lymphocytes and basophiles and a decreased delayed hypersensitivity, were limited and when statistically significant, only at $p \leq 0.05$. Total serum protein, albumin, total globulin and albumin/globulin ratio were not affected. The Panel considers that the biological significance of these data is limited.

4. Discussion

The Panel was not provided with a newly submitted dossier on Amaranth 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.

Amaranth (E 123) is a mono-azo dye authorised as a food additive in the EU and previously evaluated by the SCF in 1976, 1979 and 1983 and JECFA in 1972, 1975, 1978 and 1984 and the SCMPMD in 1998. Amaranth has also been reviewed by the IARC (1975), BIBRA (1982a) and TemaNord (2002).

Specifications have been defined in Commission Directive 2008/128/EC and by JECFA (2006). The purity is specified as not less than 85% total colouring matters. From the definition, it might be assumed that most of the remaining 15% may be accounted for by sodium chloride or sodium sulphate, but this is never mentioned explicitly. Amaranth should not contain more than 3% subsidiary colouring agents and not more than 0.5% (in total) of 4-amino-1-naphthalenesulphonic acid, 3-hydroxy-2,7-naphthalenedisulphonic acid, 6-hydroxy-2-naphthalenesulphonic acid, 7-hydroxy-1,3-naphthalenedisulphonic acid and 7-hydroxy-1,3,6-naphthalenetrisulphonic acid. In addition, Amaranth should not contain more than 0.01% unsulphonated primary aromatic amines (calculated as aniline).

The SCF allocated a temporary ADI of 0-0.75 mg/kg bw/day in 1976 (SCF, 1976). On the basis of the renal effects of Amaranth observed in the 28 day/90 day study in rats carried out by BIBRA (Ford et al., 1983), providing a NOAEL of 80 mg/kg bw/day, the SCF revised the ADI for Amaranth to 0-0.8 mg/kg bw/day (SCF, 1983). In contrast, JECFA allocated an ADI of 0-0.5 mg/kg bw/day from the long-term carcinogenicity study with *in utero* exposure in the rat carried out by BIBRA (JECFA, 1984).

Toxicokinetic data indicate that after oral dosing of mice, rats or guinea pigs, Amaranth is rapidly and extensively reduced in the gut by the microflora to naphthionic acid and 1-amino-2-hydroxy-3,6-naphthalene disulphonic acid. After azo-reduction in the gastro-intestinal tract, the absorption of metabolites of Amaranth has been reported to range from 10-20% of the dose, with faecal excretion accounting for 75-85% of total excretion. The major metabolite found in the plasma and urine is naphthionic acid. There is no report on the fate of Amaranth in humans.

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

Both JECFA and the SCF concluded that Amaranth is not carcinogenic to rats exposed *in utero* and then subsequently exposed for more than 2 years at doses up to 1250 mg/kg bw/day. The Panel agrees with this conclusion, taking into consideration other available studies on Amaranth, including the most recent, comprehensive study carried out by BIBRA (Clode et al., 1987).

In the 2-year study carried out by BIBRA (Clode et al., 1987), Amaranth caused caecal enlargement at the two highest dose levels and nephrocalcinosis is commonly associated with this, possibly due to effects on mineral absorption and increased faecal water loss (Clode et al., 1987). According to the authors the renal changes could be related to the changes seen in the gastrointestinal tract, since lactose and caramel colour produce similar gastrointestinal changes and also calcification in the kidney in both male and female rats. However, in the study with amaranth, renal pelvic nephrocalcinosis and renal pelvic epithelial hyperplasia were only observed in female rats and were observed at all dose levels, suggesting the two effects were not completely linked (Clode et al., 1987). In an additional 90-day study performed by BIBRA to investigate the renal effect of Amaranth, in which a significant increase in the number of high dose male animals with renal pelvic hyperplasia and calcification was observed, the researchers identified a NOAEL of 80 mg/kg bw/day with respect to nephrocalcinosis and hyperplasia. The same researchers also concluded that the renal changes in females in the 2-year study appeared dependent on the age-related development of glomerulonephrosis as it was not observed in females in the short-term study (Clode et al., 1987). Based on this 90-day study, the SCF (1983) established an ADI of 0-0.8 mg/kg bw/day.

In the 2-year study carried out by BIBRA, renal calcification and hyperplasia were observed at all doses, and therefore no NOAEL could be identified for Amaranth (Clode et al., 1987). Therefore, and in addition to the afore-mentioned 28-day/90-day study, a re-evaluation of the histology of the renal tissues from the 2-year study reported by Clode et al., (1987) was carried out by Butler and Conning, (1983). In the report of this re-evaluation, in respect of renal calcification and hyperplasia a NOAEL of 50 mg/kg bw/day was identified by the authors for Amaranth. JECFA used this NOAEL to establish an ADI of 0-0.5 mg/kg bw/day. The Panel has re-evaluated this study based on the available data of both sets of histopathological analyses and considers the dose of 50 mg/kg bw/day as a LOAEL, rather than a NOAEL, for renal pelvic calcification and hyperplasia in female rats. According to the Panel, the treatment-related increase in incidence of these changes in Amaranth-treated rats could be an exacerbation of developing senile nephrosis, as suggested by the authors (Butler and Conning, 1983).

The Panel considers that the nephrocalcinosis finding in female rats is not relevant for the safety assessment of Amaranth for humans because the rat is a species known to be particularly sensitive to mineralisation of the renal tubule epithelium due to dietary alteration of the calcium and phosphorus homeostasis (Ritskes-Hoitinga et al., 1989, 1991, 1992). Females are more sensitive than males to this effect, partially due to an oestrogen-induced renal mineralisation (Latendresse et al., 2001). Nephrocalcinosis in rats can be observed early during experimentation, already after 2 weeks under a diet-induced calcium: phosphorus imbalance (Cockell and Belonje, 2004). However the Panel considers that the finding of renal pelvic hyperplasia in all treated females, which was statistically significant in the paper of Clode et al. (1987) and showed a dose-related trend in the re-analysis carried out by Butler and Conning (1983) could not be disregarded for the safety assessment of Amaranth for humans.

Amaranth has undergone extensive teratogenicity and multigeneration reproduction studies. Due to methodological insufficiencies, many of them were not conclusive for the determination of any reliable NOAEL in the rat (Shtenberg and Gavrilenko, 1970; 1972; Collins et al 1976), mouse (Tanaka 1992, 1993), hamster and rabbit (FDRL, 1972). Several studies were negative in terms of reproduction toxicity in rat (Haley et al., 1972; Smith et al., 1974a,b; Collins et al., 1975a) or developmental toxicity in mouse (Larsson, 1975), rat (Khera et al., 1974; Keplinger et al., 1974; Burnett et al., 1974, Willes et al., 1980), rabbit (Keplinger et al., 1974) and dogs (Mastalski et al., 1975a,b). Consequently, the highest dose tested in these studies was considered to be the NOAEL.

In summary, there have been frequent observations of increased resorptions indicating embryotoxicity of Amaranth, but repeating the experiments with improved experimental designs has usually failed to confirm this. Taking all the reproduction and developmental studies into account, NOAELs for Amaranth can be identified in the following species tested: mouse 100 mg/kg bw/day (highest dose tested), rat 15 mg/kg bw/day, rabbit 15 mg/kg bw/day (highest dose tested), cat 50 mg/kg bw/day and dog 75 mg/kg bw/day (approximately).

In vitro genotoxicity tests with Amaranth in bacteria, fungi, insects and mammalian cells mainly produced negative results. Isolated positive results obtained in the absence of exogenous metabolic activation were marginal, poorly reproducible or associated with high doses. *In vivo*, negative results were obtained in cytogenetic tests in bone marrow and in germ cells of rodents following oral administration of Amaranth.

In an *in vivo* Comet assay performed by Tsuda et al. (2001), Amaranth induced significant increases in migration of nuclear DNA in both the glandular stomach and the colon of male mice. Necropsy and histopathological examination revealed no treatment-related effects in the colon and glandular stomach, and the authors therefore concluded that the effect observed was not likely to be due to general cytotoxicity. In contrast, in a more recent study by Poul et al. (2009) Amaranth did not reveal genotoxic effects in the micronucleus gut assay in mice at doses up to 2000 mg/kg bw. The authors commented on the results of the *in vivo* Comet assay by Tsuda et al. (2001), stating that the migration of nuclear DNA observed in the colon of mice represents transient DNA damage that is unable to be fixed in stable genotoxic lesions and might be partly explained by local cytotoxicity of the dye.

The Panel considers, in light of the negative carcinogenicity studies and negative results in standard *in vivo* genotoxicity studies, that the biological significance of the positive genotoxicity results is uncertain. Therefore, the Panel concludes that the effects of Amaranth reported in these studies are not expected to result in carcinogenicity. Furthermore, the Panel notes that a dominant lethal test in male rats was reported to be negative.

Overall, based on the weight-of-evidence of the available data, the Panel considers, in line with the opinions expressed by the SCF, JECFA and TemaNord, that there is no concern with respect to the genotoxicity of Amaranth.

The conversion of Amaranth by azo-reduction *in vivo*, results in the formation of sulphonated naphthylamines that may not be formed in the standard *in vitro* genotoxicity tests. In a review by Jung et al. (1992), a range of sulphonated aromatic amines was shown, in general, not to be associated with genotoxicity *in vitro* and *in vivo*. Since all the sulphonated aromatic amine metabolites that could in theory be formed by azo-reduction of Amaranth, including naphthionic acid, were considered in the study, the Panel concludes that the data reviewed by Jung et al. (1992) are sufficiently reassuring to support the conclusion that the sulphonated aromatic amines formed from Amaranth by azo-reduction do not give reason for concern with respect to the genotoxicity of Amaranth.

In evaluating the overall toxicological database on Amaranth, the Panel notes that several studies, including the 2-year study in rats carried out by BIBRA (Clode et al., 1987), are relevant for establishing the ADI. The Panel considers that the results of the 2-year study in rats indicate a LOAEL of 50 mg/kg bw/day for renal pelvic calcification and hyperplasia in female rats. The Panel

notes that using an additional uncertainty factor of 3 to convert the LOAEL into a NOAEL would result in a point of departure for establishment of the ADI of about 15 mg/kg bw/day. Furthermore, taking all the reproduction and developmental studies into account, the Panel notes that NOAELs can be identified in several species with the lowest value being the NOAEL of 15 mg/kg bw/day for rats (Collins and McLaughlin, 1972).

Taking both the results from the 2-year study and the reproductive and developmental toxicity studies into account and using an uncertainty factor of 100, the Panel establishes an ADI for Amaranth of 0.15 mg/kg bw/day.

A few isolated cases of sensitivity to Amaranth have been reported, usually describing urticaria, but these were frequently associated with exposure to other colours. Recent studies performed under properly controlled conditions imply that sensitivity to food additives in patients with chronic urticaria/angioedema or asthma is uncommon. The Panel considers that no conclusion on the induction of sensitivity by Amaranth could be drawn from the limited scientific evidence available.

The exposure assessment approach for Amaranth goes from the conservative estimates that form the First Tier of screening, to progressively more realistic estimates that form the Second and Third Tiers. The dietary exposure to Amaranth 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 2.7 mg/kg bw/day for adults and 0.2 mg/kg bw/day for a typical 3 year-old child.

As the maximum reported levels of use provided to the Panel by industry were similar to the MPLs of use, only the Tier 2 approach has been assessed both for children and the adult populations. Refined exposure estimates have been performed combining detailed individual food consumption information from the population with the MPLs of use and/or the maximum reported use levels.

For children (1-14 years old) exposure estimates have been calculated using data of fish roe consumption from six European countries (Cyprus, Finland, Germany, Greece, Sweden and UK). For the adult population, the Panel selected the UK population as representative of EU consumers for Amaranth intake estimates. When considering MPLs of use and/or maximum reported levels of use, the estimated mean anticipated dietary exposure to Amaranth for European children (aged 1-14 years old), ranged from 0 to 0.0006 mg/kg bw/day, and from 0 to 0.04 mg/kg bw/day for high level (95th percentile) consumers of fish roe. Estimates reported for the UK adult population give a mean anticipated dietary exposure to Amaranth of 0.008 mg/kg bw/day and of 0.88 mg/kg bw/day for high level (97.5th percentile) consumers of alcoholic beverages. The main contributors to the total anticipated exposure to Amaranth (>10%) were aperitif wine drinks (60%) for average consumers and Americano (99%) for high consumers.

The Panel notes that the specifications of Amaranth need to be updated with respect to the percentage of material not accounted for that may represent sodium chloride or sodium sulphate as the principal uncoloured components.

The Panel also notes that the JECFA specification for lead is <2 mg/kg whereas the EC specification is < 10 mg/kg.

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

CONCLUSIONS

Amaranth (E 123) is a mono-azo dye authorised as a food additive in the EU and previously evaluated by the SCF (1976, 1979, 1983) and JECFA (1972, 1975, 1978, 1984). In 1984, the SCF set an ADI for

Amaranth of 0-0.8 mg/kg bw/day based on results from a 90-day rat study. In contrast, in 1984 JECFA allocated an ADI of 0-0.5 mg/kg bw/day based on a long-term carcinogenicity study in rats.

In evaluating the overall toxicological database on Amaranth, the Panel now establishes an ADI of 0.15 mg/kg bw/day.

The Panel concludes that at the maximum permitted level of use and/or reported use levels of Amaranth (Tier 2), estimates of anticipated exposure for 1- to 14-year old children are around 30 times lower than the ADI of 0.15 mg/kg bw/day at the high percentiles (95th/97.5th). However, for adults the anticipated exposure to Amaranth at the high percentile (97.5th) can be up to 6 times higher than the ADI.

The Panel also notes that main contributors to total anticipated exposure for adults were from aperitif wine drinks and Americano. The Panel notes that anticipated exposure to these food uses have been made with the maximum permitted level of use for Americano, although no usage value was provided by industry for this beverage, and with the maximum reported levels of use for aperitif wine drinks which were reported by Industry to be at the same level as the maximum permitted level.

The Panel further notes that the specifications for Amaranth need to be updated with respect to the percentage of material not accounted for that may represent sodium chloride or sodium sulphate as the principal uncoloured components.

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

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

DOCUMENTATION PROVIDED TO EFSA

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2. CIAA (Confederation of the Food and Drink Industries of the EU), 2009. CIAA data in response to the Commission request for data: "EFSA re-evaluation of food colours" - Southampton study colours) (SANCO/E3/OS/km D 53007, May 22, 2009).
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ANNEX A

Rules defined by the Panel to deal with *quantum satis* (QS) authorisation, usage data or observed analytical data for all regulated food additives to be re-evaluated and procedures for estimating intakes using these rules

1. Decision rules taken to deal with QS authorisations for MPL: (see the decision tree in Figure 1)

- a. If the category ‘All other foodstuff’ is QS, the highest observed MPL value should be used, which is 500 mg/kg
- 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
 - ii. If many values are available for more than one colour, the highest value is used
 - iii. If there is no available value, 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 QS:** If available use the pork-based products use level; if there is no value available, the highest MPL of 500 mg/kg is used.
- **Edible cheese rinds:** The MPL of 100 mg/kg (from the flavoured processed cheese category) is used, except for E 120 (Cochineal) whose level is 125 mg/kg for red marbled cheese.

2. Rules to identify the maximum reported use levels to be used for the refined exposure assessment:

A maximum reported use level is the maximum value selected from reported usage by industry and analytical data provided to the Panel:

- a. If the identified maximum reported use level is greater than or equal to the actual MPL, then the actual MPL is used by default.
- b. If both maximum analytical and maximum current use level data are available, priority is given to the use level data, even if analytical values are lower or higher; the selected value is rounded to the nearest whole number.
- c. If no use level data are available, because either no uses were reported or industry was not asked to provide them, the choice is made between the highest analytical value or the MPL:
 - i. if more than 10 analytical data are available, the highest quantified reported value is used;
 - ii. if less than 10 analytical data are available, the MPL is used.

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

3. Tiered approach to intake estimation

The basic principles of the stepwise approach for the estimation of additives' intakes involve, for each successive Tier, a further refinement of intakes from the conservative estimates for screening (Tier 1) to more realistic estimates (Tier 2 and 3) (EC, 2001). Depending on the information on use levels data available, the three screening tiers approach must be adapted (see Figure 2 for the decision rules).

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

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

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

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

In Tier 2 and 3, the following approach is used to calculate the high percentile consumption: The high consumption should be calculated by examining the 97.5th percentile of food additive intake per food group, and selecting the highest intake* and then adding this value to the sum of the mean intakes for the remaining food groups. This approach is slightly different to the usual approach, in which the two highest food group intakes at the 97.5th percentile of additive intakes are added to the mean consumption of the other food groups. The approach was modified based on evaluation of the Expochi study, as it provides a more realistic estimate of exposure.

*High consumption value of Fruit wines (still or sparkling), Cider (except cidre bouche) and perry, Aromatized fruit wines, cider and perry from UK adult data is not taken into account for the calculation of high percentile exposure when this food category appeared to be the highest P95 exposure. In this case the second highest contributor is taken in the calculation.

GLOSSARY AND ABBREVIATIONS

ADHD	Attention-Deficit Hyperactivity Disorder
ADI	Acceptable Daily Intake
AFC	Scientific Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food
AFSSA	Agence Française de Sécurité Sanitaire des Aliments
Aluminium lakes	Aluminium lakes are produced by the absorption of water soluble dyes onto a hydrated aluminium substrate rendering the colour insoluble in water. The end product is coloured either by dispersion of the lake into the product or by coating onto the surface of the product
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
BIBRA	British Industrial Biological Research Association
CAS	Chemical Abstracts Service
CEPS	The European Spirits Organisation
CIAA	Confederation of the Food and Drink Industries of the EU
DG SANCO	The Directorate General for Health and Consumers
DFG	Farbstoffkommission der Deutschen Forschungsgemeinschaft
DMSO	dimethyl sulfoxide
EC	European Commission
EFSA	European Food Safety Authority
ELC	The Federation of European Food Additives, Food Enzymes and Food Culture Industries
ESR	Electron Spin Resonance
EXPOCHI	Refers to EFSA Article 36 2008 call for Proposals Focused on Children and Food Consumption
FMN	Flavin Mononucleotide
FDA	Food and Drug Administration
FSA	UK Food Standard Agency
FSAI	Food Safety Authority of Ireland
GHA	Global Hyperactivity Aggregate
HPLC-DAD	High-performance liquid chromatography
HPTLC	High-performance Thin Layer Chromatography
IARC	International Agency for Research on Cancer

ILSI	International Life Sciences Institute
IR	InfraRed
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
LEMM	Laboratoire d'Etudes du Métabolisme des Médicaments
LOAEL	Low-Observed-Adverse-Effect-Level
LOD	Limit Of Detection
LOQ	Limit of Quantification
MPL	Maximum Permitted Levels
MTD	Maximum Tolerated Dose
NOAEL	No Observed Adverse Effect Level
NADPH	Nicotinamide adenine dinucleotide phosphate
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic Erythrocyte
RCC	Research and Consulting Company
SCF	Scientific Committee on Food
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers
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
SCMPMD	Scientific Committee on Medicinal Products and Medical Devices
UNESDA	Union of European Beverage Associations
TWI	Tolerable Weekly Intake
WHO/FAO	World Health Organization/Food and Agriculture Organization